BRB-04613RRRR

Unilateral application of an inflammatory irritant to the rat temporomandibular joint region produces bilateral modulation of the jaw-opening reflex

Makiko Noguchi [a], Masayuki Kurose [a], Kensuke Yamamura [a], Makoto Inoue [a], Yo Taguchi [a], Barry, J. Sessle [b], Yoshiaki Yamada [a]

[a] *Division of Oral Physiology, Department of Oral Biological Science, Niigata University Graduate School of Medical and Dental Sciences, 2-5274 Gakkocho-dori, Niigata 951-8514, Japan*

[b] *Faculty of Dentistry, University of Toronto, 124 Edward Street, Toronto, Ontario M5G 1G6, Canada*

Corresponding author: Kensuke Yamamura, D.D.S., Ph.D.

Division of Oral Physiology, Department of Oral Biological Science, Niigata University Graduate School of Medical and Dental Sciences 2-5274 Gakkocho-dori, Niigata 951-8514, Japan

Tel: +81-25-227-2824

Fax: +81-25-225-0281

E-mail: yamamura@dent.niigata-u.ac.jp

Running head: Modulation of jaw-opening reflex

Title: Unilateral application of an inflammatory irritant to the rat temporomandibular joint region produces bilateral modulation of the jaw-opening reflex

Running head: Modulation of jaw-opening reflex

Abstract

The aim of this study was to determine the effect of unilateral acute inflammation of craniofacial deep tissues on the ipsilateral and contralateral jaw-opening reflex (JOR). The effects of Mustard oil (MO), injected into the temporomandibular joint region, were tested on the JOR recorded in the digastric muscle and evoked by low-intensity electrical stimulation of the ipsilateral and contralateral inferior alveolar nerve in anesthetized rats. The MO injection induced a long-lasting suppression of the amplitude of both ipsilaterally and contralaterally evoked JOR, although the latency and duration of the JOR were unaffected. The suppressive effect was more prominent for the contralaterally evoked JOR, and observed even when background activity in the digastric muscle was increased by the MO injection. The results indicate that changes in the JOR amplitude following MO injection do not simply reflect alterations in motoneuronal excitability, and suggest that inflammation of deep craniofacial tissues modulates low-threshold sensory transmission to the motoneurons.

Keywords: Jaw-opening reflex, Temporomandibular joint, Inflammatory pain, Mustard oil, Rat, Modulation

Introduction

Damage or inflammation of deep craniofacial tissues such as the temporomandibular joint (TMJ) or masticatory muscles are often considered important in the pathophysiology of temporomandibular disorders (TMD) [15]. A number of studies have shown that jaw motor functions may be altered in TMD patients [6,7,14,22,32,33]. A possible reason for such alterations in jaw movements could be modification of craniofacial sensory transmission to jaw motoneurons, since the activity of jaw motoneurons greatly depends on sensory feedback from craniofacial structures [6].

The jaw-opening reflex (JOR) is a trigeminal brainstem reflex that can be evoked by either nociceptive or non-nociceptive craniofacial stimulation (e.g., [6] [20]). The shortest reflex arc involves only two synapses; primary afferents synapse on sensory relay neurons in the spinal nucleus of the trigeminal brainstem complex, and these neurons project to and drive digastric motoneurons bilaterally [17]. The JOR involves a transient excitation of the jaw-opening muscles and inhibition of the jaw-closing muscles (e.g., [20]). Therefore, the JOR is useful for the assessment of sensorimotor processing in the trigeminal region [8,9]. Experimental models of deep craniofacial pain have reported that the inhibitory response of the jaw-closing muscles evoked by noxious stimulation to peri-oral

tissues can be suppressed in the presence of masticatory muscle pain in man, and have suggested that the inhibition occurs at the premotoneuronal level [34,38]. Indeed, a number of studies have shown that the JOR evoked by noxious stimulation can be suppressed by activation of pain-modulatory systems in anesthetized animals, with a concomitant suppression of the sensory relay neurons (e.g., [2-4,16,27,31,35,36]).

However, it is still not clear if the JOR evoked by low-intensity stimulation can be modulated by noxious conditioning stimulation of deep craniofacial tissues. Kurose et al. have recently shown that the injection of mustard oil (MO), an inflammatory irritant and small-fiber excitant, into the temporalis muscle can induce a significant suppression of the JOR evoked by low-threshold stimulation of the inferior alveolar nerve (IAN) [19]. However, the effect of the MO injection was tested only for the reflex response evoked by stimulation of the IAN ipsilateral to the MO injection. Unlike spinal reflexes, stimulation on one side of the craniofacial region evokes synchronized JOR responses on both sides (e.g., [11,12,18,24]). Clarification of the effects of unilateral inflammation of deep craniofacial tissues could require testing of the effects of unilateral noxious craniofacial stimulation on the JOR evoked by stimulation of ipsilateral and contralateral IAN. Therefore, the present study was undertaken to test the effects of unilateral injection of MO

into the rat TMJ region on the JOR evoked by low-threshold stimulation of the IAN both ipsilateral and contralateral to the MO injection.

Materials and Methods

Surgical procedures

The experiments were carried out in 27 male rats (Wistar albino, 250-270 g) in accordance with the "Principles of Laboratory Animal Care" (NIH publication #86-23, revised 1996). The animal protocols were approved by the Intramural Animal Care and Veterinary Science Committee of Niigata University. Animals were initially anesthetized with 2-3% halothane. Two percent lidocaine was injected into the skin to minimize surgical pain before incisions were made. Cannulae were inserted into the trachea and the femoral vein for respiration and for drug administration, respectively; and anesthesia was then maintained with a mixture of α-chloralose (50 mg/kg) and urethane (500 mg/kg) injected via the femoral vein. The adequacy of anesthesia was checked repeatedly throughout the experiment by pinching the paws; if a withdrawal reflex was elicited, a supplementary dose of chloralose-urethane mixture was given. Rectal temperature was measured and maintained around 37.5 °C by a servo-controlled heating pad (BWT-100, BRC, Nagoya,

Japan).

Paired copper wire electrodes (0.12 mm in diameter, 3 mm interpolar distance) with an exposed tip (1 mm) were implanted into the left masseter (Mas; a jaw-closing muscle) and the left digastric (Dig; a jaw-opening muscle) to record their electromyographic (EMG) activity. To stimulate the IAN, a pair of Teflon-coated stainless-steel wire electrodes (0.1 mm in diameter, tip exposure 0.5 mm) was inserted into each of the right and left mental foramina (1 mm deep for the anode and 3 mm deep for the cathode), and were fixed on the adjacent mandibular bone with adhesive dental acrylic (SUPERBOND C&B, SUN MEDICAL, Shiga, Japan). The animal's head was then placed in a stereotaxic frame, and the skin over the dorsal surface of the skull was reflected and four screws were implanted into the frontal and parietal bone. To facilitate access to the TMJ region without any interference, the screws were attached to a vertical support bar with dental acrylic (UNIFAST II, GC, Tokyo, Japan) and then the ear and incisor bars were removed.

