The Role of Nucleus Reticularis Pontis Caudalis (nPC) in Mastication.

by

Ziad Ali

Faculty of Dentistry

McGill University, Montreal

August, 1997

A thesis submitted to the
Faculty of Graduate Studies and Research
in partial fulfillment of the requirements of the degree of
Master of Science (M.Sc.)

Table of Contents

Acknowledgments

I would like to express my gratitude to Dr. J.P. Lund for giving me the opportunity to learn and understand how science really works. I appreciate his continuous support and understanding.

I would also like to thank Dr. G. Scott for his dynamism and enthusiasm. His support and patience were essential to my development and progress. Words fail to describe how much I appreciate him as a friend and teacher.

I would finally like to thank Sylvie Lepage, Christian Valiquette, and Nick Vrentzos for their friendship and their technical support.

ABBREVIATIONS

CMA	Cortical Masticatory area
CPG	Central pattern generator
EMG	Electromyograph
GC	Nucleus gigantocellularis
GCo	Oral portion of nucleus gigantocellularis
IAn	Inferior Alveolar Nerve
MBRF	Medial bulbar reticular formation
nPC	Nucleus reticularis pontis caudalis
n.V.mes.	Trigeminal mesencephalic nucleus
n.V.mot.	Trigeminal motor nucleus
n.V.spo.	Oral portion of the spinal trigeminal nucleus
PCRF	Parvocellular reticular formation
PGC	Nucleus paragigantocellularis
RJM	Rhythmic jaw movement

ABSTRACT

The general area of the medial bulbar reticular formation (MBRF), which includes nucleus pontis caudalis (nPC), nucleus paragigantocellularis (PGC), and nucleus gigantocellularis (GC), may include the first-relay elements of the masticatory central pattern generator (CPG). Despite good descriptions of the anatomical and functional roles of PGC and GC in the generation of mastication, the function of nPC remains largely undescribed.

The goal of this study was to determine the role of nPC in mastication. Neurons in nPC that were driven by mastication were analyzed for peripheral and cortical inputs. In addition, the effects of blocking many of these neurons with lidocaine on masticatory parameters were observed.

Nucleus pontis caudalis neurons were found to receive inputs from the cortical masticatory area as well as from the periphery. The sensory afferents to nPC included information from pinch, jaw stretch, and periodontal receptors. Some nPC neurons received one type of peripheral input while others received several. In addition, neurons in the ventral nPC were found to fire tonically during mastication while rhythmically firing neurons were found in the dorsal part of nPC. Lidocaine injections near nPC neurons resulted in mostly increases in digastric muscle burst duration and inter-burst duration, thus resulting in increases in total cycle duration. Also, lidocaine injections resulted in mostly increases in total digastric EMG activity.

The data in this study as well as data from previous anatomical studies which have demonstrated afferent nPC connections from GC and efferents to PGC and the trigeminal motor nucleus (n.V.mot.) suggest that nPC is well positioned to play an active role in the control and modulation of mastication.

RÉSUMÉ

La région de la formation réticulée bulbaire médiane, qui inclut le noyau pontis caudalis (nPC), le noyau paragigantocellularis (PGC) et le noyau gigantocellularis (GC) pourrait contenir les premiers éléments de relais du générateur central de pattron (central pattern generator, CPG). En dépit des bonnes descriptions des rôles anatomique et fontionnel du PGC et du GC dans la genèse de la mastication, la fonction du nPC demeure encore largement inconnue.

Le but de la présente étude était de déterminer le rôle du nPC dans la mastication. Nous avons analysé l'action des afférences d'origines périphérique et corticale sur les neurones du nPC mis en jeu durant la mastication. De plus, nous avons observé les effets de l'inactivation, par la lidocaïne, de plusieurs de ces neurones sur les paramètres de la mastication.

Nous avons découvert que les neurones du noyau pontis caudalis recevaient un grand nombre d'afférences provenant autant de la région masticatrice corticale que de la région périphérique. Les afférences sensorielles reçues par le nPC provenaient des récepteurs au pincement, à l'étirement de la mâchoire et des récepteurs *périodontal*. Certains neurones du nPC recevaient un seul type d'afférence périphérique tandis que d'autres en recevaient plusieurs. De plus, nous avons découvert que les neurones de la région ventrale du nPC déchargeaient de façon tonique durant la mastication alors que ceux de la région dorsale déchargeaient de façon rythmique. L'injection de lidocaïne à proximité du nPC induit essentiellement une augmentation de la durée des bouffées de décharge du muscle digastrique et de la durée inter-bouffées, et par là même une augmentation de la durée totale du cycle. De la même façon, l'injection de lidocaïne induit une augmentation de l'activité EMG digastrique totale.

Les données de cette étude, tout comme les données issues d'étude précédentes qui ont montrées que le nPC reçoit des connexions afférentes provenant du GC et en établit d'autres, efférentes, vers le PGC et le noyau moteur trigeminal suggèrent que le nPC est bien positionné pour jouer un rôle important dans le contrôle et la modulation de la mastication.

INTRODUCTION

Mastication is a rhythmic act in which the muscles of the jaw, tongue, and face act together to place the food properly in the mouth, cut it up, and prepare it for swallowing. Certain common features in the mastication of mammals, like the alternation of jaw-opening and jaw-closing, as well as the changes in the pattern of movement during a masticatory sequence to fit the properties of the food, suggest a common neural mechanism for mastication in mammals. Rabbits, which have been previously used in the study of mastication, will be used in the present study.

In a typical masticatory sequence, mastication is achieved by the alternate activation of jaw-closing and jaw-opening muscles (Lund and Enomoto, 1988) (figure 1). In the rabbit, jaw opening is produced using the lateral pterygoid, digastric, and mylohyoid muscles. The masseter, medial pterygoid, and temporalis muscles are used to close the jaw (Lund and Enomoto, 1988) (figure 4). The specific pattern of muscle activation results in jaw movement that is characterized by three-dimensional (3-D) vertical, lateral, and anterior-posterior components (Schwartz et al., 1989) (figure 1).

Based on the involvement of different muscles as well as on the 3-D pattern of jaw movement, mastication in the rabbit can be divided into three types of cycles (Schwartz et al., 1989) (Figure 1). In type 1 cycle (preparatory), the rabbit moves the food backward with little lateral movement. In addition, the masseter muscle, unlike the digastric, is not very active. Also, two main phases exist here, a fast closing (FC) phase

and an opening (O) phase (Figure 2a). In type 2 cycle (reduction), the posterior teeth begin to reduce the food and the masseter muscle is very active as the food is being crushed. The cycles here are composed of three phases: FC, slow opening (SO), and O (Fig 2b). Finally, in type 3 cycles (pre-swallowing), the food is being prepared for swallowing. This cycle type is composed of five phases: FC, SC, O_1 (rapid), O_2 (pause), and O_3 (rapid) (Figure 2c).

The masticatory cycles within a masticatory sequence can be described on the basis of the electromyographic (EMG) activity (Figure 3). A single cycle, measured by the total cycle length, is made up of a muscle burst (digastric or masseteric) and an inter-burst interval (Lund, 1991). The inter-burst interval is the time between one muscle burst and the next, where as the burst duration is the length of the muscle burst itself. Another important parameter, the EMG area, is the total EMG activity of a single muscle burst.

As seen in the masticatory sequence from an awake rabbit, mastication is not a stereotyped act (Figure 1). The muscles involved, the extent of muscle involvement, and the various masticatory parameters change throughout the masticatory sequence. This variability depends on sensory feedback pertaining to the location and properties of the food during each particular cycle. Sensory afferents relay the information back to the other neural elements involved in mastication, thus leading to the modification of the basic masticatory pattern and parameters. For example, several studies have demonstrated that an increase in the size or toughness of the food, as detected by sensory afferents, leads to an increase in cycle duration (Thexton et al., 1980; Luschei et al., 1974). Moreover, the

importance of this sensory feedback on the proper functioning of the system has been confirmed repeatedly. For example, when the maxillary and inferior alveolar nerves, which supply the upper and lower jaw with sensory feedback, are damaged, the masticatory sequence was longer, the movements were irregular, and jaw-closing EMG activity were reduced (Inoue et al., 1989).

There are several mechanoreceptors involved in the relay of sensory information back to the brainstem. Each type of receptor is more active in certain phases of the masticatory cycle than others (Figure 4). Epithelial afferents from hair and mucosal receptors have been recorded in the mandibular division of the trigeminal ganglion of the rabbit (Appenteng et al., 1982; Lund et al., 1982). Mucosal afferents with receptive fields on the lip and elsewhere in the mouth were most active during the SC phase of a typical type II cycle (Lund and Olsson, 1983). Periodontal afferents, which were found to be very active during the SC phase of a typical type II cycle (Lund and Olsson, 1983), play a very important role in sensory feedback because they are sensitive to the force applied to the crown of the tooth (Ness et al., 1954; Hannam et al., 1969; Hannam et al., 1982). The cell bodies of these afferents were found in the trigeminal ganglion as well as in the trigeminal mesencephalic nucleus (n.V.mes) (Byers et al., 1986; Collier et al., 1987; Aigouy et al., 1988), and the firing frequency of these afferents was found to be proportional to the force or rate of change of force applied to the crown (Amano and Iwasaki, 1982). In addition, the cell bodies of masseter muscle spindle afferents are found in the n.V.mes (Matsunami et al., 1972; Goodwin et al.,1975) and some of these afferents fire maximally during the

slow closing phase as well as at the end of the jaw-opening phase (Lund et al., 1979). A lesion of n.V.mes that eliminates the feedback from muscle spindles as well as from periodontal afferents in monkeys induces the animal to chew preferentially on the side opposite to the deafferentiated side (Goodwin and Luschei, 1974). Also, a procedure that selectively removes the feedback from periodontal pressoreceptors in humans reduced the force output of maximal voluntary biting by as much as 40% suggesting that these afferents may be the source of an important positive feedback action on jaw-closing motoneurons (Lund and Lamarre, 1973). It is therefore clear that masticatory neural elements receive several types of sensory feedback that they can use as a guide to control masticatory pattern and parameters. One of the important nerves containing these sensory afferents is the inferior alveolar nerve (IAn) which supplies the lower jaw and teeth.

Mastication can be elicited in anesthetized animals by electrically stimulating the cortical masticatory area (CMA, in the 1° motor cortex) or its efferent corticobulbar tract at 10-60 Hz (Rioch et al., 1960; Kawamura et al., 1960; Sumi et al., 1969). CMA stimulation will eventually lead to the firing of digatric and masseteric motoneurons in the trigeminal motor nucleus (n.V.mot.; at the mid-pons level; figures 6,7). Movements evoked by CMA stimulation in rabbits were found to closely resemble natural mastication (Lund et al., 1984). Studies of brain stem circuits that are fundamental to mastication use CMA-induced mastication, as is the case in this study. Mastication can also be elicited in decerebrate animals by sensory stimulation. For example, rhythmic jaw movement has been produced by touching or rubbing the mucosa of the teeth (Bremer et al., 1923) or by

applying tonic pressure on the hard palate (Van Willigen et al., 1984; Juch et al., 1985). Finally, mastication can be elicited in paralyzed animals where neuromuscular junctions are blocked thus resulting in what is known as fictive mastication. Essentially, movement is eliminated despite stimulating the corticobulbar tract and despite rhythmic motoneuronal activity. Therefore, any changes in sensory feedback are eliminated despite normal rhythmic motoneuronal firing in the n.V.mot.

Despite the fact that mastication can be induced by cortical or sensory stimulation, studies have shown that the minimal neural elements that generate the basic masticatory rhythm are located in the brain stem (Dellow and Lund, 1971). This was shown using a model in which the brainstem was functionally isolated by both paralysis and decerebration. After paralyzing and decerebrating animals, it was found that elements in the isolated brainstem were sufficient to generate the basic features of mastication. These elements are collectively known as the masticatory central pattern generator (CPG). A CPG can be defined as an ensemble of neural elements whose properties and connectivity can give rise to a pattern of rhythmic activity in the absence of external feedback from either periphery or CMA (Rossignol and Dubuc, 1994). The CMA exerts its actions on the CPG via the cortico-bulbar tract (Bazett and Penfield, 1922; Miller, 1920) mainly contralaterally (Nozaki et al., 1986; Tal, 1987).

The pattern of mastication has two main components : rhythm generation and burst generation (Lund and Enomoto, 1988). The rhythm, or timing component,

determines the length of the cycle. Masseteric and digastric motoneuronal activity in n.V.mot. determines the duration and pattern of muscle firing (burst component), as seen in muscle EMG activity. It is currently believed that these two components are generated at two different stages by two separate groups of neurons in the CPG (Lund and Enomoto, 1988).

Rhythm generation is believed to be generated first by groups of cells in the medial bulbar reticular formation (MBRF) located between the n.V.mot. and the inferior olive including the nucleus reticularis paragigantocellularis (PGC) and nucleus reticularis gigantocellularis (GC). Indeed, lesioning these nuclei abolishes mastication (Nozaki et al., 1986; Chandler and Tal, 1986). The CMA was found to project contralaterally to PGC (Nozaki et al., 1986), which in turn projects to GC. In addition, the PGC neurons were found to fire tonically, not rhythmically when the cortex is stimulated. On the other hand, despite receiving tonic firing from the PGC, the oral part of GC (GCo) produces a rhythmic output to the bulbar parvocellular reticular formation (PCRF) (Nozaki et al., 1986). Consequently, last-order interneurons and trigeminal motor neurons also fire rhythmically during CMA-induced mastication. Thus, GCo is believed to contain the oscillatory circuitry responsible for the production of cortically-induced rhythmical jaw movement (RJM) within the CPG (Nozaki et al., 1986) (figure 5). In addition, some have suggested that GCo also encodes the parameters of the motoneuron burst (burst duration, pattern, area) (Nakamura, 1985). However, this is unlikely because anatomical studies have not provided much evidence that these areas have strong monosynaptic projection to n.V.mot.,

thus suggesting that other last-order interneurons generate trigeminal motor neuron bursts (Gurahian et al., 1989).

Last-order interneurons that transmit the pattern of mastication and muscle bursts to trigeminal motor neurons were found using horseradish peroxidase (HRP) studies (Landgren et al., 1986). The areas found most likely to be involved include regio h (border zone surrounding n.Vmot.; figure 6) and the rostral part of the spinal trigeminal nucleus oralis (n.V.spo; figure 7) (Landgren et al., 1986). Neurons found in these nuclei fired rhythmically in phase with the rhythm induced by cortical stimulation (Donga and Lund, 1991). In addition, neurons in n.V.spo project to the digastric motoneuronal pool in n.V.mot (Olsson and Westberg, 1989). The fact that these areas project to n.V.mot and fire in phase with the muscle bursts strongly qualifies them as candidates for burst generation. A summary of the CPG and its components is found in figure 8.

Many have suggested that neurons in the general area of the medial bulbar reticular formation (MBRF), which includes nucleus pontis caudalis (nPC), nucleus paragigantocellularis (PGC) and nucleus gigantocellularis (GC) may be the first-relay elements of the CPG (Nozaki et al., 1986; Nakamura et al., 1980). Indeed, neurons in the nucleus PGC were found to fire tonically during mastication and to receive monosynaptic inputs from the CMA (Nozaki et al., 1986). Interestingly, despite good descriptions of the anatomical and functional role of PGC and GC, the function of nPC remains largely undescribed.

Several anatomical facts suggest a role, as of yet not understood, of nPC in mastication. For example, nPC in rat is known to receive input from GC (Shammah-Lagnado et al., 1987) and PGC (Shammah-Lagnado et al., 1987). In addition to these afferents, nPC has been shown to project to PGC (Andrezik et al., 1981) as well as n.V.mot (Vornov and Sutin, 1983; Li et al., 1993). Interestingly, nPC has been shown to receive input from mesencephalic reticular formation (Shammah-Lagnado et al., 1987) which when stimulated has been shown to induce rhythmic jaw movements in the guinea pig (Hashimoto et al., 1989). Another interesting study (Gurahian et al., 1989) found that rhythmically occurring depolarizing membrane potentials in jaw opener motoneurons and long-duration hyperpolarizing membrane potentials in jaw-closing motoneurons were completely suppressed by GC and nPC stimulation. It was therefore proposed that this suppression is due to nPC and/or GC's suppression of activity in neurons responsible for masticatory rhythm generation. All this information suggests an active role of nPC in mastication.