Stimulations and recordings

To evoke the JOR, the right and left IAN were alternately stimulated (single pulse, 0.2 ms duration) at an interval of 5 s (test stimuli). The threshold of the JOR was

determined as the minimum IAN stimulus current that consistently evoked detectable EMG reflex responses. During data recording, the stimulus current was set at 1.5 times the reflex threshold (T). EMG activity was amplified with custom-built AC amplifiers (band pass: 0.1-3 kHz), and the signals were fed into a computer equipped with a CED Power 1401 board and analysis software (Spike2; Cambridge Electronic Design Ltd., Cambridge, UK). The sampling rate for the EMG activity was 5000/s. Recorded EMG activity was stored electronically and analyzed offline. The stimulus pulses for the IAN test stimuli were also fed into a computer with a CED Power 1401 board as event signals.

The 27 animals were divided into two groups; one (n=17) consisted of animals in which MO (Wako Pure Chemical Industries Ltd, Osaka, Japan, 20% in mineral oil, 40 μl) was injected into the left TMJ region, and the other (n=10) consisted of animals in which the same amount of mineral oil (the vehicle for MO, 40 μl). In each animal group, a 30-gauge needle connected to a 50 μl Hamilton syringe with a polyethylene tube was passed through the TMJ capsule into the left TMJ and fixed in place. After an interval of at least 30 min, repetitive IAN test stimuli were started; baseline EMG activity and evoked JOR responses were recorded for 10 min (control period), and then the MO (or vehicle) was injected into the TMJ region over 10-20 s. After the MO (or vehicle) injection, EMG

recordings were continuously made for 120 min. Preliminary experiments had revealed that consecutive test stimuli of the right and left IANs in an alternating fashion lasting more than 2 hours did not induce any detectable effects on the JOR.

At the completion of the experiment, brilliant blue dye (40 μl) was injected into the TMJ region through the same needle used for the MO or vehicle injection. Next, the animal was administrated a lethal dose of sodium pentobarbital, and the injection site and EMG electrode locations were confirmed by post-mortem dissection. The posterior part of the TMJ capsule usually was most densely stained by brilliant blue dye, but the dye also spread to adjacent connective tissues. If the TMJ capsule was not stained densely, the data was excluded from the analyses.

Data analysis

In the present experiment, only the JOR evoked in the Dig ipsilateral to the MO injection was assessed. Parameters of the JOR that were assessed were mean latency, amplitude (peak to peak) and duration. Peak to peak values were calculated using raw data. To assess latency and duration, EMG signals were full-wave rectified and smoothed (time constant 20 ms) with the Spike2 analysis software. To define the onset and offset of each JOR, baseline EMG activity in each muscle was calculated for 2 min during the control

period, and the onset of the JOR was defined as the point in time when the evoked EMG activity exceeded 2 SD from baseline EMG activity. Likewise, the offset was defined as the point in time when the evoked EMG activity fell below 2 SD from the baseline EMG activity. To elucidate the sequential effect of the injection of MO or vehicle on the JOR, mean values of 60 reflexes during the control period were calculated for each parameter and were considered to be the control values. Then, the mean values of five consecutive JOR responses at each time point of 0, 1, 2, 3, 4, 5, 6, 10, 15, 20, 30, 45, 60, 90, 120 min after the initiation of the chemical injection were calculated. The mean values for each time point were normalized to the control value and compared with the control.

In addition to the reflex analyses, the effect of the injection of MO or vehicle into the TMJ on baseline EMG activity was analyzed. Increases in EMG activity after the injection were regarded as significant if EMG activity exceeded 2 SD from the baseline EMG activity before the chemical injection. When a significant increase in EMG activity was noted, its duration and amplitude (area) were calculated.

Effects of the injection on the JOR were statistically evaluated with a Friedman one-way repeated measures ANOVA on ranks and post-hoc comparisons (Tukey test). A Mann-Whitney Rank Sum Test was used to compare the parameters between the JOR

responses evoked by left IAN stimulation (ipsilaterally evoked JOR: the JOR evoked by the stimulation of the IAN ipsilateral to the MO injection and EMG recordings) to those evoked by right IAN stimulation (contralaterally evoked JOR) at each time point. Also for comparisons of the parameters between muscles, a Mann-Whitney Rank Sum Test was used. Values were expressed as mean +/- SD, and P values less than 0.05 were regarded as significant.

Results

Properties of the jaw-opening reflex before the MO injection

The JOR was evoked by stimulation of the IAN either ipsilateral (left) or contralateral (right) to the EMG recordings. The reflex threshold was 25.6 +/- 17.1 μA (n=27) for the ipsilaterally evoked JOR and 27.1 +/- 13.3 μA (n=27) for the contralaterally evoked JOR. When the stimulus current was 1.5T, the reflex latency was 4.7 +/- 0.1 ms (n=27) for the ipsilaterally evoked JOR and 5.0 +/- 0.4 ms (n=27) for the contralaterally evoked JOR. The latency of the ipsilaterally evoked JOR was significantly shorter than that of the contralaterally evoked JOR. The amplitude of the reflex was 3.4 +/- 1.2 mV (n=27) for the ipsilaterally evoked JOR and 3.0 +/- 1.3 mV (n=27) for the contralaterally evoked

JOR. The duration of the reflex was 3.9 +/- 0.8 ms (n=27) for the ipsilaterally evoked JOR and 3.8 +/- 0.8 ms (n=27) for the contralaterally evoked JOR. No significant difference was noted for amplitude and duration between the sides.

Effects of the MO injection

The injection of vechicle into the TMJ region did not cause any significant changes in the baseline EMG activity in each muscle, or in latency, amplitude and duration of the JOR throughout the recording period (n=10, see Figure 2A for JOR amplitude). On the other hand, the injection of MO into the TMJ region evoked a sustained reflex increase in EMG activity in both Dig and the Mas muscles (Figure 1A). Such sustained EMG activity was evoked immediately after the injection of MO in all animals tested (n=17). The duration of sustained EMG activity was 136 +/- 92 s for the Dig and 109 +/- 59 s for the Mas (n=17, Fig. 2B). The area under the EMG response curve was 772 +/- 653 A/D unit for the Dig and 1060 +/- 987 A/D unit for the Mas (n=17). No significant difference was noted in either duration or amplitude (area) of sustained EMG activity between the muscles (Mann-Whitney Rank Sum Test).