The goal of this study is to examine the role of nucleus reticularis pontis caudalis (nPC) in mastication. More specifically, we intend to :-

a) Determine whether nPC neurons are among the first relay neurons in the sequence of events that determine the pattern of mastication.

b) Determine the extent of peripheral or sensory inputs to these cells.

c) Determine the role of nPC neurons in mastication, including their role in determining burst length, inter-burst interval, and EMG area.

In order to achieve these goals, extracellular recording of neurons in nPC will first be used to find neurons that discharge as a result of CMA stimulation. These neurons will then be thoroughly characterized. For example, the CMA will be stimulated with short pulses in order to assess the latencies of cortical inputs to these areas. This will help assess if nPC neurons are indeed first-relay neurons or if they play a role in subsequent stages. In addition, electrical stimulation of the IAn as well as a range of peripheral stimulation paradigms such as pinch (to determine pain inputs), pressure on skin, stretch of jaw (to determine muscle spindle input), as well as tapping of teeth (to determine periodontal input) will be used to assess sensory afferents to nPC neurons. Also, these neurons will then be blocked by the local anesthetic lidocaine so that mastication parameters like cycle length, burst length, inter-burst interval, and EMG area could be compared before, during, and after lidocaine injection. The various changes to these parameters will hopefully reveal any specific role that nPC may play in the modification of the basic rhythm.

MATERIALS AND METHODS

A. Anesthesia and Pre-medication.

Seventeen adult male New Zealand rabbits (2.0-3.5 Kg) were anesthetized by halothane (1.0-3.5%; Fluothane, Wyeth-Ayerst, Canada) in a gas mixture (1:1) of O_2 and NO_2. Anesthesia was continued with urethane (1.5 g/kg intravenously) and then maintained thereafter with supplementary doses of urethane (0.2 mg/kg intravenously). Ketamine (25 mg/kg intramuscularly; Ketalean, MTC pharmaceuticals, Canada) was given prior to injection of anesthesia.

B. Surgery and Arrangements for Stimulating and Recording

1. Tracheotomy

An incision was made just inferior to the larynx and the trachea was exposed by separating away the surrounding muscle and fat tissue. An incision was then made in the trachea thus allowing the insertion of the three-way tracheal tube to which the anesthesia machine was connected.

2. Femoral catheter

An incision in the skin of the inner thigh area was made. The femoral vein was exposed and isolated by separating away the surrounding muscle and fat. Two thin threads were placed untied approximately 2-3 cm apart. The more distal thread was tied off to block blood flow. A small incision was then made between the two threads and the catheter tube was inserted in the vein. The proximal thread was then tied off.

The catheter tube was connected to a three way valve. One of the two other connections delivered a hypertonic solution (0.15% KCl in 5% dextrose & 0.45% NaCl solution; Abbott Laboratories Ltd, Montreal, Canada) which was continuously administered throughout the experiment. The other connection had two purposes. Initially, it was connected to a saline-containing syringe. This was used to draw blood from the catheter to ensure that it is properly placed in the femoral vein. Later, the connection was used to systemically inject the animal with urethane.

3. Inferior Alveolar Nerve and Digastric/Masseter Muscle Electrodes

An incision was made in the neck area below the chin. The digastric muscles as well as the mandibular bone were exposed by separating away the surrounding muscle and fat. The inferior alveolar nerve (IAN) in the mandibular canal was exposed by drilling through the mandibular bone. The IAN was stimulated (0.2-20 V; 0.5 Hz; 0.5 ms) with a pair of needle electrodes (free tips ~ 1.5mm) at the level of the second molar tooth (figure

9). Two wire hook electromyographic (EMG) electrodes were implanted into the digastric and masseter muscles of both sides. The latter was easily accessible without making any skin incisions. Two screws were drilled in the jawbone and were wrapped with the electrodes to stabilize them.

4. Stereotaxic Apparatus and Recording/Stimulating Setup

The masseter muscle was cut with a blade in order to expose the two holes at the end of the zygomatic arch. Using these two holes, the head of the animal was positioned and fixed in a stereotaxic apparatus. The head was stabilized by drilling two screws in the skull and attaching a metal rod to the screws with dental acrylic. The head was tilted to a standard position using the height difference, angle, and distance between bregma and lambda. An incision in the scalp was then made from the area between the two orbits (thus exposing the top of the skull) all the way to the dorsal aspect of the neck (thus exposing the underlying muscle and occipital part of the skull).

After drilling through the left skull area anterior to the bregma position, concentric bipolar varnish-insulated stimulating electrodes were lowered into the cortical masticatory area (CMA) at bregma coordinates of approximately A 2.0 mm and L 5.0 mm. Repetitive square wave pulses (50Hz, 0.5ms duration) were delivered to induce rhythmic jaw movements (figure 9).

A metal bar holding an incandescent filament lamp in front of the lower incisor teeth was attached to the mental symphisis of the mandible with screws and dental acrylic. Movement of the light bulb, and thus the jaw, in the vertical and horizontal planes were measured by a light-sensitive position transducer (figure 9). These signals together with the microelectrode outputs and EMG data were amplified, displayed on an oscilloscope, and recorded on magnetic tape.

The brainstem was exposed by drilling off the occipital protuberance and by separating away the surrounding muscles and dura mater. Zero coordinates for the recording electrode were taken from the obex (figure 7). The recording electrode was inserted through the cerebellum and into the pons. Extracellular recordings were made in the contralateral nucleus reticularis pontis caudalis (nPC) (Tungsten electrode; AP = 6.5-7.5 mm; Depth = 9.0 - 12.5 mm; Lat = 0.5-1.5 mm). The neurons chosen for recording were those that responded to either single pulse CMA stimulation or mastication induced by stimulating the CMA with a train of pulses. Micro-injections (200nl-500nl) of lidocaine were made into the area of nPC. For these injections, a microcannula (30-gauge) filled with 2% lidocaine hydrochloride (Astra, Canada) was introduced into the nPC. To verify and monitor the effects of these local anesthetic injections, cellular activity was continuously recorded in the region of microinjection from a 50 μm epoxy-coated tungsten microelectrode that extended 1mm beyond the orifice of the microinjection cannula (Crist Instruments, USA). This technique has been described in detail previously (Crist et al., 1988; Duncan et al. 1993; Hikosaka and Wurtz, 1986).

In order to characterize nPC neurons, a 2 minute stimulation program was used. The program consisted of two separate parts. In the initial 15 seconds, 0.5 ms pulses (f = 50 Hz; Amplitude = 30-300 μA.) were delivered to the CMA in order to elicit mastication. The rest of the two minutes consisted of two alternating signals. The first was a single pulse stimulation of the inferior alveolar nerve (duration = 0.5 ms; every two seconds) to assess the sensory inputs to nPC. The other signal stimulated the CMA (1-4 pulses; f = 500 Hz; duration = 0.05 ms). The latter revealed the existence of short-latency cortical inputs to the nPC. Once the existence of short-latency responses in the nPC, one, two, three, and four pulses were delivered in order to determine which of the four CMA-stimulating pulses was responsible for the short-latency response.

In addition to the IAn, a range of peripheral stimulation paradigms such as pinch (to determine pain inputs), stretch of skin, stretch of jaw (to determine muscle spindle input), as well as tapping of teeth (to determine periodontal input) were used to assess sensory afferents to nPC neurons.

C. Histology

At the conclusion of the experiment, rabbits were anesthetized with an overdose of urethane, and perfused intracardially with 1 liter of PBS followed by 1 liter of 10% paraformaldehyde (4°C). Brains were removed, post-fixed for 48 hours and cut on a cryostat into 20mm coronal section. Sections were stained with Cresyl violet and

examined under a microscope to identify the location of the electrode tracks. The reticular and trigeminal nuclei were located and defined using the terminology of Messen and Olszewski (1949).

D. Data Analysis & Statistics

Data was recorded on magnetic tape (Honeywell 101) and simultaneously displayed on a digital storage oscilloscope (Gould 1604). Latencies of neuronal responses to stimulation were measured directly from the oscilloscope print-out. Selected records were analyzed on an IBM compatible PC. In these cases, neuronal activity, jaw displacement, and EMG activity were digitized at 10kHz and stored on the computer. All analysis were done on digastric muscle bursts. Mastication parameters like cycle duration (CD), burst duration (BD), inter-burst duration (IBD), and digastric burst area were calculated using custom-made software. Results were compared before, during, and after lidocaine injection.

Mann-Whitney Rank Sum tests were performed for comparisons of variables before, during, and after lidocaine injection, and P values less than 0.05 were considered significant. Tests that did not show complete recovery to pre-lidocaine levels were excluded from the analysis.

RESULTS

Recordings were made in various areas of the right nucleus reticularis pontis caudalis (nPC) while mastication was induced by stimulating the contralateral CMA. Stimulating the ipsi-lateral CMA did not activate neurons. The right inferior alveolar nerve (IAn) was stimulated to reveal any sensory inputs. A total of 51 cells responded during cortically-induced mastication and were thus analyzed for CMA and Ian latency. Further analysis was only performed on data from injections that were followed by a full recovery of mastication and neuronal activity. In addition, 7 cells in the area surrounding nPC were chosen as controls for lidocaine analysis. A sample analysis is shown in figure 10 while figure 11 contrasts the firing of a tonically active neuron with a rhythmically firing neuron.

A. Latency Measurements

1. Cortical Inputs

Of the 51 neurons analyzed, 40 (78%) responded to 4 pulse (500Hz, 0.05ms) stimulation of the CMA. Also, 27 neurons (53%) received short-latency cortical inputs (latency <5 ms; average = 3.60 ms ± 0.17 ms) while 13 neurons (25%) received long-latency cortical input (latency >5ms; average = 10.86 ms ± 2.38 ms). Distribution of short and long-latency cortical inputs appeared randomly distributed along the dorsal-ventral axis of nPC (figure 12a).

2. Inferior Alveolar nerve (IAn) Inputs

Stimulation of the IAn resulted in a response in 16 neurons (31%; figure 13). Thirteen of those received relatively short-latency IAn input (average = 8.47 ms ± 0.19 ms) while 3 received long-latency IAn input (average = 28.53 ms ± 3.90 ms).

3. Peripheral Inputs

A subset of 32 neurons were manually tested for various peripheral receptive fields. The neurons that responded to a specific peripheral stimulus displayed no anatomical clusters, instead appearing randomly throughout nPC (figure 14). Twelve of the 32 neurons (38%) responded to noxious pinch in the upper left lip area (right lip was not tested). These responses tended to be slowly adapting with sustained activity throughout the period of stimulation. An example of this activity is demonstrated in Figure 15.

Ten cells (31%) responded to manual vertical displacement of the mandible (figure 14). As seen in figure 16a, these neurons fired during both sustained and phasic vertical jaw displacement. Upon closer examination of the latter situation, it was found that this same neuron was active during the jaw-opening phase of rapid stretch of the mandible (figure 16b).

Nine cells (28%) responded to mechanical torsion of the upper incisors (Figure 14). Out of these nine cells, 4 responded to pressure on the upper right incisor only, two responded to the upper left incisor only, one responded to the upper left and right as well as bottom right incisor, and one cell responded to upper left and right incisor. Finally, two cells responded to non-noxious mechanical indentation of the skin of the left upper lip (figure 14).

When neurons with multiple inputs were studied, it was found that twelve responded to both cortical and peripheral inputs (either IAn or manual stimulation). Also, eleven cells received multiple peripheral inputs including IAn inputs. Again, the distribution of all these cells seemed random over the dorsal-ventral aspect of nPC (figures 17,18), except for the area in the middle of the dorsal-ventral axis, which seemed to have a marked reduction in multi-input neurons.

B. Neuronal Firing Characteristics

Out of the 58 cells recorded, 51 cells fired tonically while 7 cells fired rhythmically during mastication. All rhythmically firing cells were found at depths ranging from 9.5 mm to 11.6 mm thus placing them in the dorsal aspect of nPC. On the other hand, the majority of tonically firing cells (69%) were found in the ventral part of nPC. It is noteworthy that while looking around nPC for active neurons, we found very few active neurons in PGC and GC during mastication. Interestingly, latencies of both

tonically and rhythmically active neuronal responses to CMA stimulation were very similar (mean = 5.88 ± 0.93 ms and 5.77 ± 1.42 ms respectively) (fig 12b).

C. Lidocaine Injections

Lidocaine injection near the selected neurons in nPC affected several parameters associated with digastric muscle bursts. Analysis was only performed on injections that were eventually followed by a full recovery of mastication and neuronal activity. Consequently, 15 cells were analyzed for digastric burst duration, inter-burst interval, and total EMG area. Interestingly, lidocaine injection near 2 neurons resulted in a complete block of mastication (figure 19). One of the 2 neurons was found in the dorsal part of nPC, while the other was found in the ventral part of nPC.

1. Burst Duration

Lidocaine injections in the areas of 8 cells (53%) resulted in a significant increase in muscle burst length. Burst durations ranged from 162-240 ms. Six of these eight cells were located in the ventral part of nPC (figure 18). Lidocaine injections in the areas of 2 cells (13%), both in dorsal nPC, resulted in a significant decrease in burst duration. Both cells were found in the dorsal part of nPC. Finally, lidocaine injections in the areas of 3 cells (20%) found near the mid dorso-ventral area had no effect on burst length (figure 19).

2. Inter-Burst Interval

Lidocaine injections in the areas of 5 cells (33%) resulted in a significant increase in inter-burst interval. Also, lidocaine injection in the area of 2 cells (13%) resulted in a significant decrease in inter-burst interval. Finally, lidocaine injections in the areas of 6 cells (40%) resulted in no significant change in inter-burst interval. The cells of these three groups were well distributed with no apparent grouping in either part of the nPC (figure 20). Cycle durations (burst duration + inter-burst interal) ranged from 240-609 ms.

3. EMG Area

Lidocaine injections in the areas of 7 cells (47%) resulted in a significant increase in EMG area. All seven cells were located near the mid dorso-ventral line or deeper in the ventral part of nPC (figure 21). Injections in the area of one cell in the dorsal part of nPC resulted in a decrease in EMG area. Also, injections in the areas of 4 cells (27%; 2 in ventral nPC, 2 in dorsal nPC) resulted in no significant changes in EMG area.

D. Control Injections

Lidocaine was injected into the area of 7 cells surrounding nPC. Indeed, the effects on masticatory cycle parameters were very minimal. One of the exceptions was an injection near the facial nucleus that caused a decrease in burst duration (figure 18).

Another injection near the fasciculus longitudinalis posterior (Flp) caused an increase in burst length, inter-burst interval, and EMG area (figures 19,20,21).

DISCUSSION

A. NEURONAL INPUTS & FIRING CHARACTERISTICS

In the present study, neurons throughout the medial part of nucleus reticularis pontis caudalis (nPC) were found to receive various cortical and peripheral (including inferior alveolar nerve IAn) inputs. For example, 78% of neurons driven by mastication received short to intermediate latency cortical input. Of those, 68% were found to receive short-latency cortical input (average 3.60 ms ± 0.17 ms) while 32% received longer-latency cortical input (average 10.86 ms ± 2.38 ms). Short-latency cortical input to nPC neurons is most probably polysynaptic since other studies have obtained latencies for similar distances in the order of 1.7 ms (Nozaki et al., 1986; Westberg and Olsson, 1991). This suggests that CMA-driven neurons in nPC do not receive direct input from the CMA and therefore implies that nPC is not the first-relay in the transmission of information from the CMA to the CPG.

Many of the mastication-driven nPC neurons also received peripheral input. In fact, 69% received at least one type of sensory input (i.e. IAn stimulation, pinch, stretch, periodontal, or mechanical). Several interesting observations were made during the characterization of these inputs. For example, neuronal activity due to vertical displacement of the jaw was both slowly adapting and phasically activated by jaw opening, suggesting that these inputs may come from slowly adapting muscle spindle afferents (Lund and Olsson, 1983, Fig 2).