A significant alteration also occurred to the amplitude of the JOR after the MO

injection, although its latency and duration were not significantly affected (Figure 1B-E). Figure 2 shows the time course of the amplitude of the JOR after the MO injection. The amplitude of both the ipsilaterally evoked JOR and the contralaterally evoked JOR was significantly decreased immediately after the MO injection (76.3 +/- 29.7% of control for the ipsilaterally evoked JOR, 53.7 +/- 34.6% for the contralaterally evoked JOR, n=17, see Figure 1C). This immediate suppression was observed even during the sustained increase in the Dig EMG activity (see Figures 1A, C). The suppressive effect on the JOR was interrupted between 2 to 6 min after the MO injection, and then reappeared. Thus, two peaks were noted for the suppressive effect; one appearing just after the MO injection and the other at 30 min after the MO injection for the ipsilaterally evoked JOR (83.1 +/- 18.2% of control, n=17) or at 10 min after the MO injection for the contralaterally evoked JOR (53.5 +/- 32.2% of the control, n=17) (Figure 2B). The suppression then gradually declined with time, with activity returning to 93.0 +/- 10.4% of the control level for the ipsilaterally evoked JOR (n=17) or to 103.7 +/- 23.1 % for the contralaterally evoked JOR (n=17) at 120 min after the MO injection (see Figure 2B, also see Figure 1E). A significant decrease in the JOR amplitude was noted from immediately after the MO injection and up to 30 min after the MO injection for the contralaterally evoked JOR or at 0, 15 and 30 min after the MO

injection for the ipsilaterally evoked JOR ($p<0.05$, Friedman one-way repeated measures ANOVA on ranks followed by Tukey post-hoc test)(Figure 2). While the time course of the MO-induced suppressive effect on the JOR was generally the same between sides, the effect was more prominent for the contralaterally evoked JOR than that for the ipsilaterally evoked JOR. A significant difference in the strength of the suppressive effect between the ipsilaterally evoked JOR and contralaterally evoked JOR was noted at the time points of 4, 5 and 10 min after the MO injection ($p<0.05$, Mann-Whitney Rank Sum Test).

Discussion

The present study has shown that noxious stimulation of deep craniofacial tissues by the injection of MO into the TMJ region modulates the JOR evoked by low-intensity stimulation of the IAN either ipsilateral or contralateral to the MO injection. The JOR is known to be evoked by either nociceptive or non-nociceptive craniofacial stimulation (e.g., [6,20]). The afferent fibers mediating the non-nociceptive JOR are large myelinated fibers, since the reflex can be evoked by a stimulus slightly higher, or even equal to, the threshold of the trigeminal afferent fibers (e.g., [6]). In this study, we cannot entirely reject the possibility that afferent fibers other than large myelinated fibers were activated. However,

the intensity of the IAN stimulus (<80 μA) was lower than that used in awake animals (at most 130 μA) and could be considered "not painful" from a behavioral basis (see [13,39]). This suggests that the majority of the afferent fibers mediating the IAN-evoked JOR in the present study were those innervating peri- or intra-oral mechanoreceptors (i.e., large myelinated fibers).

The MO injection into the TMJ region induced a sustained increase in baseline EMG activity both in the Dig (jaw-opening muscle) and Mas (jaw-closing muscle). Such sustained increase in EMG activity following the MO injection into the TMJ region has been reported in many studies (e.g., [1,37,45]). The trigeminal subnucleus caudalis (Vc) has been shown to be a critical element in the neural pathways underlying such sustained activity [37]. The muscle synergies encountered in the sustained response (co-activation of jaw-opening and jaw-closing muscles induced by noxious stimulation of deep tissues) differed from those encountered in the JOR (activation of jaw-opening muscles and inhibition of jaw-closing muscles induced by low-intensity stimulation), suggesting that these two responses may involve different neural pathways, i.e. the former principally involving Vc [37] and the latter more rostrally located trigeminal sensory nuclei (e.g., subnucleus oralis) [17,25,40].

It was notable that the JOR was suppressed even during the MO-evoked sustained increase in baseline EMG activity in the Dig and Mas. This finding indicates that the JOR was suppressed even when Dig motoneuronal excitability was increased by nociceptive sensory inputs from the TMJ region, and suggests that changes in the JOR amplitude do not simply reflect Dig motoneuronal excitability. In addition, a significant difference in the suppressive effect was noted between the JOR evoked by stimulation of the IAN ipsilateral to the MO injection and that evoked by stimulation of the IAN contralateral to the MO injection. Since these evoked reflex responses were recorded from the same muscle (i.e., the Dig ipsilateral to the MO injection), we can rule out the possibility that the difference in the modulatory effects between the stimulation sides was due to differences in motoneuronal excitability. This suggests that suppression on the JOR was the result of a decrease in bilateral IAN excitatory sensory inputs to Dig motoneurons ipsilateral to the MO injection.

Inhibition of sensory transmissions at the sensory relay neurons on each side could explain the changes in the bilateral IAN excitatory inputs to the Dig motoneurons. Previous studies have shown that stimulation of the periaqueductal gray matter or the nucleus raphe magnus, major elements of the descending pain-modulatory system, suppresses the JOR [3]

and the responsiveness of sensory relay neurons in the trigeminal spinal nucleus to both noxious and non-noxious stimuli [5,27,29], and that the suppressive effect can be reversed only partially by systemic administration of the opiate antagonist naloxone [29]. In addition, we have recently shown that injection of MO into the temporalis muscle, a deep craniofacial tissue, suppressed the JOR evoked by non-nociceptive IAN stimulation, and that the effect was naloxone insensitive [19]. Furthermore, in the spinal system, local application of 5-HT *in vitro* increased the incidence of primary afferent-evoked long-term depression in rat deep dorsal horn neurons where a considerable number of non-nociceptive as well as nociceptive primary afferent neurons terminate [10]. These various findings suggest that the injection of MO into the TMJ region may have activated the descending pain-modulatory system that caused the suppression of sensory transmission from the IAN to sensory relay neuron (i.e., in the trigeminal spinal nucleus), and thereby suppression of the IAN-evoked JOR, but that an endogenous opioid may not have played a crucial role in these effects.

Although the suppression was a major MO-induced effect on the JOR, it was notable that the MO-induced suppressive effect was phasically reduced from 2 to 6 min after the MO injection for the reflex evoked by either side of the IAN; this suggests that

facilitation of the JOR had been also induced by the MO injection. In this respect, Yu et al. showed that the injection of MO into the tongue muscle [44] or TMJ [46] induced an expansion of low-threshold as well as high-threshold cutaneous mechanoreceptive fields in trigeminal sensory relay neurons responding to both nociceptive and non-nociceptive stimulation of cutaneous and deep tissues. The finding indicates that the MO injection into deep craniofacial tissues induces an increase in excitability in such neurons. Thus, the present demonstration that the MO-induced suppressive effect was more prominent for the contralaterally evoked JOR could be interpreted as follows: 1) the responsiveness to low-threshold stimulation in the so-called WDR neurons receiving convergence from cutaneous (i.e., IAN) and deep (i.e., TMJ) tissues and projecting to the Dig motoneurons was increased by the MO injection into the TMJ region; 2) the increase in excitability was more prominent in neurons ipsilateral to the MO injection, but 3) the effect was generally "masked" by the suppressive effect on the sensory transmission, as discussed above.

The present results showed that unilateral MO-induced acute inflammation in the TMJ region induces long-lasting modulatory effects on the JOR evoked by low-intensity stimulation. The suppression may prevent rapid jaw movements evoked by low-threshold orofacial sensory inputs, and with a synergistic increase in baseline EMG activity in both

jaw-opening and jaw-closing muscles in the presence of deep tissue inflammation may help "muscle splinting" and thereby protect the masticatory system from excessive damage [26,28]. However, such modulatory effects on trigeminal sensorimotor processing might impair orofacial movements such as mastication. Unexpected perturbations and changing environmental conditions (e.g. the consistency of ingested food) frequently occur during mastication. Anatomical and electrophysiological studies have shown that a number of trigeminal premotor neurons receive non-nociceptive craniofacial sensory inputs (e.g., [30, 41, 42, 43]). The findings suggest that low-threshold craniofacial sensory inputs play important roles in reflexly guiding jaw movements during masticatory movements so that the resultant movements can be adapted for ongoing mastication. In addition, there is considerable evidence that the JOR is modulated in a phase-dependent manner during mastication (i.e. opening, fast-closing and slow-closing phases) [13,21,39], and that such a modulatory system plays an important role in regulating jaw movements during mastication (see [20,23] for review). The modulatory mode of the JOR during mastication in the presence of deep tissue inflammation may be different from that in normal conditions, which can result in the impairment of movements. However, further experiments will be required in functional settings that more closely relate to such pathophysiological

Acknowledgements

This study was partly supported by Grants-in-Aid for Scientific Research from the Ministry of Education, Culture and Science of Japan (#14771019 to K.Y. #14571760 to M.I. and # 14207077 to Y.Y.).

Figure legends

Figure 1.

Example of recordings before and after the injection of MO into the left TMJ region. A shows the effect of a MO injection on baseline EMG activity. The bar in the uppermost part indicates the period of the MO injection. Sustained increases in baseline EMG activity were induced by the injection of MO in both the masseter and digastric muscles. B-E show examples of the jaw-opening reflex evoked by stimulation of the inferior alveolar nerve during the control period (B), immediately after the MO injection (C: indicated by a dotted line in A), 10 min after the MO injection (D) and 60 min (E) after the MO injection in the same animal shown in A. Lower traces show the reflex responses at selected time points in the upper traces (indicated by open or filled circles) with an expanded time base. Arrowheads in the lower traces indicate stimulus artifacts. Thresholds of the jaw-opening reflex evoked were 31 μA for the left (ipsilateral to the EMG recordings and the MO injection) IAN stimulation and 21 μA for the right (contralateral to the EMG recordings and the MO injection). The MO injection induced suppressive effects on the reflex, but the effect was more prominent for the reflex evoked by the right IAN stimulation than that evoked by the left IAN stimulation. Note that the magnification of the EMG is much higher in A than B-E, so most of the reflex responses in the left digastric muscle are out of range in A. Also note that stimulus artifacts are included in the EMG activity in the left masseter. R-stim: right inferior alveolar nerve stimulation, L-stim: left inferior alveolar nerve stimulation, L-Mas: EMG of the left masseter muscle, L-Dig: EMG of the left digastric muscle.

Figure 2.

Effect of an injection of mineral oil (A) or MO (B) into the TMJ region on the JOR. Time courses of the amplitude (peak to peak) of the JOR evoked by stimulation of the IAN ipsilateral to the MO injection (Ip IAN) and that by stimulation of the IAN contralateral to the MO injection (Ct IAN) are shown. Each point represents the mean + SD for Ip IAN and mean – SD for Ct IAN. The horizontal dotted line represents the mean reflex amplitude during the control period. Two horizontal bars on the top of the graph in B indicate the duration (mean + SD) of the sustained increase in the baseline activities in the Dig and Mas.

*: A significant difference was noted when the value was compared with the control ($P<0.05$, Friedman one-way repeated measures ANOVA on ranks and Tukey post-hoc test).

†: A significant difference was noted between the Ip-IAN and Ct-IAN ($p<0.05$, Mann-Whitney Rank Sum Test). See text for details.

References

[1] J.G Broton, B.J. Sessle, Reflex excitation of masticatory muscles induced by algesic chemicals applied to the temporomandibular joint of the cat, Arch. Oral Biol. 33 (1988) 741-747.

[2] C.Y. Chiang, I.C. Chen, J.O. Dostrovsky, B.J. Sessle, Inhibitory effect of stimulation of the anterior pretectal nucleus on the jaw-opening reflex, Brain Res. 497 (1989) 325-333.

[3] C.Y. Chiang, J.O. Dostrovsky, B.J. Sessle, Periaqueductal gray matter and nucleus raphe magnus involvement in anterior pretectal nucleus-induced inhibition of jaw-opening reflex in rats, Brain Res. 544 (1991) 71-78.

[4] J.O. Dostrovsky, B.J. Sessle, J.W. Hu, Presynaptic excitability changes produced in brain stem endings of tooth pulp afferents by raphe and other central and peripheral influences, Brain Res. 218 (1981) 141-160.

[5] J.O. Dostrovsky, Y. Shah, B.G Gray, Descending inhibitory influences from periaqueductal gray, nucleus raphe magnus, and adjacent reticular formation. II. Effects on medullary dorsal horn nociceptive and nonnociceptive neurons, J. Neurophysiol. 49 (1983) 948-960.

[6] R. Dubner, B.J. Sessle, A.T. Storey, The neural basis of oral and facial function, Plenum Press, New York, 1978.

[7] S.F. Dworkin, K.H. Huggins, L. LeResche, M. Von Korff, J. Howard, E. Truelove, E. Sommers, Epidemiology of signs and symptoms in temporomandibular disorders: clinical signs in cases and controls, J. Am. Dent. Assoc. 120 (1990) 273-281.