Another interesting observation was made when studying neuronal responses to torsion of the upper incisors. It is unlikely that stimulation resulted in neuronal responses that were only due to activation of periodontal mechanoreceptors for a number of reasons. Firstly, periodontal mechanoreceptors are very sensitive to direction of stimulation and require only modest application of force (5-200mg) to elicit activity (Hannam, 1976; Mei *et al.*, 1977; Olsson *et al.*, 1988). In our experiments, the stimuli were applied manually and we were therefore unable to control the direction and force of stimulation in a precise manner. The responses evoked required large amounts of pressure and did not respond to light tapping of the teeth. In addition, neuronal responses did not appear directionally sensitive. It is therefore possible that many of these responses were due to activation of periodontal mechanoreceptors sensitive to nociceptive or intense mechanical stimuli. Indeed, it has been demonstrated that mechanoreceptors (type II receptors) distinct from classical periodontal receptors were activated selectively by strong mechanical and nociceptive stimulation (Mei *et al.*, 1977). This may explain the fact that IAn latencies to nPC neurons were divided into a short-latency group (average = 8.47 ms ± 0.19 ms) and a long-latency group (average = 28.53 ms ± 3.90 ms). Certain nPC neurons may have received inputs from relatively fast fibers carrying non-painful sensory information like stretch and non-painful periodontal receptors, while others may have received input from relatively slow unmyelinated pain fibers carrying painful information from torsion-sensitive periodontal receptors, among others. Another explanation for the longer-latency group may be that information was relayed several times before reaching nPC.

One important finding in this study was that 22% of the nPC neurons analyzed received multiple peripheral inputs. The fact that some nPC neurons receive one type of peripheral input while others receive several may help us understand the functional role of nPC in mastication. More specifically, neuronal sub-populations may exist within nPC with some neurons receiving one type of information while others receive multiple types. The latter sub-populations could thus perform an integrative function in which they process information from various peripheral inputs in order to determine the appropriate changes to mastication. In addition, 24% received cortical input and at least one type of peripheral input. Therefore, it seems possible that higher-order nPC neurons integrate peripheral as well as cortical inputs. Interestingly, nPC neurons have been shown to project to PGC (Andrezik et al., 1981) as well as n.V.mot. (Vornov and Sutin, 1983; Li et al., 1993) thus making these neuronal sub-populations suitable candidates to directly or indirectly affect masticatory pattern and parameters.

Several mastication-driven nPC neurons (12%) were found to fire rhythmically during mastication, and were located in the dorsal part of nPC. The rest of the cells (88%) were tonically firing and were found mostly in the ventral part of nPC. The presence of mastication-driven cells that fire tonically in the ventral nPC may be explained by this area's bi-directional connection of the ventral nPC (Andrezik et al., 1981; Shammah-Lagnado et al., 1987) with the tonically firing PGC (Nozaki et al., 1986). Another explanation may be masticatory tonic input from the neighboring

pyramidal tract. Similarly, the presence of rhythmically firing cells in the dorsal part of nPC may be explained by dorsal nPC input from the rhythmically firing GC neurons (Shammah-Lagnado, 1987; Nozaki et al., 1986). It is interesting that these neurons share the same neurophysiological properties as the PGC and GC, except that nPC neurons recorded in this study are more rostral on the rostro-caudal axis.

B. LIDOCAINE INJECTIONS

Lidocaine injection near mastication-driven nPC neurons affected several parameters associated with digastric muscle bursts. Cycle duration was mostly increased due to increases in burst duration and inter-burst intervals. More specifically, a significant increase in muscle burst duration was found upon lidocaine injection near 8 neurons in mostly the ventral part of nPC. In addition, a significant decrease in muscle burst duration was found when lidocaine was injected near 2 cells in the dorsal part of nPC. Inter-burst interval increases occurred following lidocaine injections and these effects appeared randomly throughout nPC. Similarly, an increase in normalized total EMG activity resulted from lidocaine injection near neurons in the ventral aspect of nPC. Increased muscle burst duration and total EMG activity suggests a role for the ventral aspect of nPC in inhibiting and fine-tuning muscle burst generation.

In this study, total EMG activity was normalized. Therefore, increases in total activity were due to increases in motoneuronal activity, not to increases in burst duration. Also, the fact that inhibition of nPC cells caused increases in burst and inter-

burst duration suggests that nPC may cause tonic inhibition of motor activity. This inhibition may be achieved through either direct inhibition of motoneurons or excitation of inhibitory premotor neurons.

Interestingly, lidocaine injections near 2 neurons caused an abolition of mastication, a fact that may have functional significance or it may simply be due to blockage of passing fibers. Also, control injections just outside of nPC resulted in no changes to masticatory parameters except for 2 cells in which there was an increase in burst duration and an increase in EMG activity. These two exceptions may be explained by the fact that other areas outside of nPC may be involved in the generation of mastication and these two nuclei may well play a role in this genesis. Another possibility may be that lidocaine simply blocked passing fibers that were important in the genesis of mastication.

C. THE ROLE OF nPC WITHIN THE CURRENTLY ACCEPTED MODEL OF MASTICATION.

The data obtained in the present study suggests an active role of nPC in the modulation and adaptation of mastication. In the proposed model (figures 22), nPC integrates 1) sensory information including information coming from periodontal, pinch, and jaw stretch receptors, 2) sensory information relayed by the IAn, and 3) cortical information from the CMA. In addition to its role in the integration of sensory and cortical information, nPC may also be involved in the modulation of masticatory output. Indeed, lidocaine injection near mastication-driven neurons in nPC resulted in changes

to the parameters associated with rhythm and burst generation. More specifically, blocking nPC neurons resulted in increases in digastric burst duration, EMG, and interburst intervals. This suggests that nPC may be involved in the tonic inhibition and/or downgrading of digastric muscle activity both in frequency and motoneuronal firing.

The way that nPC actually helps modulate cortical input remains unexplored. One possibility may be related to its bi-directional connection with PGC (Andrezik et al., 1981; Shammah-Lagnado et al., 1987) and its input from GC (Shammah-Lagnado et al., 1987) (figure 22). By receiving information from the tonically active PGC and rhythmically active GC, nPC may be able to compare the integrated sensory/cortical information with the rhythmic program as encoded by PGC and more importantly the rhythm-generating GC. After all, in order for nPC to correctly modulate mastication, it must somehow receive the basic rhythmic program that it must modify. It is therefore conceivable that nPC compares the intended motor program with what is actually occurring in the periphery in a similar way to the cerebellum in limb locomotion.

One major question remains unanswered: If nPC serves as an integrative center for mastication by integrating information from sensory afferents, CMA, PGC, and GC (figure 22), what output system does it use to modify the motor program appropriately? Several possibilities exist: Firstly, nPC does have an output to PGC but

has significant effects on the masticatory pattern, including increases in BD, normalized EMG area, and to a lesser extent IBI. All this suggests a tonic inhibitory influence of these nPC neurons over digastric motoneurons.

Although our results are consistent with the currently accepted model of mastication (Nakamura et al., 1980; Nozaki et al., 1986a,b; Chandler et al., 1990) in that the neurons in the medial bulbar reticular formation (MBRF) participate in the genesis and patterning of mastication, a number of differences are noteworthy. For example, it was reported that neurons in and dorsal to PGC (an area that includes nPC) fire tonically and receive monosynaptic inputs from the CMA (Nozaki et al., 1986a,b). However, in the present study, even cortical inputs with short latencies are too long for a monosynaptic relay (average 3.60 ms ± 0.17 ms). This suggests that nPC cannot be part of the first-relay system in the corticobulbar pathway, something that was indirectly implied in the Nozaki studies.

Another concept that was proposed by Nozaki et al. (1986a,b) was that tonically firing neurons found in the ventral MBRF (an area that includes PGC and ventral nPC) activate phasically firing neurons in the dorsal MBRF (an area that includes GCo and dorsal nPC). Our results are only partially consistent with this concept because although we have found the ventral/tonic versus dorsal/rhythmic division, we were unable to find many neurons in PGC and GC that were active during mastication. In fact, the anatomical locations of these events appear displaced along the

this connection is not very extensive (Shammah-Lagnado et al., 1987). Indeed, affecting PGC might be efficient since it is PGC that is the first relay and thus provides the driving force for GC firing (figure 22). Secondly, nPC may have an output to GC but this area remains unexplored. Thirdly, anatomical studies have shown nPC output to n.V.mot. (Vornov and Sutin, 1983) (figure 22). However, despite the fact that jaw-opening is phasically inhibited during the jaw closing phase of real rhythmic jaw movement (RJM) (Lund et al, 1981), the digastric motoneurons are not inhibited leading many to believe that central inhibitory influences are exerted on premotoneuronal sites (Nakamura and Katakura, 1995). In addition, unpublished data from this laboratory have demonstrated that using a retrograde tracer (dextran conjugated to rhodamine or texas red), neurons within nPC are not labeled when the tracer is injected into a localized area confined within n.V.mot. (Kolta et al, unpublished). However, when the injection site overlaps the boundary of n.V.mot. and also contains regio h (pre-motor area surrounding n.V.mot.), a large number of neurons in nPC are retrogradely labeled. Therefore, nPC modulation of the basic masticatory rhythm might occur by influencing premotor areas (figure 22), not the digastric motoneurons directly.

D. COMPARISON BETWEEN PRESENT RESULTS AND RESULTS FROM PREVIOUS STUDIES

The present study clearly demonstrates that lidocaine injection into nPC modifies cortically-induced mastication. More specifically, figures 19-21 demonstrate that lidocaine injections in the medial part of nPC, especially the tonically firing ventral part,

rostro-caudal plane. Close inspection of the anatomical sections illustrated in Nozali et al (1986a. fig 5Ae & 5Af, fig6; 1986b, fig 2I) reveals that PGC is depicted as encompassing almost the entire medial area ventral to GC, extending rostrally from the facial nucleus (NVII) to n.V.mot. Stereotaxic atlases for rabbit (e.g. Messen and Olszewski, 1949) demonstrate that this region depicted is actually nucleus gigantocellularis pars alpha (GCα), and that the rostral part of GCα is located at the level of NVII, meeting the caudal pole of nPC in this area. Therefore, according to this atlas as well as anatomical studies in other animals (e.g. rat:Andrezik et al., 1981), the location of PGC depicted by Nozaki et al. is displaced medially and rostrally to that previously observed, and is more consistent with the location of rostral GCα and ventral nPC. Thus, it is possible that the responses reported in PGC were, in fact, located in ventral nPC.

In the study by Chandler et al. (1990) in the guinea pig, lidocaine injections into the dorsal aspect of nPC had very small effects on total cycle duration. Also, a small decrease in EMG amplitude was observed. In contrast, our study showed the opposite, with mostly increases in cycle duration and EMG amplitude. Another important finding by the same group (Gurahian et al., 1989) was that electrical stimulation of nPC/GC actually suppressed mastication. This is unusual since GC, according to the presently accepted model (Nakamura and Katakura, 1995), is the nucleus that produces the oscilllations necessary for rhythm generation. In addition, This clearly contradicts their study showing that lidocaine injection into nPC slightly

attenuated mastication, and supports the findings in this study which implicate nPC in the tonic inhibition of digastric motoneurons.

E. OTHER ANATOMICAL CONNECTIONS OF nPC

Nucleus pontis caudalis (nPC) has several other anatomical connections. For example, retrograde studies have demonstrated nPC afferents from vestibular neurons (Strutz and Schmidt, 1982), Therefore, nPC might be involved in balance control. Anatomical connections also suggest a role for nPC in pain. For example, nPC has connections with areas that are known to be involved in nociception such as afferents and efferents from/to periaqueductal gray (PAG; Mantyh, 1983; Marchand and Hagino, 1983), as well as afferents from raphe magnus (Shammah-Lagnado et al., 1987). In addition, stimulating nPC with the neurotransmitter neurotensin (NT) results in an increase in nociception threshold (Kalivas et al., 1982). Therefore, data from the present study which suggest direct painful input from peripheral receptors (e.g. torsion of the incisors) to nPC, as well as data from the above mentioned studies suggest that nPC might attenuate mastication under the influence of painful input.

F. METHODOLOGY LIMITATIONS AND FUTURE STUDIES

The present study strongly suggests a very active role of nPC in the integration of various information describing masticatory program and output. However, in order to fully understand how nPC uses this integrated information to modulate the motor

output, it is imperative to study its outputs to various structures like GC and the various premotor areas.

In addition, although using lidocaine was a very important first step in the characterization of nPC, not using other drugs to block nPC neurons limited the number of definite conclusions that can be made. Lidocaine blocks both cell bodies and passing fibers, rendering it impossible to tell whether the effects obtained were due to blocking nPC neurons or due to blocking the action of other brainstem areas. Therefore, in future studies, it is important to also use other drugs like Glutamate-receptor blockers CNQX (non-NMDA antagonist) and AP5 (NMDA antagonist). Any changes to masticatory parameters in the glutamate-blockers' experiments would confirm, if any, the role of nPC neurons in mastication.

G. FINAL THOUGHTS

A study by Kogo et al., showed that when a coronal section is made at the level of the facial colliculus, between the trigeminal and facial nuclei, rhythmic activity can still be recorded in the trigeminal motor nucleus (n.V.mot.) (Kogo et al., 1995). In such a section, both nucleus GCo and PGC are cut off while preserving nPC, regio h, and n.V.mot. This shows that masticatory rhythmogenesis can occur without GCo and PGC. Also, the present study clearly showed that a) lidocaine microinjection in the ventral nPC, an area that it presently thought to be involved in rhythmogenesis, nearly always fails to completely block mastication. This is unexpected since blocking the

process at an early stage should completely block mastication. b) all masticatory components (BD, IBI, EMG area) were altered by lidocaine injection, suggesting that this area contributes to all aspects of mastication, not just rhythm generation. c) cortical inputs to tonically and rhythmically active neurons had similar latencies (fig 12b), which is unexpected since the tonically active neurons are supposedly driving the rhythmically active neurons. Therefore, based on the results from this study as well as the others mentioned above, it seems unlikely that the process of rhythm generation is a simple serial process as was previously proposed (figure 5; Nakamura et al., 1980; Nozaki et al., 1986a,b; Chandler et al., 1990).

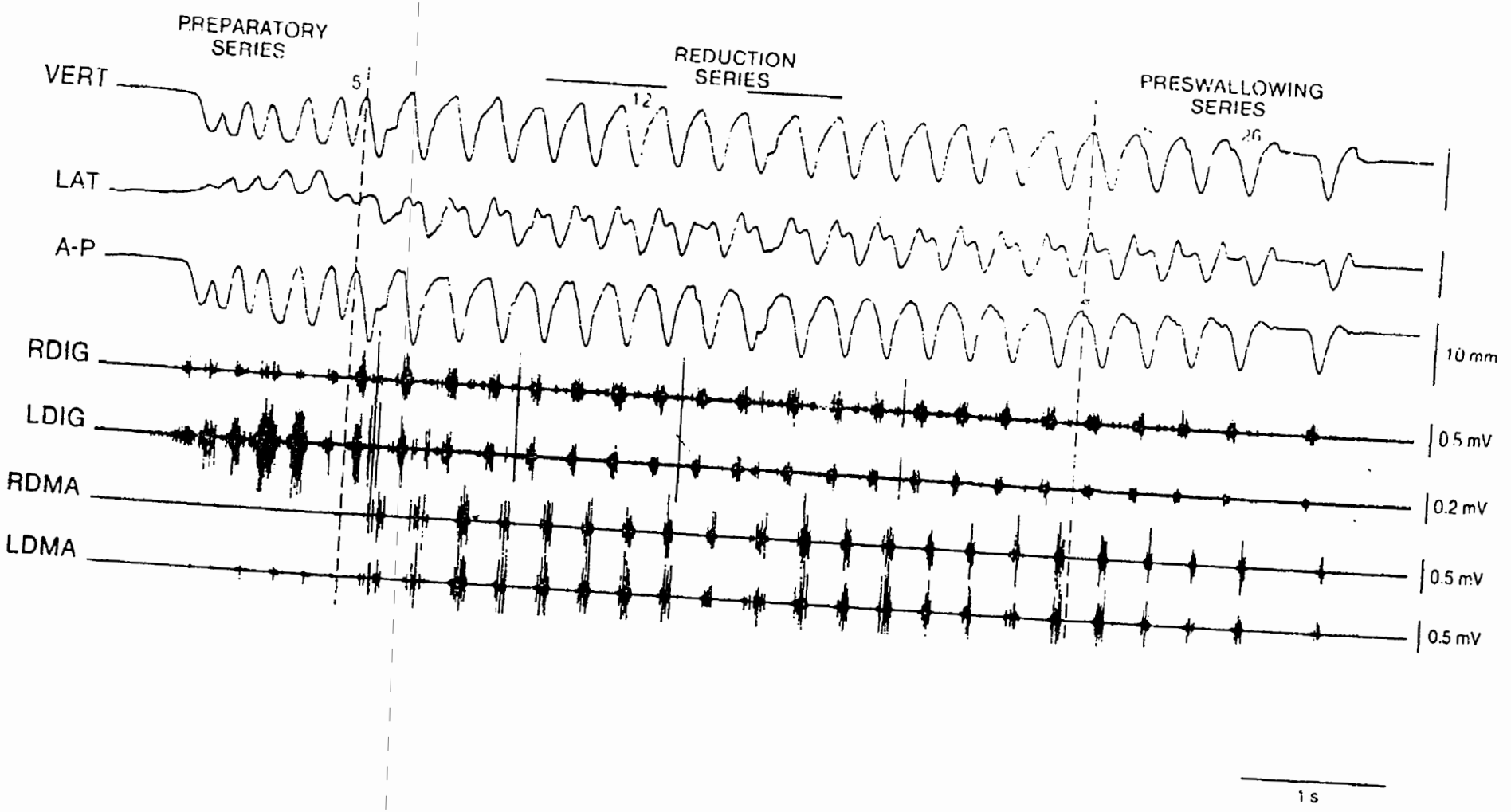
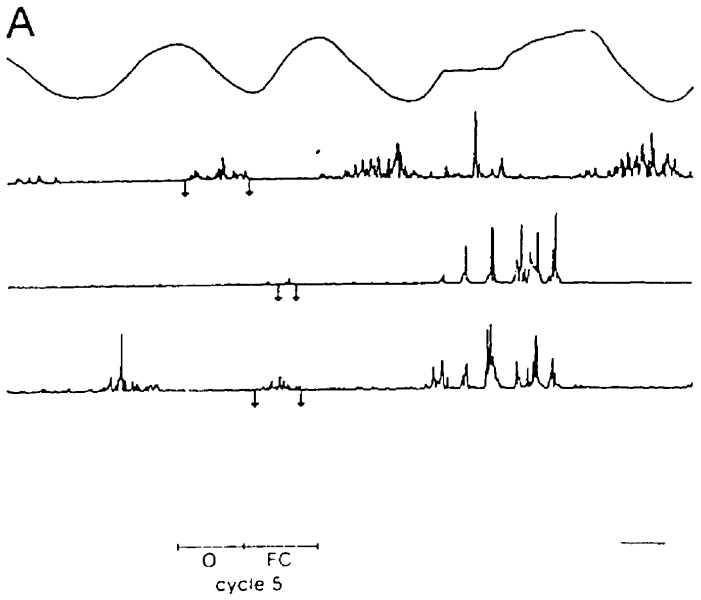