[8] J. Ellrich, Electric low-frequency stimulation of the tongue induces long-term depression of the jaw-opening reflex in anesthetized mice, J. Neurophysiol. 92 (2004) 3332-3337.

[9] J. Ellrich, M. Wesselak, Electrophysiology of sensory and sensorimotor processing in mice under general anesthesia, Brain Res. Protoc. 11 (2003) 178-188.

[10] S.M. Garraway, S. Hochman, Serotonin increases the incidence of primary afferent-evoked long-term depression in rat deep dorsal horn neurons, J. Neurophysiol. 85 (2001) 1864-1872.

[11] L.J. Goldberg, Excitatory and inhibitory effects of lingual nerve stimulation on reflexes controlling the activity of masseteric motoneurons, Brain Res. 39 (1972) 95-108.

[12] L.J. Goldberg, Y. Nakamura, Lingually induced inhibition of masseteric motoneurones, Experientia 24 (1968) 371-373.

[13] N. Haraguchi, Y. Yamada, H. Furusawa, K. Matsuo, K. Oi, T. Sato, A. Mizuno, Variation of the jaw-opening reflex during spontaneous mastication in rabbits, Brain Res. Bull. 35 (1994) 93-95.

[14] M. Helkimo, Studies on function and dysfunction of the masticatory system. IV. Age and sex distribution of symptoms of dysfunction of the masticatory system in Lapps in the north of Finland, Acta Odontol. Scand. 32 (1974) 255-267.

[15] J.W. Hu, C.-M. Tsai, M. Bakke, K. Seo, C.H. Tambeli, H. Vernon, D.A. Bereiter, B.J. Sessle, Deep craniofacial pain: Involvement of trigeminal subnucleus caudalis and its modulation., In Proceedings of the 8th world congress on pain, eds. T.S. Jensen, J. A. Turner and Z. Wiesenfeld-Hallin, IASP Press, Seattle, WA, 1997, pp. 497-506.

[16] A. Iriki, K. Toda, Suppression of tooth pulp evoked responses by activation of raphe-trigeminal neurons in rat, Brain Res. Bull. 8 (1982) 777-780.

[17] Y. Kidokoro, K. Kubota, S. Shuto, R. Sumino, Possible interneurons responsible for reflex inhibition of motoneurons of jaw-closing muscles from the inferior dental nerve, J. Neurophysiol. 31 (1968) 709-716.

[18] Y. Kidokoro, K. Kubota, S. Shuto, R. Sumino, Reflex organization of cat masticatory muscles, J. Neurophysiol. 31 (1968) 695-708.

[19] M. Kurose, K. Yamamura, M. Noguchi, M. Inoue, S. Ootaki, Y. Yamada, Modulation of jaw reflexes induced by noxious stimulation to the muscle in anesthetized rats, Brain Res. 1041 (2005) 72-86.

[20] J.P. Lund, Mastication and its control by the brain stem, Crit. Rev. Oral Biol. Med. 2 (1991) 33-64.

[21] J.P. Lund, S. Enomoto, H. Hayashi, K. Hiraba, M. Katoh, Y. Nakamura, Y. Sahara, M. Taira, Phase-linked variations in the amplitude of the digastric nerve jaw-opening reflex response during fictive mastication in the rabbit, Can. J. Physiol. Pharmacol. 61 (1983) 1122-1128.

[22] J.P. Lund, B.J. Sessle (Eds.), Temporomandibular joint and masticatory muscle disorders, Neurophysiological mechanisms, Munksgaard, Copenhagen, 1994, pp.188-207.

[23] Y. Nakamura, N. Katakura, Generation of masticatory rhythm in the brainstem, Neurosci. Res., 23 (1995) 1-19.

[24] Y. Nakamura, H. Nagashima, S. Mori, Bilateral effects of the afferent impulses from the masseteric muscle on the trigeminal motoneuron of the cat, Brain Res. 57 (1973) 15-27.

[25] K.A. Olsson, K.G Westberg, Integration in trigeminal premotor interneurones in

the cat. 2. Functional characteristics of neurones in the subnucleus-gamma of the oral nucleus of the spinal trigeminal tract with a projection to the digastric motoneurone subnucleus, Exp. Brain Res. 84 (1991) 115-124.

[26] B.J. Sessle, Masticatory Muscle Disorders: Basic Science Perspectives. In B.J. Sessle, P.S. Bryant, R.A. Dionne (Eds.), Temporomandibular disorders and related pain conditions, Vol. 4, IASP, Seattle, 1995, pp. 47-61.

[27] B.J. Sessle, J.W. Hu, Raphe-induced suppression of the jaw-opening reflex and single neurons in trigeminal subnucleus oralis, and influence of naloxone and subnucleus caudalis, Pain 10 (1981) 19-36.

[28] B.J. Sessle, J.W. Hu, Mechanisms of pain arising from articular tissues, Can. J. Physiol. Pharmacol. 69 (1991) 617-626.

[29] B.J. Sessle, J.W. Hu, R. Dubner, G.E. Lucier, Functional properties of neurons in cat trigeminal subnucleus caudalis (medullary dorsal horn). II. Modulation of responses to noxious and nonnoxious stimuli by periaqueductal gray, nucleus raphe magnus, cerebral cortex, and afferent influences, and effect of naloxone, J. Neurophysiol. 45 (1981) 193-207.

[30] Y. Shigenaga, A. Yoshida, K. Tsuru, Y. Mitsuhiro, K. Otani, C.Q. Cao, Physiological and morphological characteristics of cat masticatory

motoneurons--intracellular injection of HRP, Brain Res. 461 (1988) 238-256.

[31] M.L. Sotgiu, The effects of periaqueductal gray and nucleus raphe magnus stimulation on the spontaneous and noxious-evoked activity of lateral reticular nucleus neurons in rabbits, Brain Res. 414 (1987) 219-227.

[32] C.S. Stohler, Craniofacial pain and motor function: pathogenesis, clinical correlates, and implications, Crit. Rev. Oral Biol. Med. 10 (1999) 504-518.

[33] C.S. Stohler, J.A. Ashton-Miller, D.S. Carlson, The effects of pain from the mandibular joint and muscles on masticatory motor behaviour in man, Arch. Oral Biol. 33 (1988) 175-182.

[34] P. Svensson, A.S. McMillan, T. Graven-Nielsen, K. Wang, L. Arendt-Nielsen, Modulation of an inhibitory reflex in single motor units in human masseter by tonic painful stimulation, Pain 83 (1999) 441-446.

[35] H. Tanaka, K. Toda, Inhibition of the tooth pulp-evoked jaw opening reflex by stimulation of raphe nuclei in the rat, Exp. Neurol. 77 (1982) 102-112.

[36] K. Toda, A. Iriki, H. Tanaka, Jaw opening reflex affected by electroacupuncture in rat, Neurosci. Lett. 25 (1981) 161-166.

[37] C.M. Tsai, C.Y. Chiang, X.M. Yu, B.J. Sessle, Involvement of trigeminal

subnucleus caudalis (medullary dorsal horn) in craniofacial nociceptive reflex activity, Pain 81 (1999) 115-128.

[38] K. Wang, P. Svensson, L. Arendt-Nielsen, Modulation of exteroceptive suppression periods in human jaw-closing muscles by local and remote experimental muscle pain, Pain 82 (1999) 253-262.

[39] K. Yamamura, M. Inoue, N. Igarashi, Y. Takahashi, Y. Yamada, Effects of food consistency on the modulatory mode of the digastric reflex during chewing in freely behaving rabbits, Brain Res. 796 (1998) 257-264.

[40] A. Yoshida, Y.C. Bae, Y. Shigenaga, B.J. Sessle, Physiology and morphology of the neuronal circuits of jaw reflexes in cats. In A. Taylor, M.H. Gladden, R. Durbaba (Eds.), Alpha and Gamma Motor Systems, Plenum Press, New York, 1995, pp. 57-59.

[41] A. Yoshida, H. Fukami, Y. Nagase, K. Appenteng, S. Honma, L.F. Zhang, Y.C. Bae, Y. Shigenaga, Quantitative analysis of synaptic contacts made between functionally identified oralis neurons and trigeminal motoneurons in cats, J. Neurosci. 21 (2001) 6298-6307.

[42] A. Yoshida, T. Hiraga, M. Moritani, K. Chen, Y. Takatsuki, Y. Hirose, Y.C. Bae, Y. Shigenaga, Morphologic characteristics of physiologically defined neurons in the cat

trigeminal nucleus principalis, J. Comp. Neurol. 401 (1998) 308-328.

[43] A. Yoshida, K. Yasuda, J.O. Dostrovsky, Y.C. Bae, M. Takemura, Y. Shigenaga, B.J. Sessle, Two major types of premotoneurons in the feline trigeminal nucleus oralis as demonstrated by intracellular staining with horseradish peroxidase, J. Comp. Neurol. 347 (1994) 495-514.

[44] X.M. Yu, B.J. Sessle, J.W. Hu, Differential effects of cutaneous and deep application of inflammatory irritant on mechanoreceptive field properties of trigeminal brain stem nociceptive neurons, J. Neurophysiol. 70 (1993) 1704-1707.

[45] X.M. Yu, B.J. Sessle, H. Vernon, J.W. Hu, Effects of inflammatory irritant application to the rat temporomandibular joint on jaw and neck muscle activity, Pain 60 (1995) 143-149.

[46] X.M. Yu, B.J. Sessle, D.A. Haas, A. Izzo, H. Vernon, J.W. Hu, Involvement of NMDA receptor mechanisms in jaw electromyographic activity and plasma extravasation induced by inflammatory irritant application to temporomandibular joint region of rats, Pain 68 (1996) 169-178.