PREPARATORY SERIES
REDUCTION SERIES
PRESWALLOWING SERIES
5
12
26
VERT
LAT
A-P
RDIG
LDIG
RDMA
LDMA
10 mm
0.5 mV
0.2 mV
0.5 mV
0.5 mV
1 s

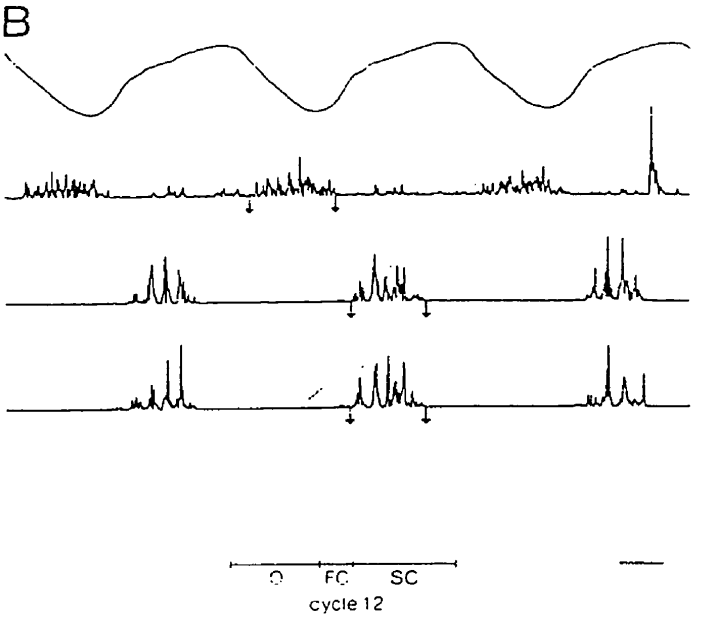
Figure 1. **Masticatory Sequence**

In a typical masticatory sequence, mastication is achieved by the alternate activation of jaw-closing and jaw-opening muscles. In the rabbit, jaw opening is produced using the lateral pterygoid, digastric (DIG), and geniohyoid muscles. The masseter (DMA) and temporalis muscles are used to close the jaw. The specific pattern of muscle activation results in jaw movement that could be characterized by three-dimensional (3-D) vertical (VERT), lateral (LAT), and anterior-posterior (A-P) components. Also, based on the involvement of different muscles as well as on the 3-D pattern of jaw movement, mastication can be divided into three types of cycles : preparatory, reduction, and preswallowing. R= right ; L= left. *Modified from Schwartz et al., 1989.*

PREPARATORY SERIES



REDUCTION SERIES



PRESWALLOWING SERIES

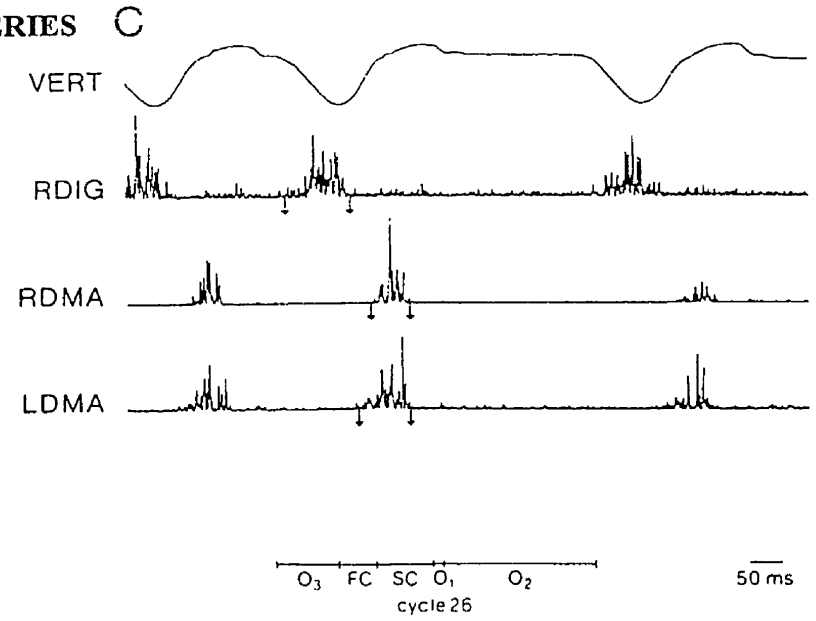


Figure 2. **The Three Types of Masticatory Cycles in the Rabbit**

In this figure, we take a closer look at the three types of masticatory cycles seen in figure 1. In type 1 cycle (preparatory; A), the rabbit moves the food backwards with little lateral movement. The masseter muscle, unlike the digastric, is not very active. Also, two main phases exist here, a fast closing (FC) phase and an opening (O) phase. In type 2 cycle (reduction; B), the posterior teeth begin to reduce the food. The masseter muscle is very active as the food is being crushed. The cycles are composed of three phases : FC, slow opening (SO), and O (Fig 2b). Finally, in type 3 cycle (pre-swallowing; C), the food is being prepared for swallowing. This cycle type is composed of five phases: FC, SC, O_1 (rapid), O_2 (pause), and O_3 (rapid) (Figure 2c). *Modified from Schwartz et al., 1989).*

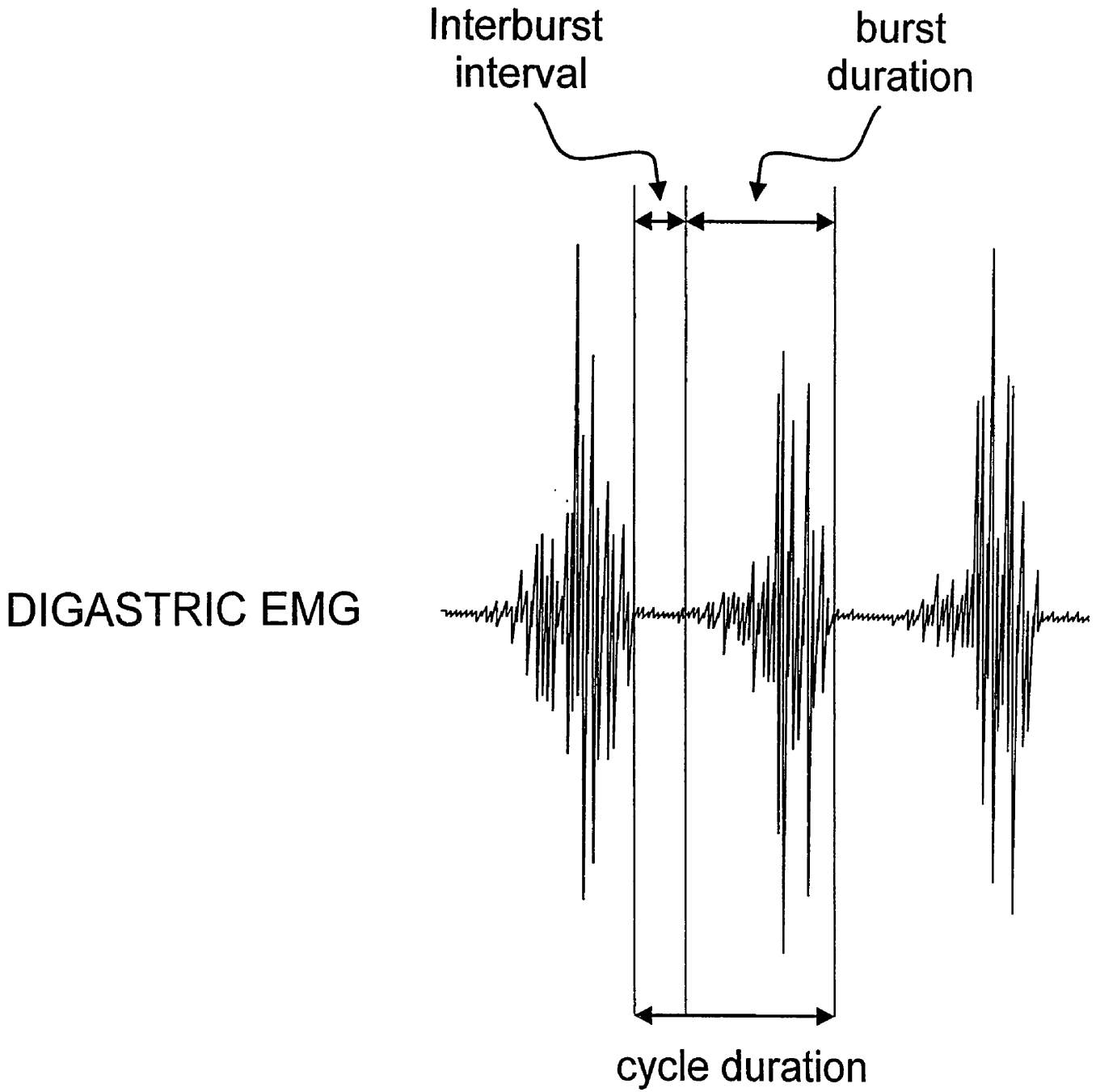

Interburst interval
burst duration
DIGASTRIC EMG
cycle duration

Figure 3. **Masticatory Cycle Parameters**

The masticatory cycles of EMG activity within a masticatory sequence can be described using different parameters. A single cycle, measured by the total cycle length, is made up of a muscle burst (digastric or masseteric) and an inter-burst interval (Lund, 1991). The inter-burst interval is the time between one muscle burst and the next, where as the burst duration is the length of the muscle burst itself.

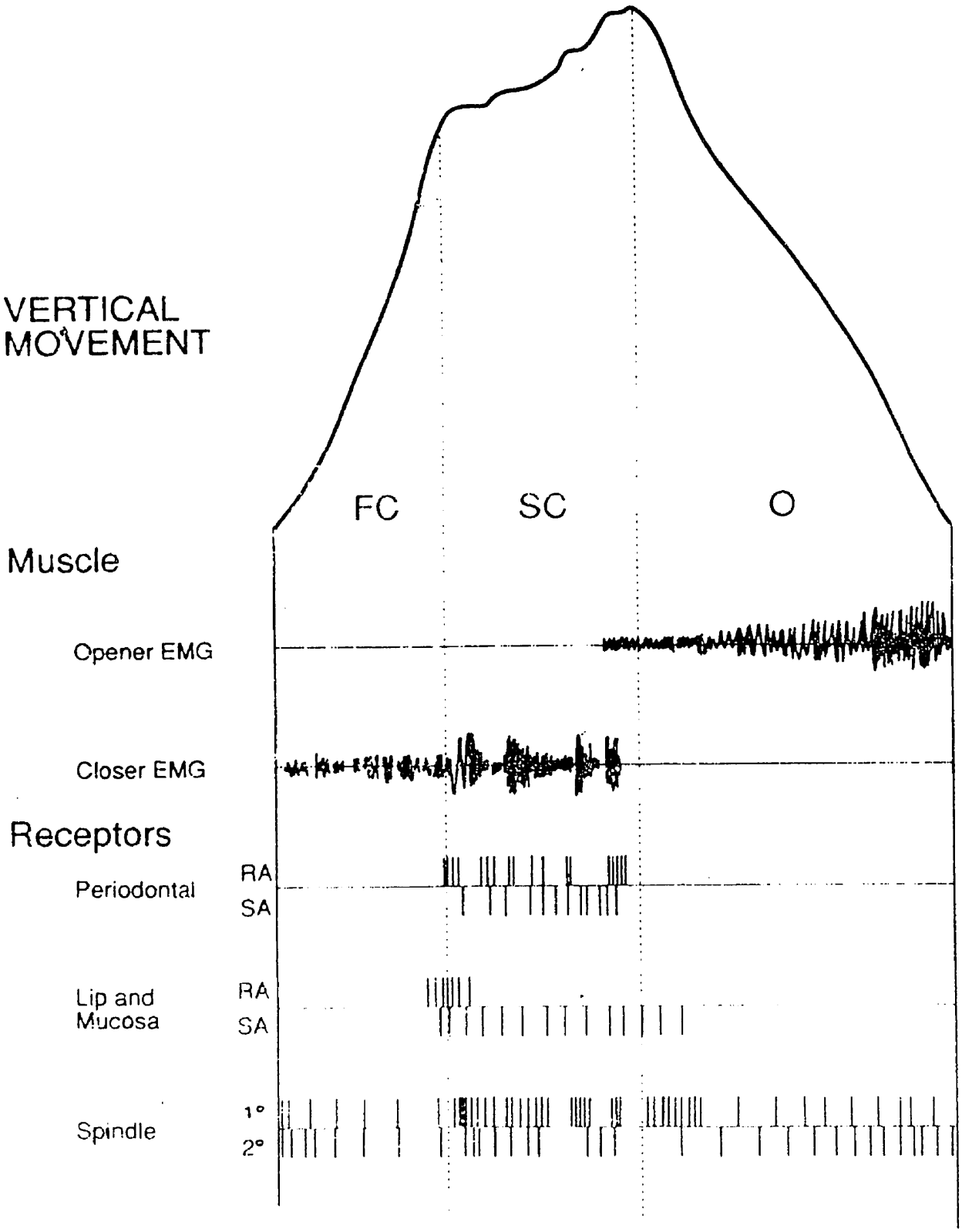
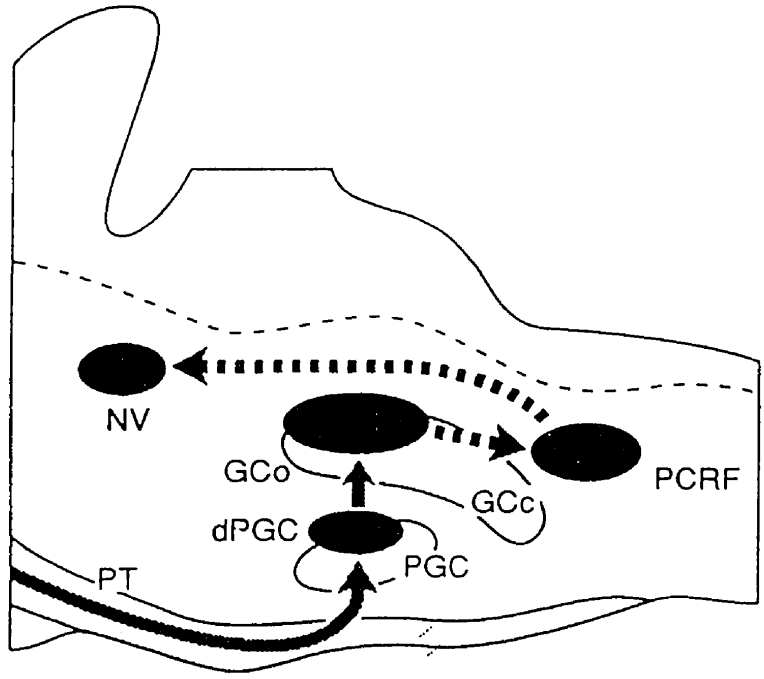