Figure 1

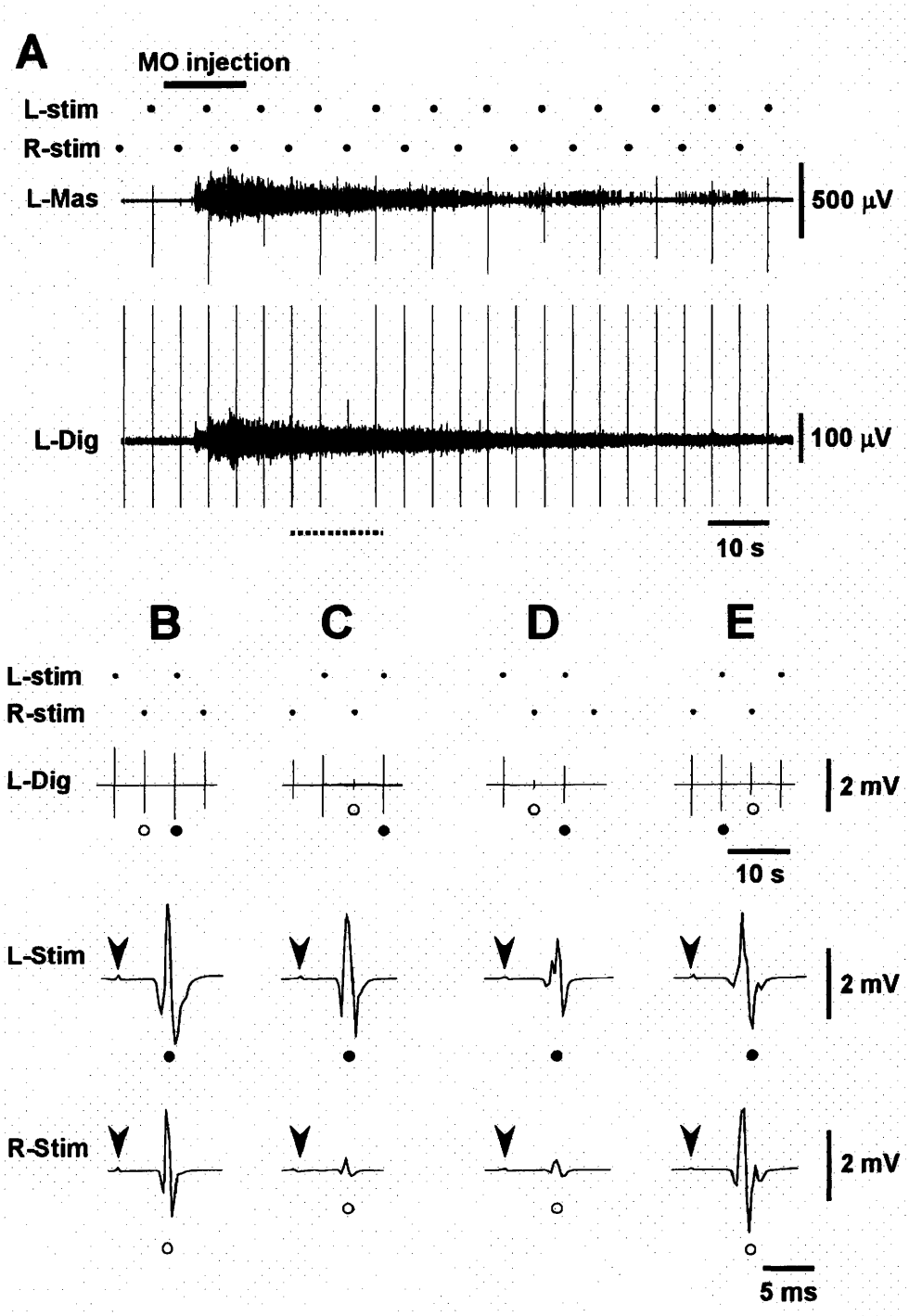
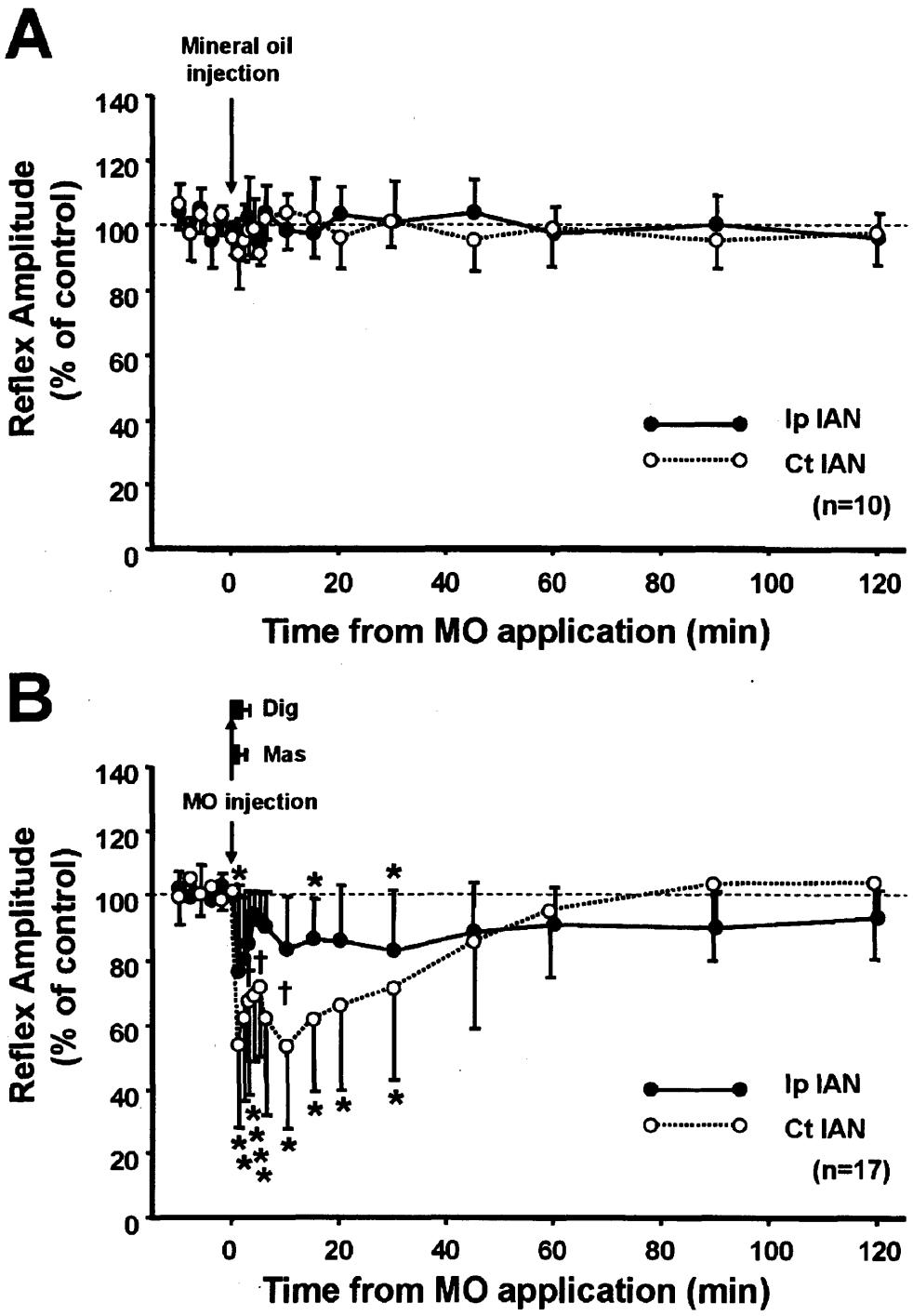
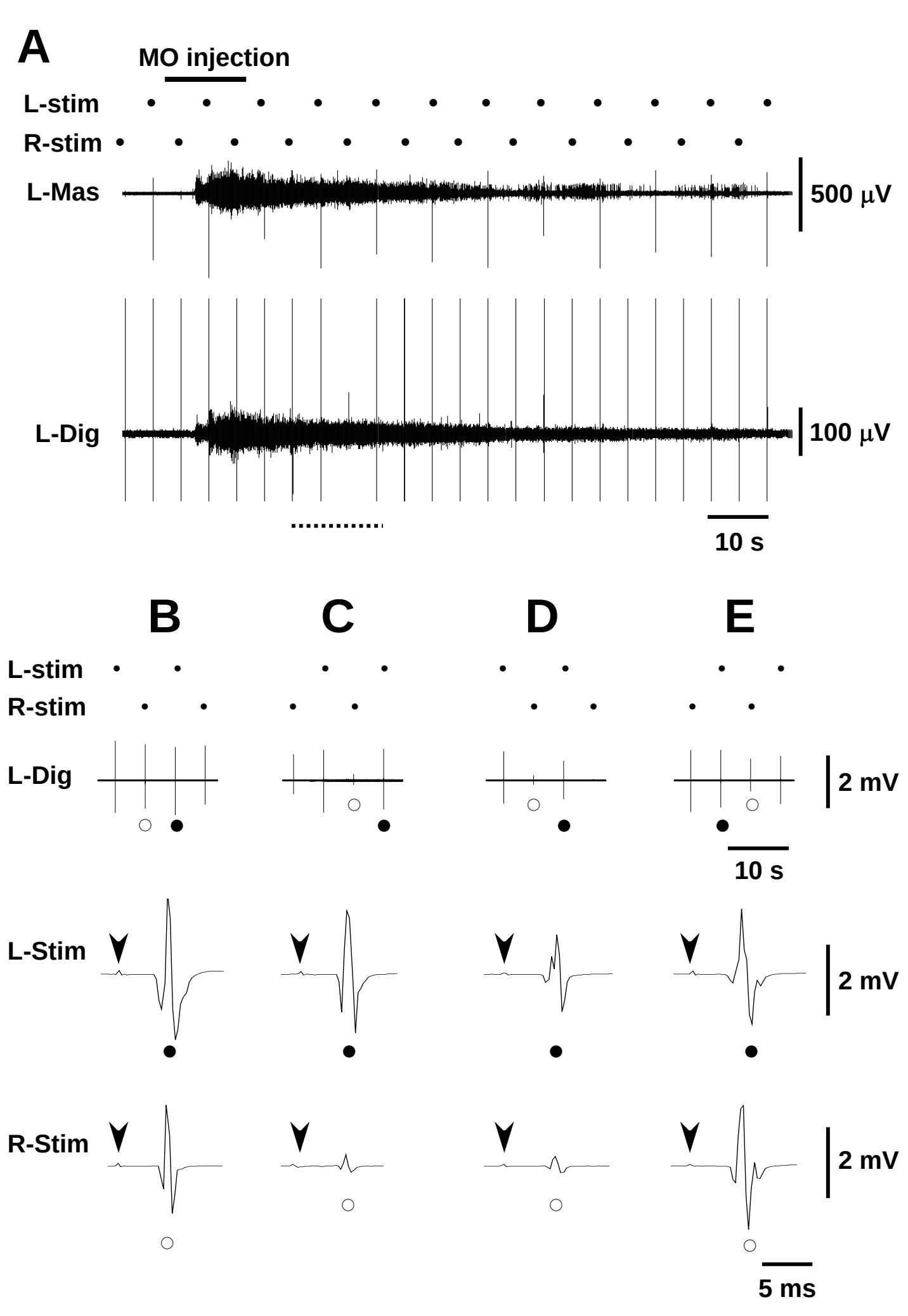
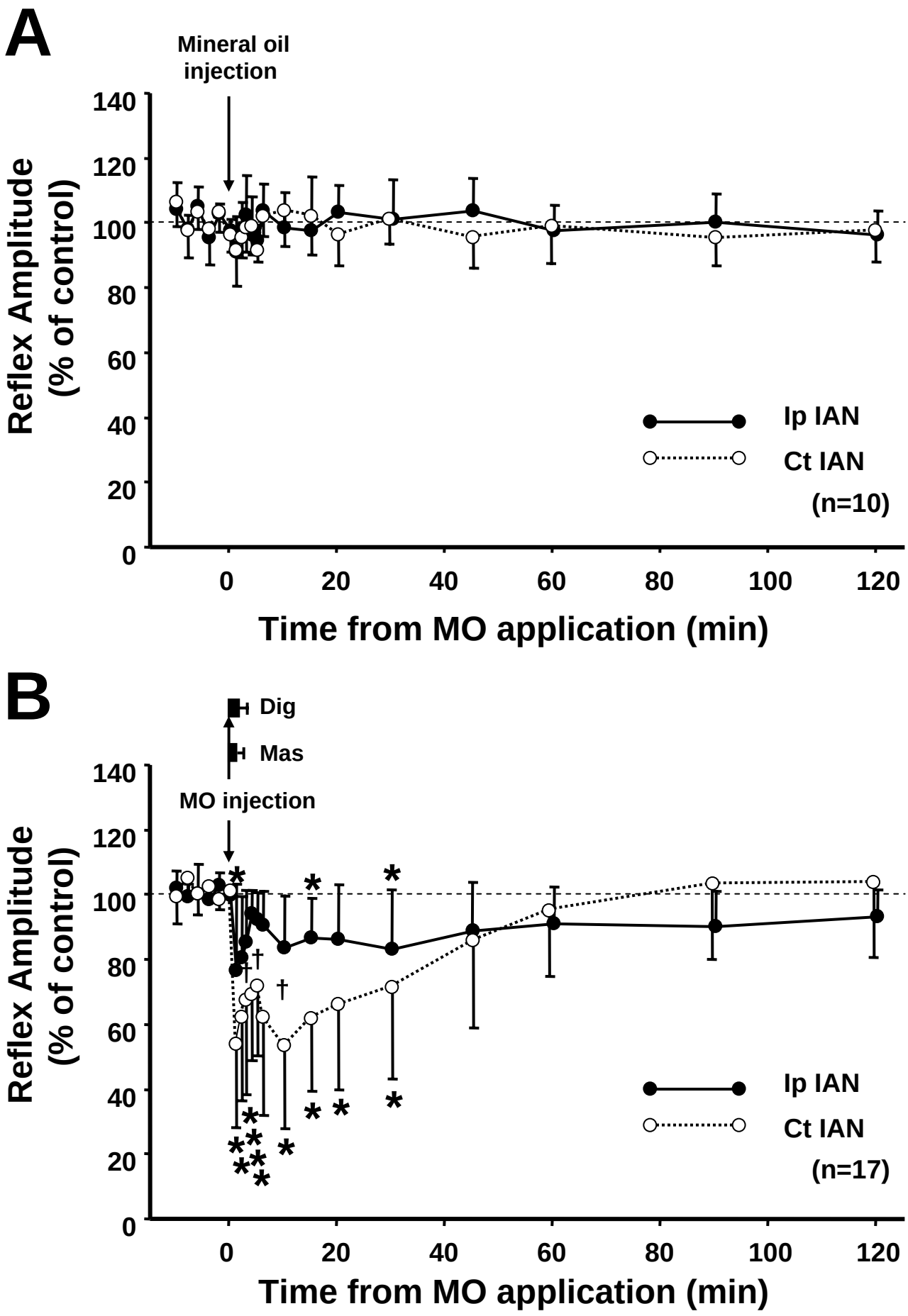

A
MO injection
L-stim
R-stim
L-Mas
500 μV
L-Dig
100 μV
10 s
B
C
D
E
L-stim
R-stim
L-Dig
2 mV
10 s
L-Stim
2 mV
R-Stim
2 mV
5 ms

Figure 2


A
Mineral oil injection
Reflex Amplitude (% of control)
140
120
100
80
60
40
20
0
0
20
40
60
80
100
120
Time from MO application (min)
Ip IAN
Ct IAN
(n=10)
B
Dig
Mas
MO injection
Reflex Amplitude (% of control)
Time from MO application (min)
Ip IAN
Ct IAN
(n=17)


A
MO injection
L-stim
R-stim
L-Mas
500 μV
L-Dig
100 μV
10 s
B
C
D
E
L-stim
R-stim
L-Dig
2 mV
10 s
L-Stim
2 mV
R-Stim
2 mV
5 ms


A
Mineral oil injection
Reflex Amplitude (% of control)
140
120
100
80
60
40
20
0
0
20
40
60
80
100
120
Time from MO application (min)
Ip IAN
Ct IAN
(n=10)
B
Dig
Mas
MO injection
Reflex Amplitude (% of control)
Time from MO application (min)
Ip IAN
Ct IAN
(n=17)