VERTICAL MOVEMENT
FC
SC
O
Muscle
Opener EMG
Closer EMG
Receptors
Periodontal
RA
SA
Lip and Mucosa
RA
SA
Spindle
1°
2°

Figure 4. **Sensory Afferent Activity During Mastication**

This figure demonstrates how each type of mechanoreceptor involved in the relay of sensory information back to the masticatory neural elements is more active in certain phases of the masticatory cycle than others. Mucosal afferents with receptive fields on the lip and mouth (Lip and Mucosa) were most active during the slow closing (SC) phase of a typical type II cycle (Lund and Olsson, 1983). Periodontal afferents (Periodontal) were found to be very active during this phase as well (Lund and Olsson, 1983). On the other hand, masseter muscle spindle afferents (Spindle) fired maximally during the slow closing phase as well as at the end of the jaw-opening phase (Lund et al., 1979). Note also opener and closer EMG activity during the opening and closing phases respectively. *Adapted from Lund, 1991.*


NV
GCo
GCc
PCRF
dPGC
PGC
PT

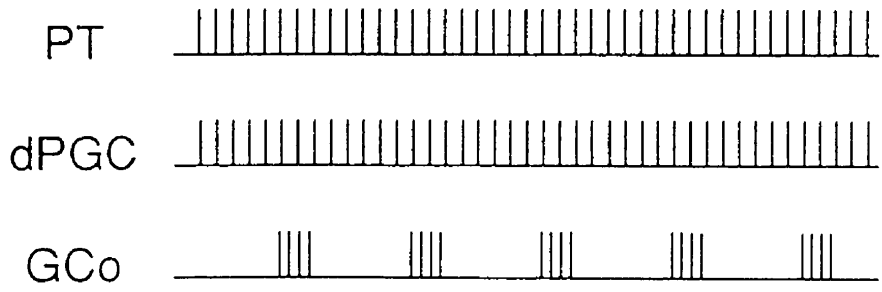

PT
dPGC
GCo

Figure 5. **Location of Masticatory Rhythm Generator**

This figure summarizes the presently accepted model of masticatory rhythm generation. Rhythmicity is believed to be generated first by groups of cells in the medial bulbar reticular formation including the nucleus reticularis paragigantocellularis (PGC) and nucleus reticularis gigantocellularis (GC). The CMA projects via the pyramidal tract (PT) contralaterally to PGC (Nozaki et al., 1986), which in turn projects to the oral part of GC (GCo). In addition, the PGC neurons were found to fire tonically, not rhythmically when the cortex is stimulated. On the other hand, despite receiving tonic firing from the PGC, GCo produces a rhythmic output to the bulbar parvocellular reticular formation (PCRF) (Nozaki et al., 1986), which in turn projects to the trigeminal motor nucleus (n.V.mot.). Thus, GCo is believed to be the site of the first rhythmically firing neurons within the CPG. *Modified from Nakamura and Katakura, 1995.*

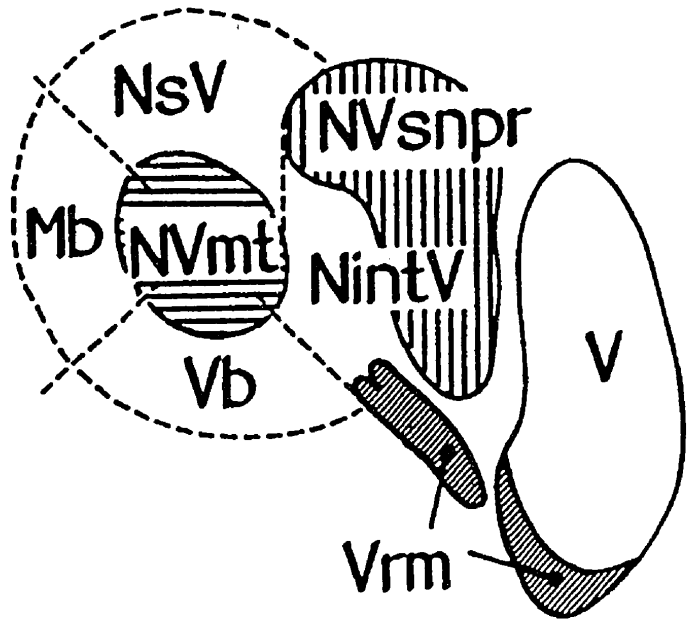

NsV
NVsnpr
Mb
NVmt
NintV
V
Vb
Vrm

Figure 6. **Trigeminal Last-Order Interneurons**

Last-order interneurons that transmit the pattern of mastication and muscle bursts to trigeminal motor neurons were found using horseradish peroxidase (HRP) (Landgren et al., 1986). The areas found most likely to be involved include regio h (border zone surrounding n.V.mot. "NVmt") which includes the supratrigeminal area (NsV), the intertrigeminal area (NintV), the medial border zone (Mb), and ventral border zone (Vb). Note that NVsnpr = Main Sensory Trigeminal Nucleus; V = Trigeminal nerve/tract. *Modified from Olsson and Westberg, 1989.*

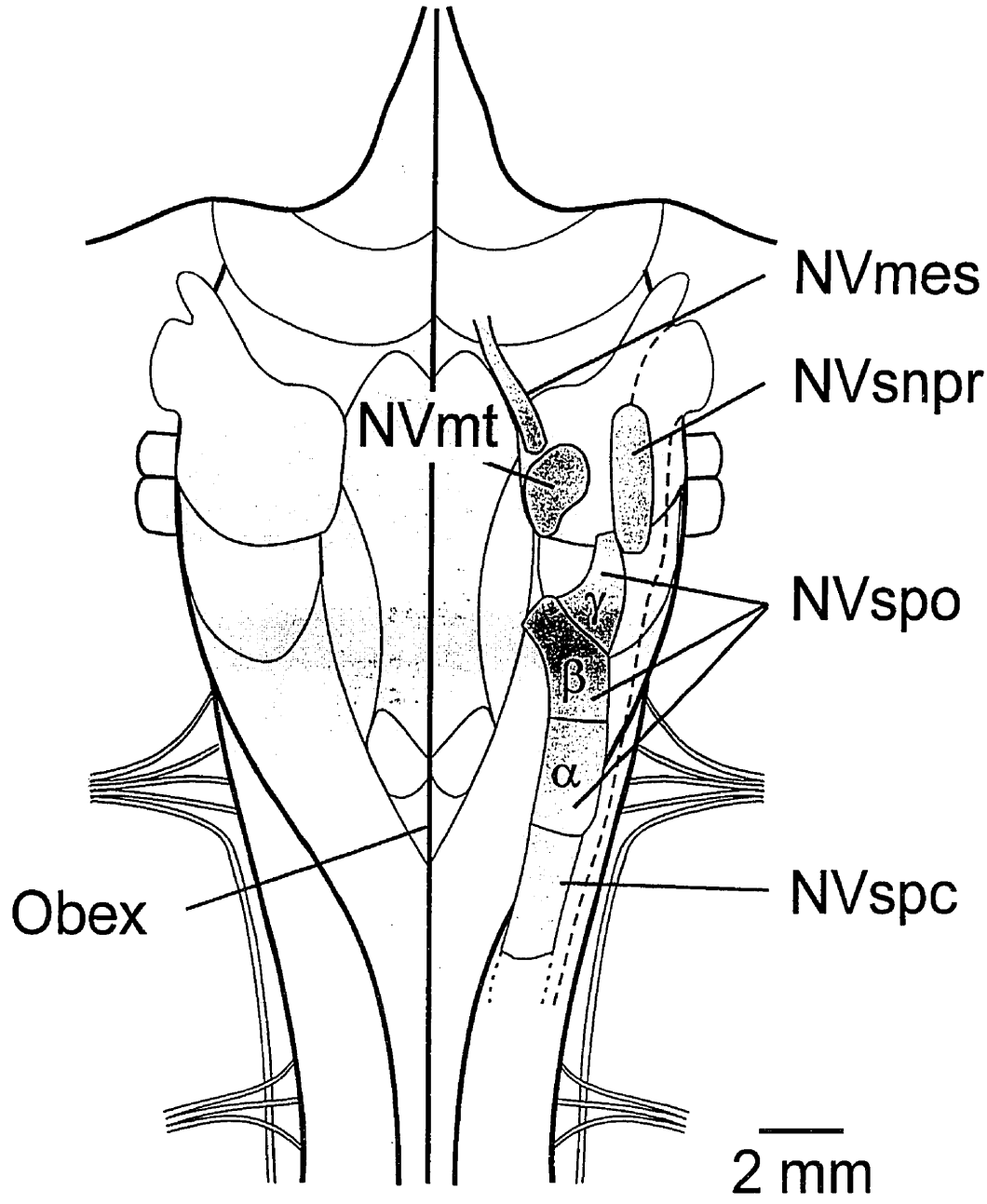

NVmes
NVsnpr
NVmt
NVspo
γ
β
α
Obex
NVspc
2 mm

Figure 7. **Trigeminal Last-Order Interneurons**

Other areas that might act as last-order interneurons include the spinal trigeminal nucleus oralis Nvspo which is subdivided into α, β, and γ components. Note the location of the obex which is used as the zero coordinates for the recording electrode.

NVmes = Trigeminal mesencephalic nucleus; NVsnpr = Main sensory trigeminal nucleus.

NVspc= Spinal trigeminal nucleus caudalis.

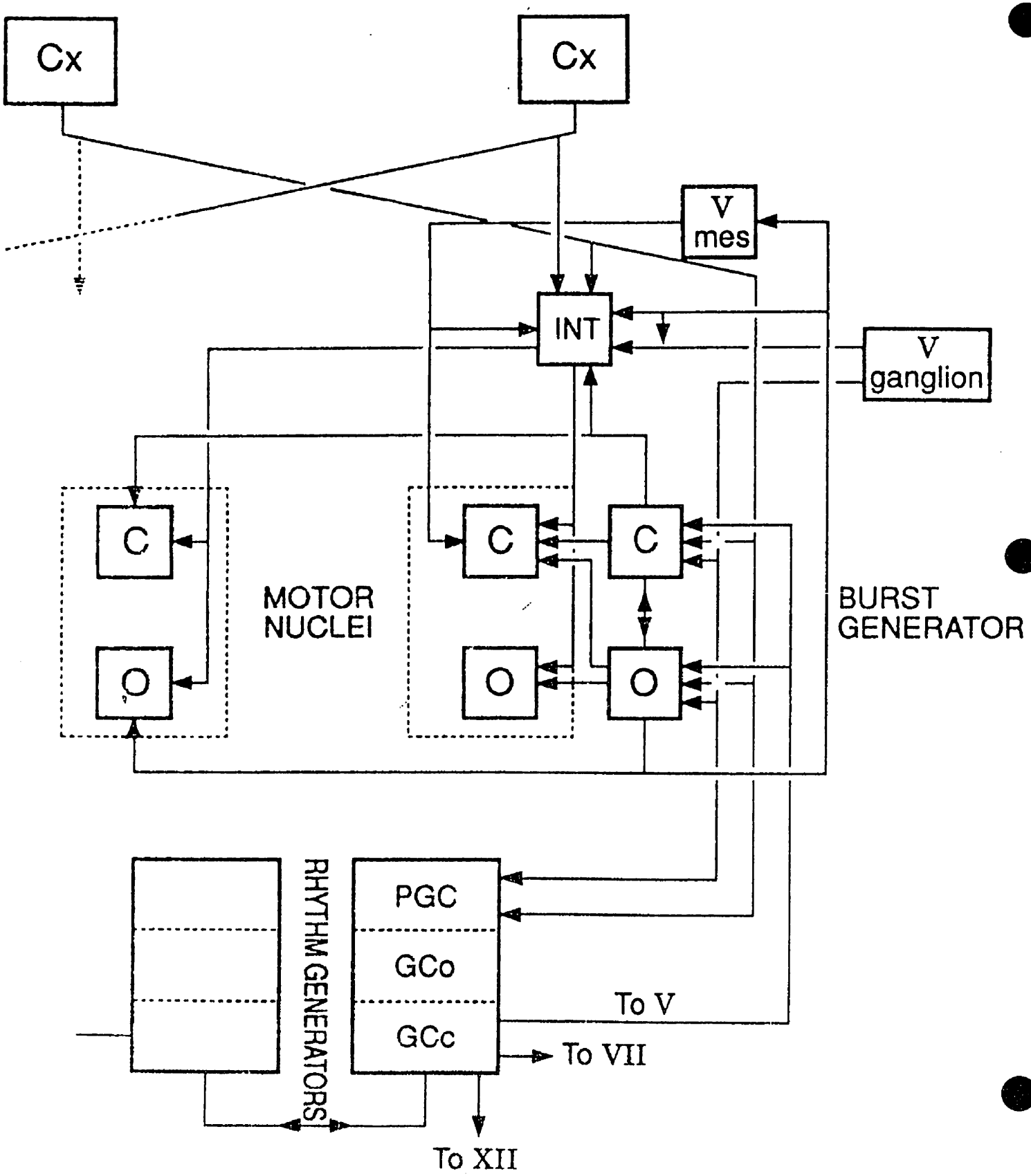

Cx
Cx
V
mes
INT
V
ganglion
C
C
C
O
O
O
MOTOR
NUCLEI
BURST
GENERATOR
RHYTHM GENERATORS
PGC
GCo
GCc
To V
To VII
To XII

Figure 8. **Summary of Brainstem Networks Controlling Mastication.**
Input from the masticatory cortex (Cx) or oral cavity, coming through the trigeminal ganglion (V ganglion), drives the rhythm generators (including nucleus gigantocellularis oralis, GCo, and nucleus paragigantocellularis, PGC), which in turn control burst generators. Burst generators themselves then control the different pools of motoneurons (closing "C" and opening "O"), interneurons (INT), and the primary afferents (V mes). The trigeminal motoneurons are the output of the system and they project to the opener and closer muscles including the digastric and masseteric muscles. *Adapted from Lund, 1991.*

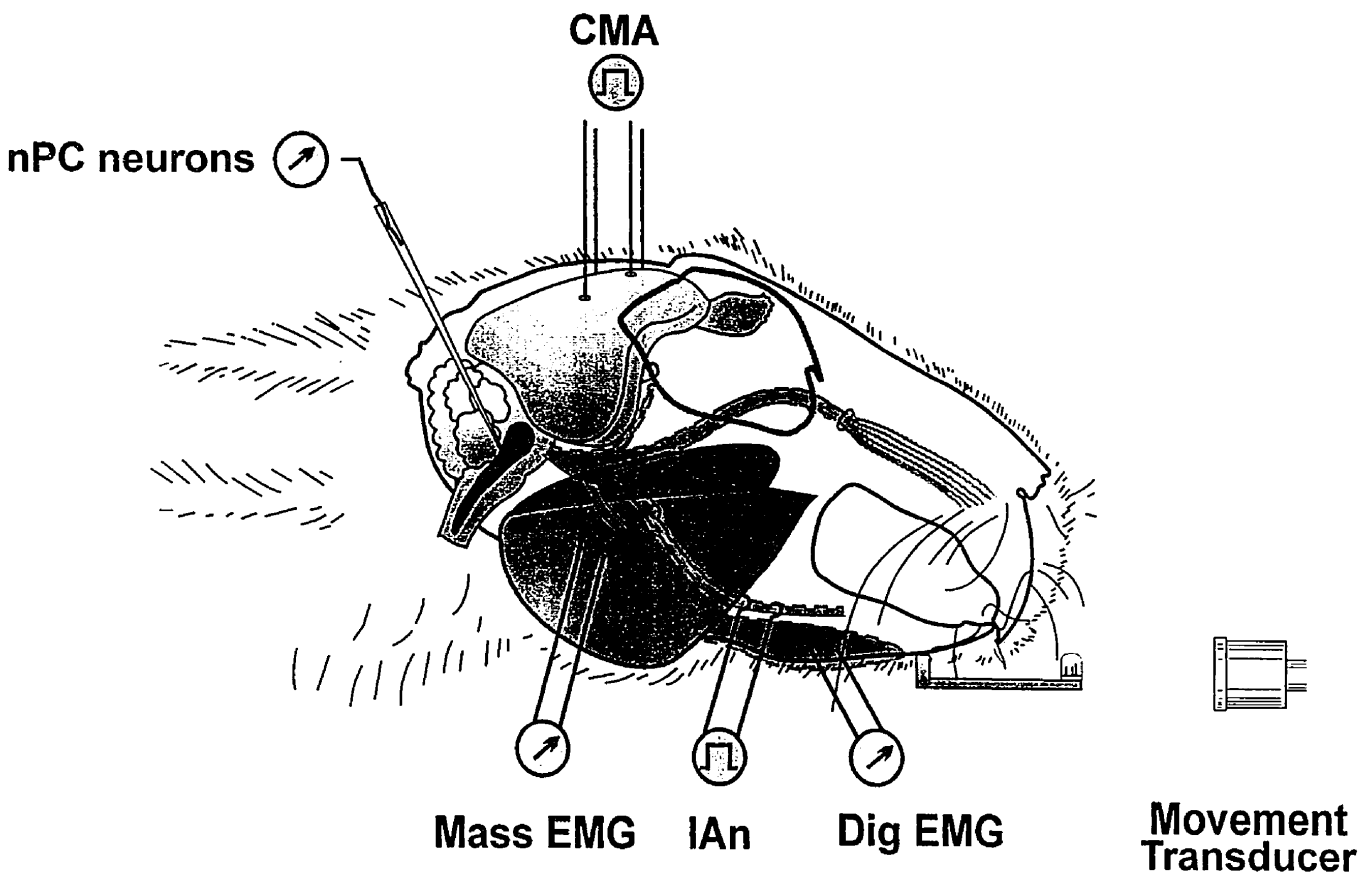
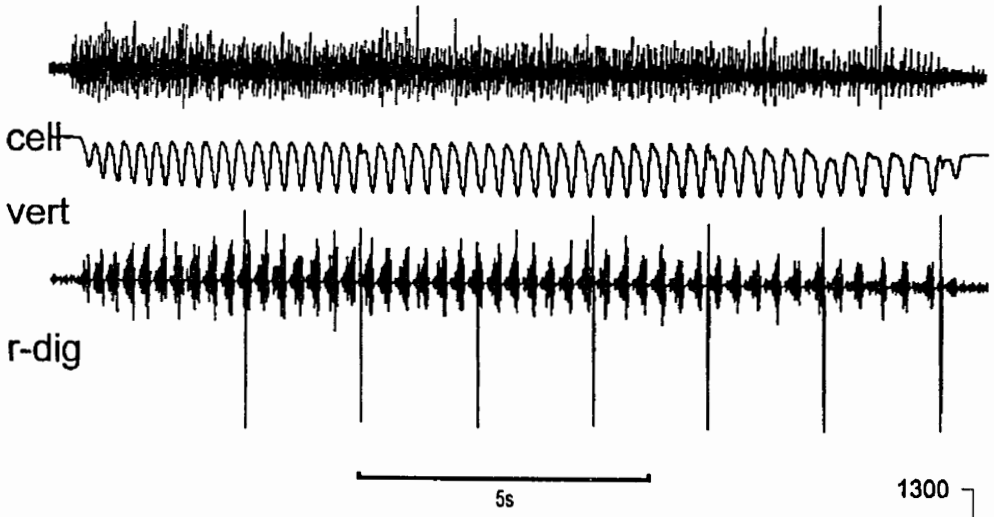
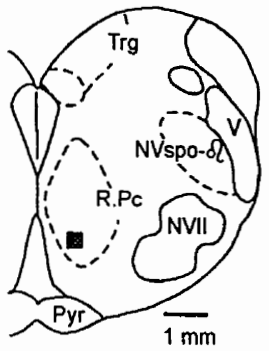

CMA
nPC neurons
Mass EMG
IAn
Dig EMG
Movement
Transducer

Figure 9. **Experimental Setup**

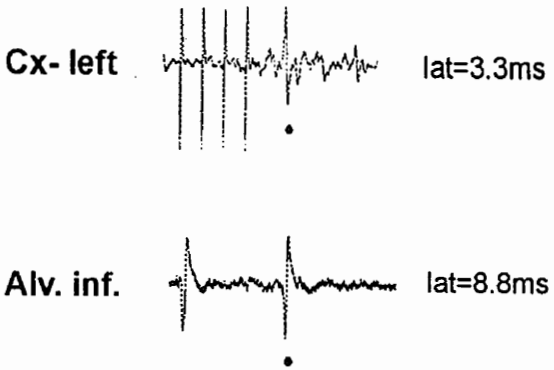
The inferior alveolar nerve (IAn) was stimulated (f= 0.5 Hz) with a pair of needle electrodes (free tips ~ 1.5mm) at the level of the second molar tooth. In addition, two wire hook electromyographic (EMG) electrodes were implanted into the digastric (Dig EMG) and masseter (Mass EMG) muscles on both sides. Also, concentric bipolar varnish-insulated stimulating electrodes were lowered into the cortical masticatory area (CMA) at bregma coordinates of approximately A 2.0 mm and L 5.0 mm. A metal bar holding an incandescent filament lamp in front of the lower incisor teeth was attached to the mental symphisis of the mandible with screws and dental acrylic. Movement of the light bulb, and thus the jaw, in the vertical and horizontal planes were measured by a light-sensitive position transducer. Finally, extracellular recordings were made in the contralateral nucleus reticularis pontis caudalis (nPC) (AP = 6.5-7.5 mm; Depth = 9.0 - 12.5 mm; Lat = 0.5-1.5 mm). Microinjections (200nl-500nl) of lidocaine were made into the area of nPC while the 50μm epoxy-coated tungsten recording microelectrode extended 1 mm beyond the orifice of the microinjection cannula.



RECORDING SITE



SHORT LATENCY INPUTS



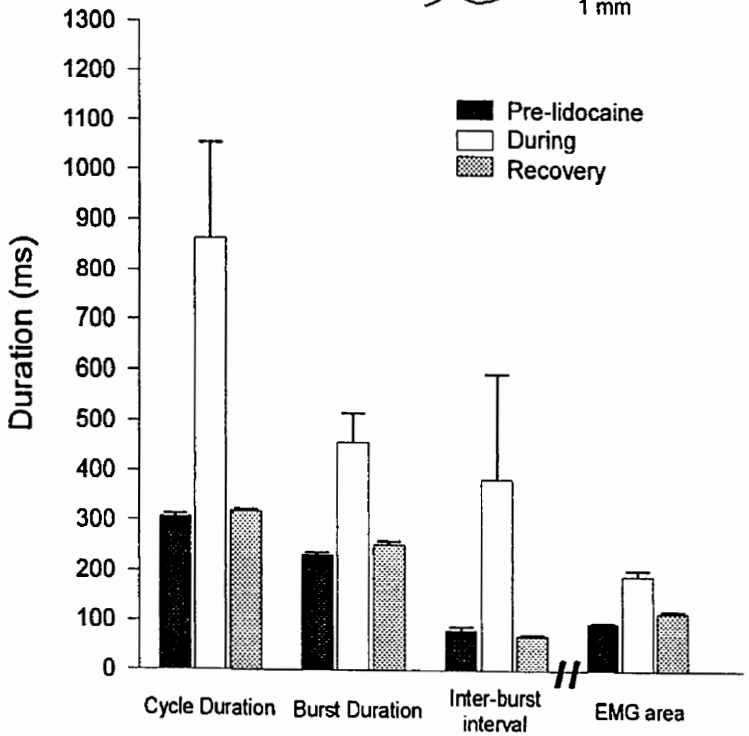


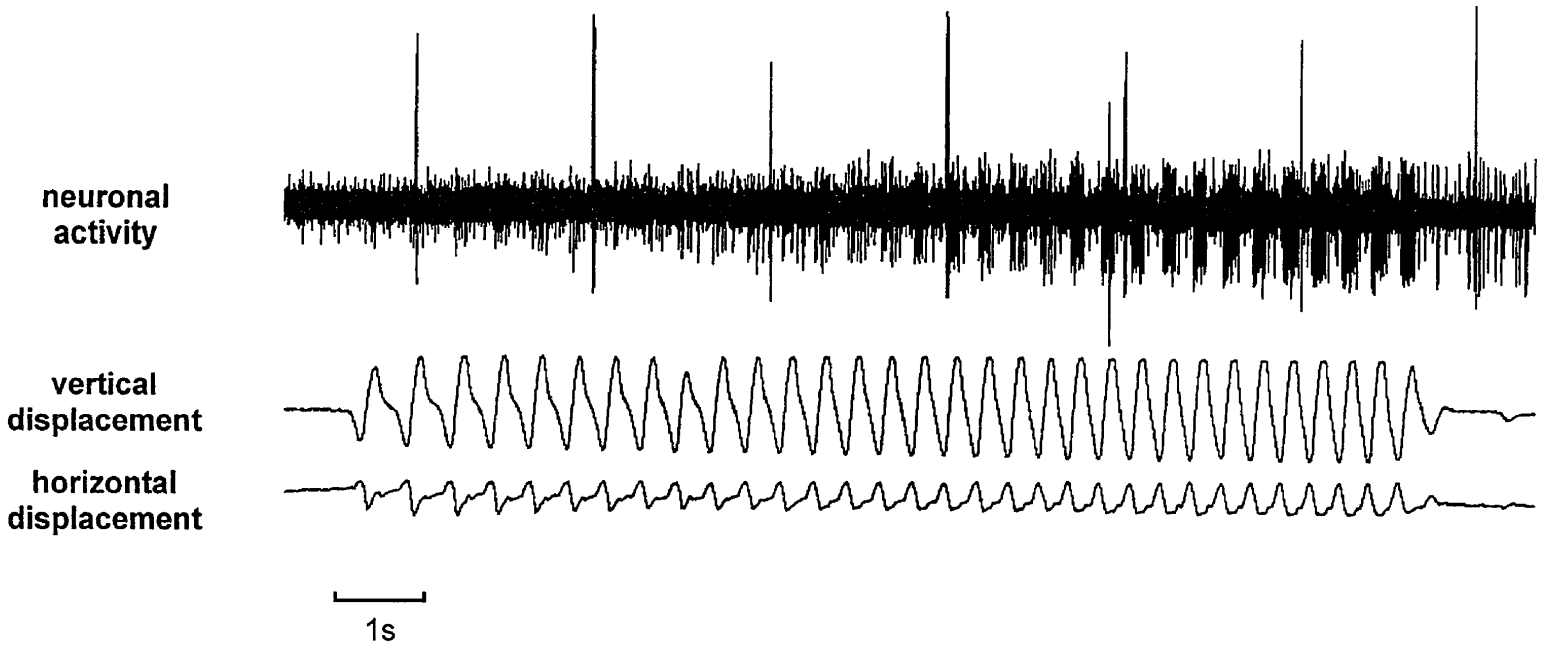
Figure 10. **Sample Neuron**

A sample nPC neuron used in this study is shown here. This tonically*-firing neuron was found in the ventral part of nPC at the level of the facial nucleus. During mastication (shown here by the rhythmic bursts of the right digastric muscle), it received short-latency inputs from the left cortex (latency = 3.3 ms) as well as from the Ian (latency = 8.8 ms).

The graphic in the lower right corner illustrates the effect of blocking the neuron with lidocaine. This blockage caused an increase in burst duration, inter-burst interval, as well as EMG area. Cycle duration naturally increased since it is a combination of burst duration and inter-burst interval.

* Tonic firing is demonstrated in the upper-most trace; please see figure 11 for comparison of tonically and rhythmically firing neurons.

rhythmically active neuron



tonically active neuron

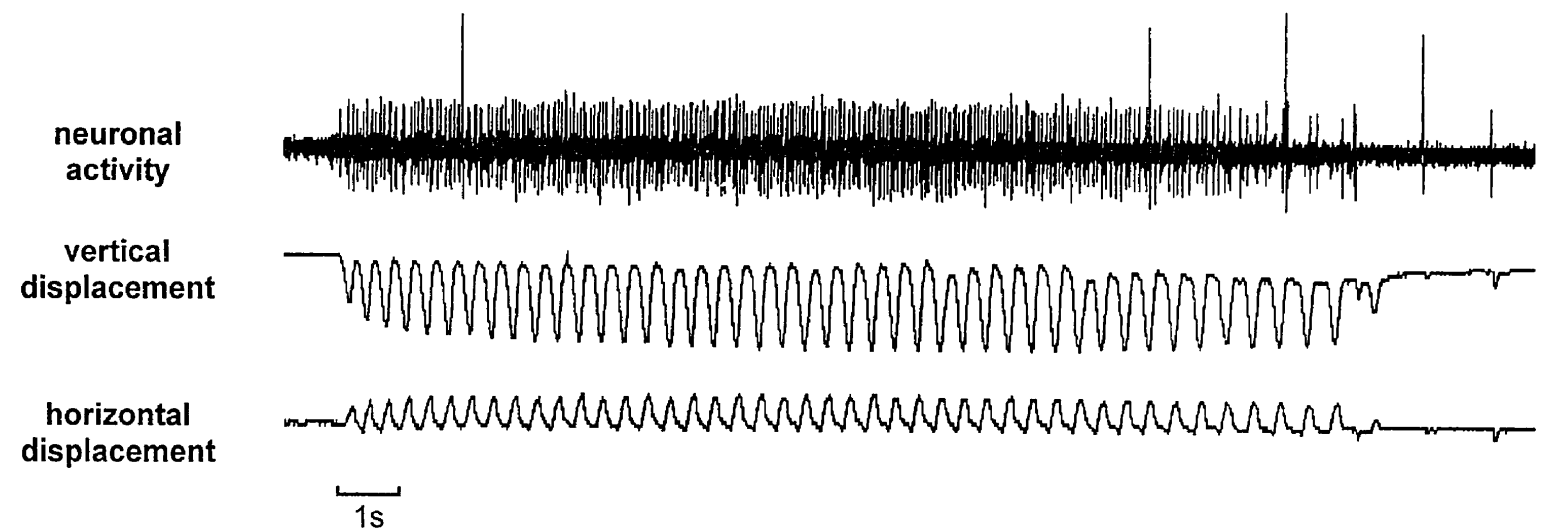


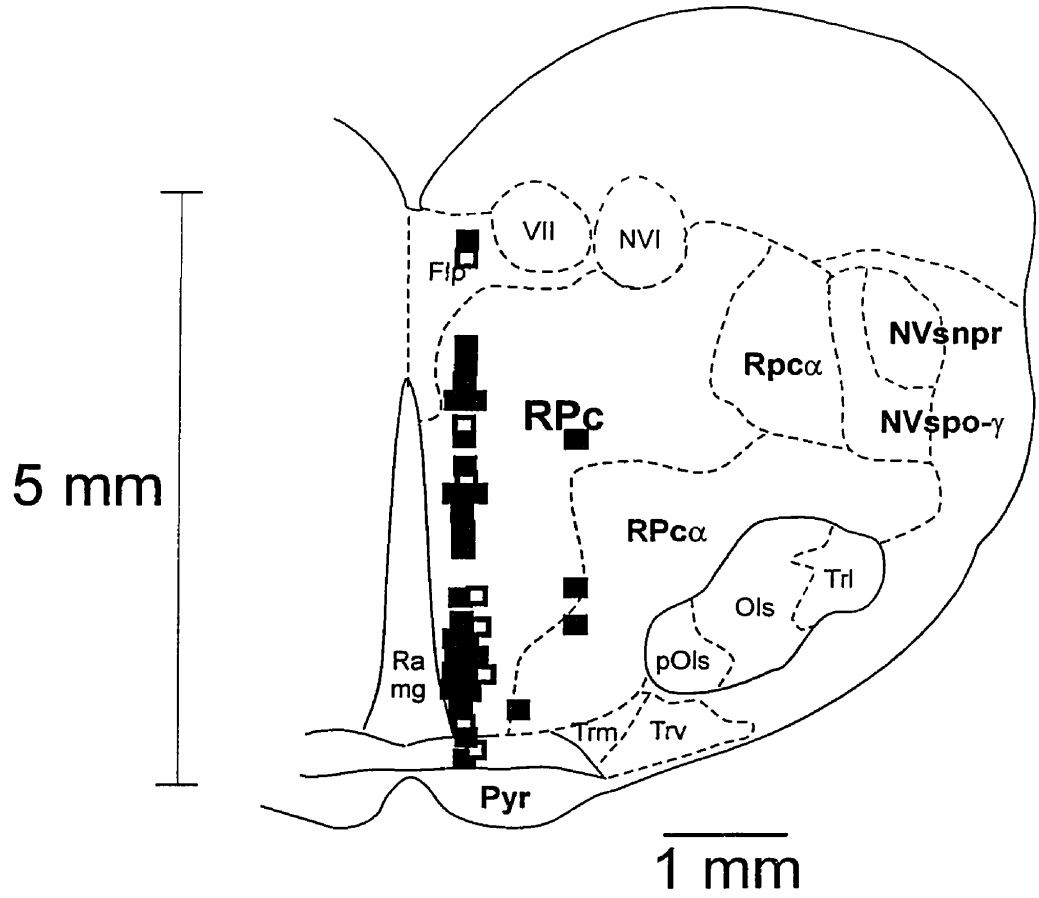
Figure 11. **Tonically and Rhythmically Firing nPC Neurons During Mastication.**

Shown here are the patterns of tonically and rhythmically-firing neurons during mastication, which is represented here as vertical and horizontal displacements of the jaw. Note how the rhythmically active neuron fired more tonically at first but started to organize its firing until it became rhythmic with very little random firing between its rhythmic bursts. On the other hand, the other neuron fired tonically throughout the masticatory sequence.

Cortically-evoked responses

■ ctx <5ms

□ ctx >5ms



Flp - fasciculus longitudinalis posterior
NVspoγ - subnucleus tractus spinalis trigemini oralis gamma
NVsnpr - nucleus trigemini sensibilis principalis
NVI - nucleus nervi abducentis
Ols - nucleus olivaris superior
pOls - nucleus paraolivaris superior
Pyr - tractus pyramidalis
Ramg - nucleus raphes magnus
RPc - nucleus reticularis pontis caudalis
RPcα - pars alpha nuclei reticualris pontis caudalis
Rpcα - pars alpha nuclei reticularis parvocellularis
Trl - subnucleus trapezoides lateralis
Trm - subnucleus trapezoides medialis
Trv - subnucleus trapezoides ventralis
VII - genu nervi facialis

Figure 12a. **Cortical Inputs Map**

Representative sections, like this one, of nucleus reticularis pontis caudalis (nPC) were taken just rostral to the facial nucleus, at the level of the pars α nuclei reticularis parvocellularis and pars α nuclei reticularis pontis caudalis (Rpcα and RPcα respectively)*.

In this particular section, location of nPC neurons that received cortical inputs is shown with a distinction between short and long-latency neurons. A total of 40 neurons (78%) responded to 4 pulse (500Hz, 0.05ms) stimulation of the CMA including 27 (53%) that received short-latency cortical input (filled squares; latency <5 ms; average = 3.60 ms ± 0.17 ms) and 13 (25%) that received long-latency cortical input (open squares; latency >5ms; average = 10.86 ms ± 2.38 ms). The distribution of short and long-latency cortical inputs appeared random along the dorsal-ventral axis of nPC.

* These abbreviations apply to all the representative maps shown hereafter.

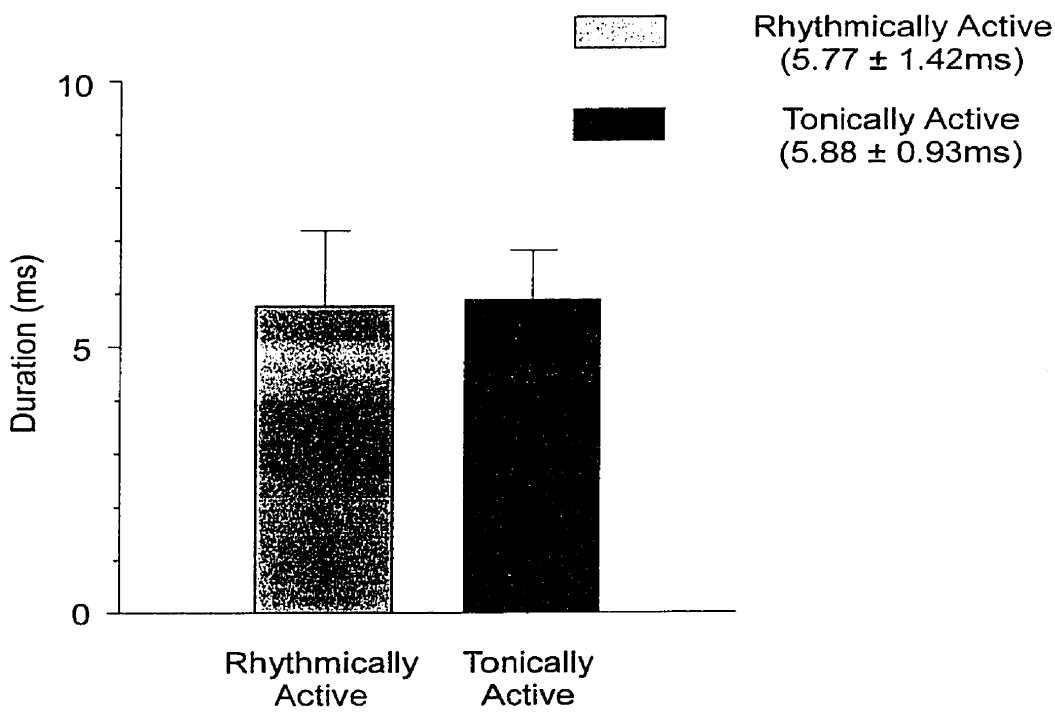
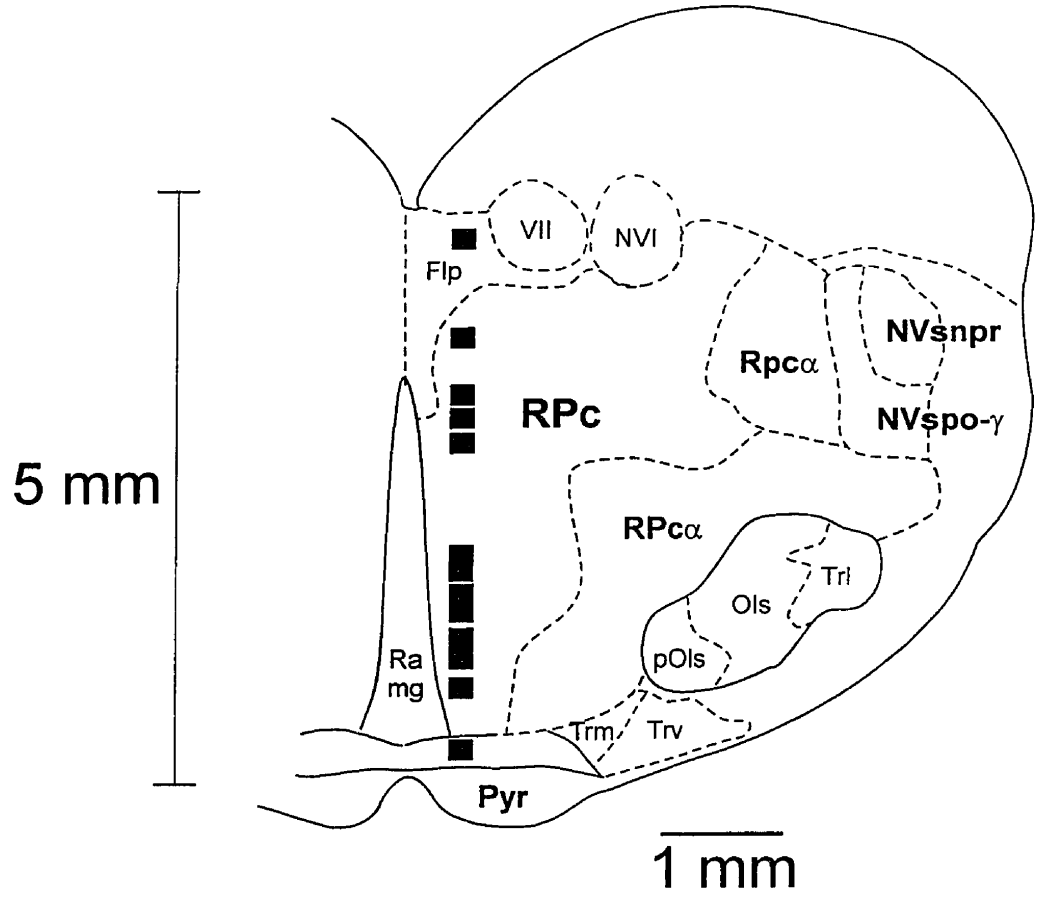

Rhythmically Active
(5.77 ± 1.42ms)
Tonically Active
(5.88 ± 0.93ms)
10
5
0
Duration (ms)
Rhythmically Active
Tonically Active

Figure 12b. **Latencies of nPC neuronal responses to CMA stimulation**

This plot demonstrates that cortical inputs to rhythmically and tonically active neurons had the same latencies.

IAN-evoked responses



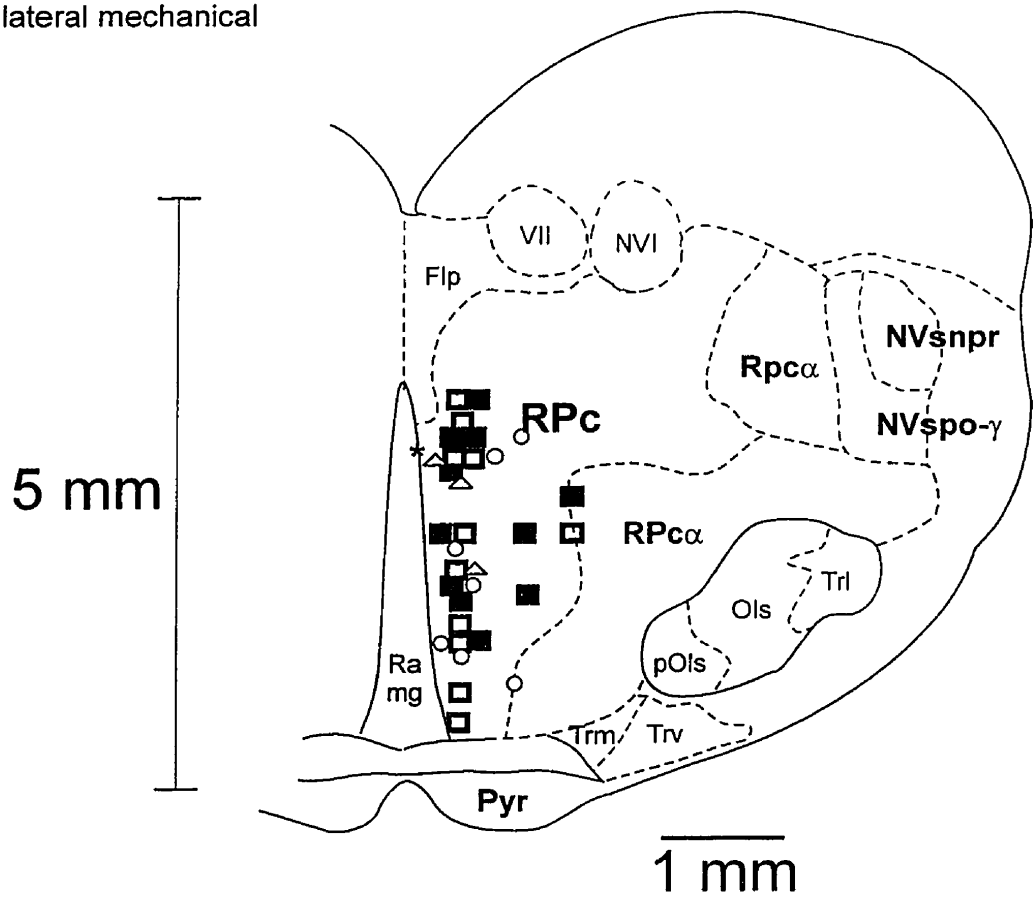
Flp - fasciculus longitudinalis posterior
NVspoγ - subnucleus tractus spinalis trigemini oralis gamma
NVsnpr - nucleus trigemini sensibilis principalis
NVI - nucleus nervi abducentis
Ols - nucleus olivaris superior
pOls - nucleus paraolivaris superior
Pyr - tractus pyramidalis
Ramg - nucleus raphes magnus
RPc - nucleus reticularis pontis caudalis
RPcα - pars alpha nuclei reticualris pontis caudalis
Rpcα - pars alpha nuclei reticularis parvocellularis
Trl - subnucleus trapezoides lateralis
Trm - subnucleus trapezoides medialis
Trv - subnucleus trapezoides ventralis
VII - genu nervi facialis

Figure 13. **Inferior Alveolar Inputs Map**

In this section, the location of the sixteen neurons (31%) that responded to stimulation of the Ian are represented by the filled squares.

Responses to Peripheral stimuli

- ■ Stretch
- ▣ Contralateral pinch
- ○ Periodontal
- △ Contralateral mechanical



Flp - fasciculus longitudinalis posterior
NVspoγ - subnucleus tractus spinalis trigemini oralis gamma
NVsnpr - nucleus trigemini sensibilis principalis
NVI - nucleus nervi abducentis
Ols - nucleus olivaris superior
pOls - nucleus paraolivaris superior
Pyr - tractus pyramidalis
Ramg - nucleus raphes magnus
RPc - nucleus reticularis pontis caudalis
RPcα - pars alpha nuclei reticualris pontis caudalis
Rpcα - pars alpha nuclei reticularis parvocellularis
Trl - subnucleus trapezoides lateralis
Trm - subnucleus trapezoides medialis
Trv - subnucleus trapezoides ventralis
VII - genu nervi facialis

Figure 14. **Peripheral Inputs Map**

Thirty-two cells were manually tested for peripheral inputs. Twelve of these neurons (38%) responded to a noxious pinch in the upper left lip area (open squares). Ten neurons (31%) responded to a stretch of the mandible (filled squares). Nine neurons (28%) responded to mechanical pressure on the incisors (open circles). Finally, two neurons responded to non-noxious mechanical indentation of the skin of the upper lip (open triangles).

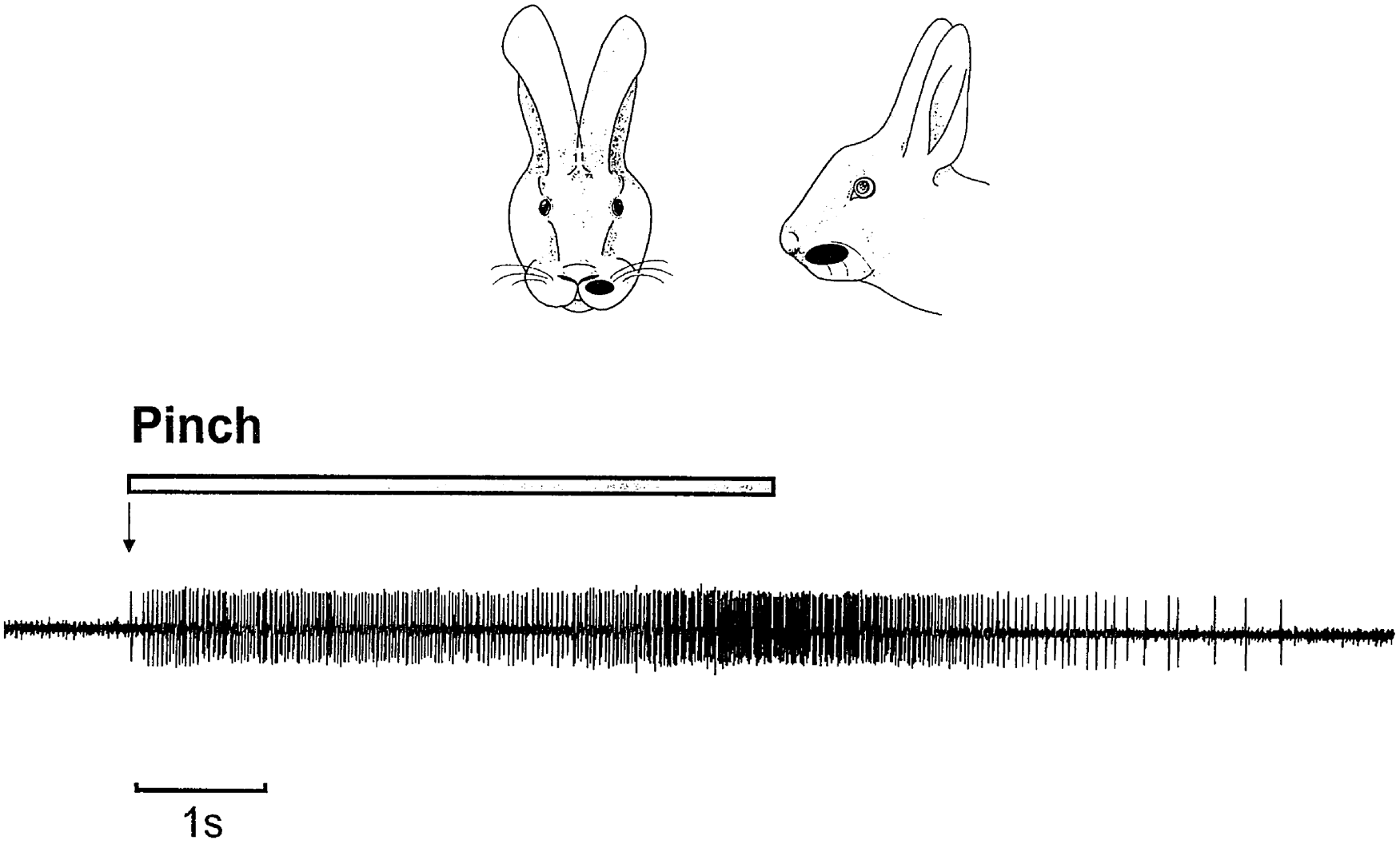

Pinch
1s

Figure 15. **Pinch Sensory Input to nPC Neurons**

Twelve cells (38 %) in nPC responded to a noxious pinch in the upper left lip area. Neuronal activity increased upon pinching of the upper lip area and was slowly adapting. The shaded area represents the pinched area.

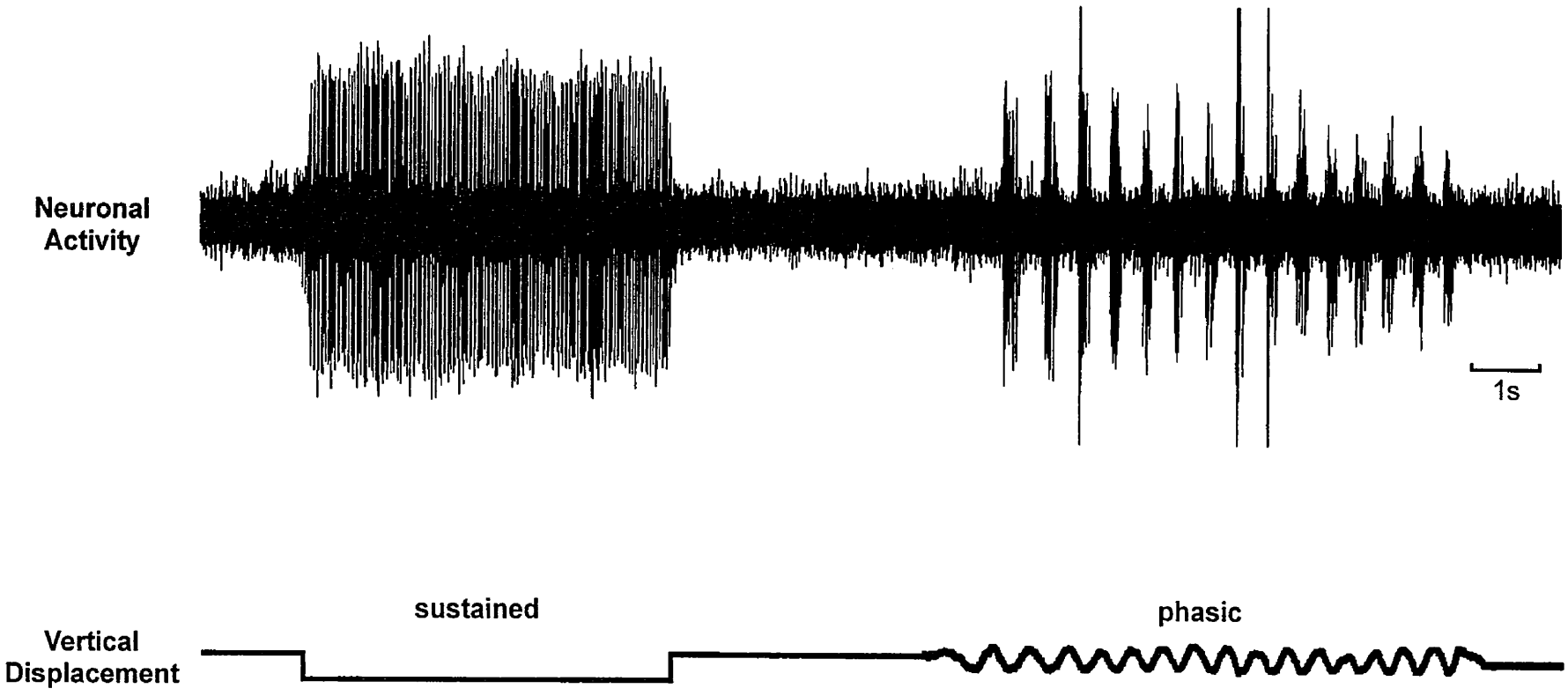

Neuronal Activity
1s
sustained
phasic
Vertical Displacement

Figure 16a. **Stretch Sensory Input to nPC Neurons**

Ten cells (31 %) responded to a stretch of the mandible. In this example, this nPC cell fired continuously with sustained vertical displacement of the jaw and phasically with phasic vertical displacement of the jaw.

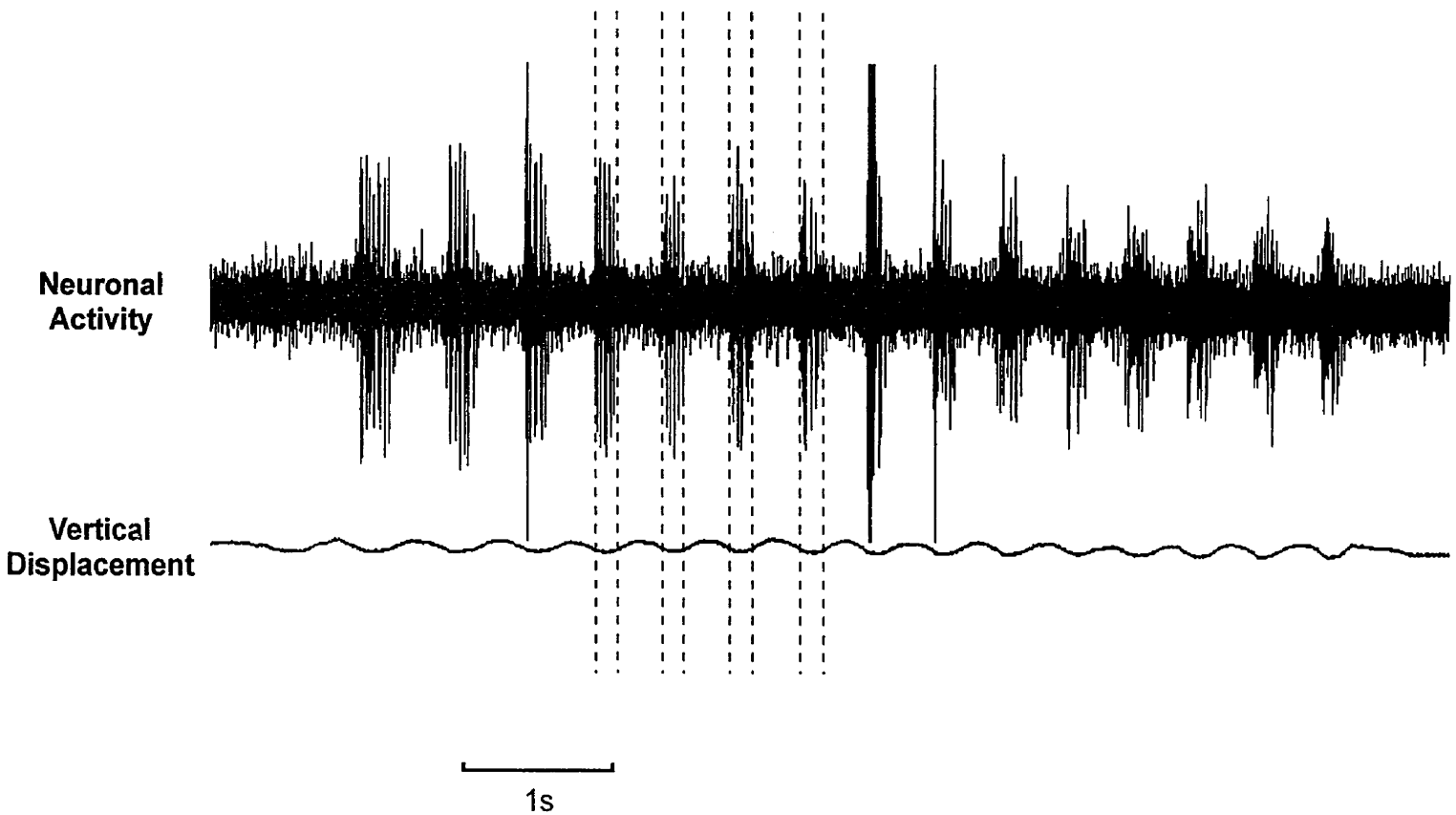
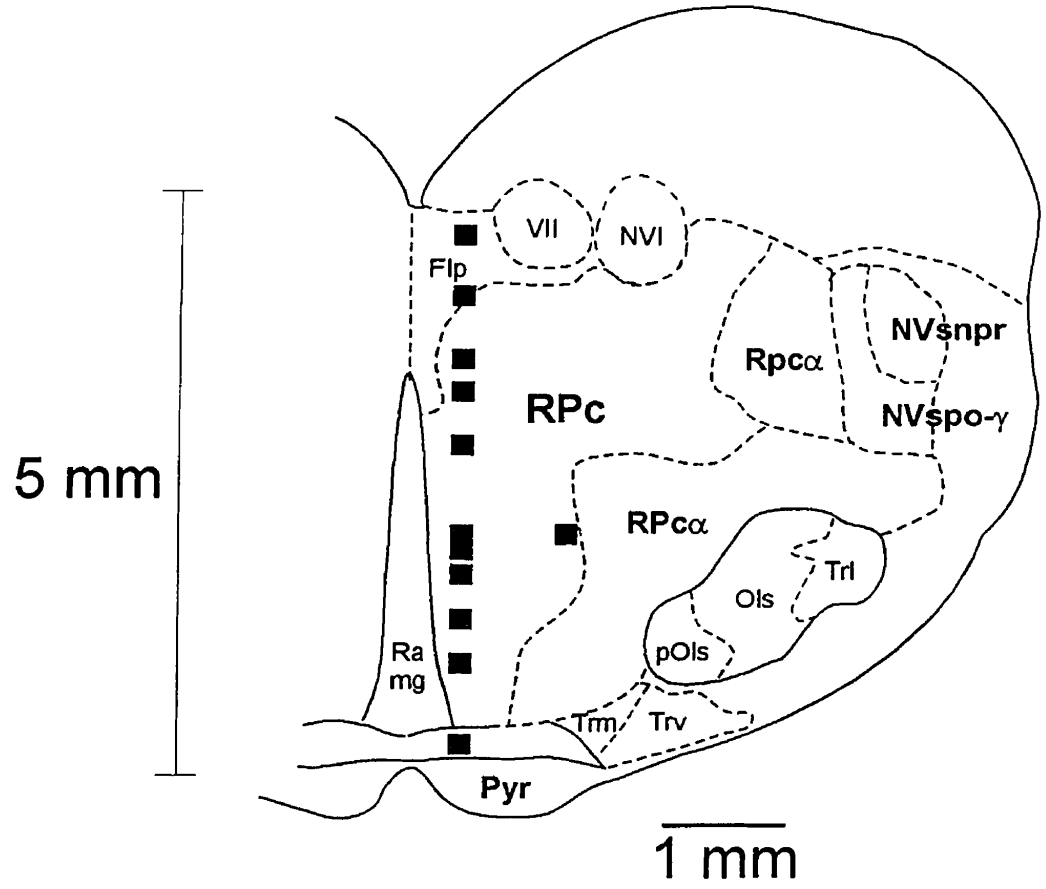

Neuronal Activity
Vertical Displacement
1s

Figure 16b. **Stretch Sensory Input to nPC Neurons**

A nPC cell firing phasically upon phasic vertical displacement of the jaw. Neuronal activity is observed during the jaw-opening and initial jaw-closing phases.

Cortical and peripheral inputs

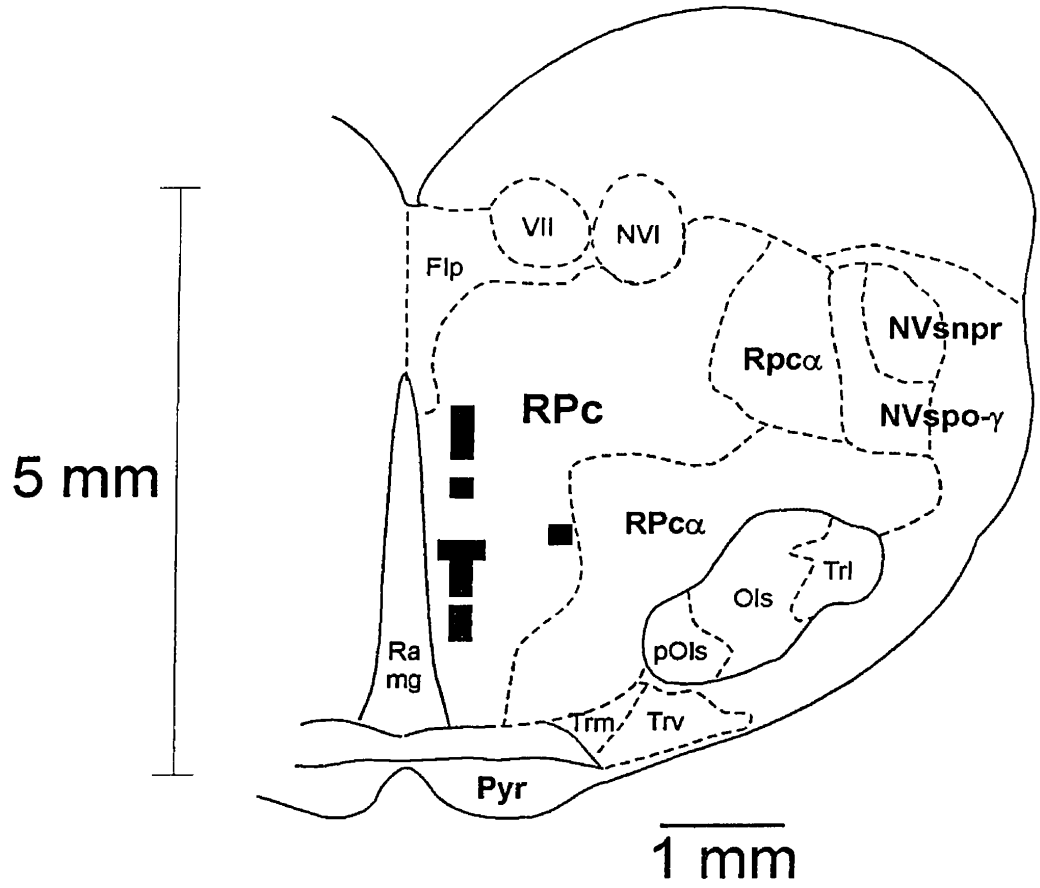


Flp - fasciculus longitudinalis posterior
NVspoγ - subnucleus tractus spinalis trigemini oralis gamma
NVsnpr - nucleus trigemini sensibilis principalis
NVI - nucleus nervi abducentis
Ols - nucleus olivaris superior
pOls - nucleus paraolivaris superior
Pyr - tractus pyramidalis
Ramg - nucleus raphes magnus
RPc - nucleus reticularis pontis caudalis
RPcα - pars alpha nuclei reticualris pontis caudalis
Rpcα - pars alpha nuclei reticularis parvocellularis
Trl - subnucleus trapezoides lateralis
Trm - subnucleus trapezoides medialis
Trv - subnucleus trapezoides ventralis
VII - genu nervi facialis

Figure 17. **Neurons with Both Cortical and Peripheral Inputs**

As shown in this section, twelve cells responded to both cortical and peripheral inputs (either IAn or manual stimulation; filled squares).

Multiple peripheral inputs



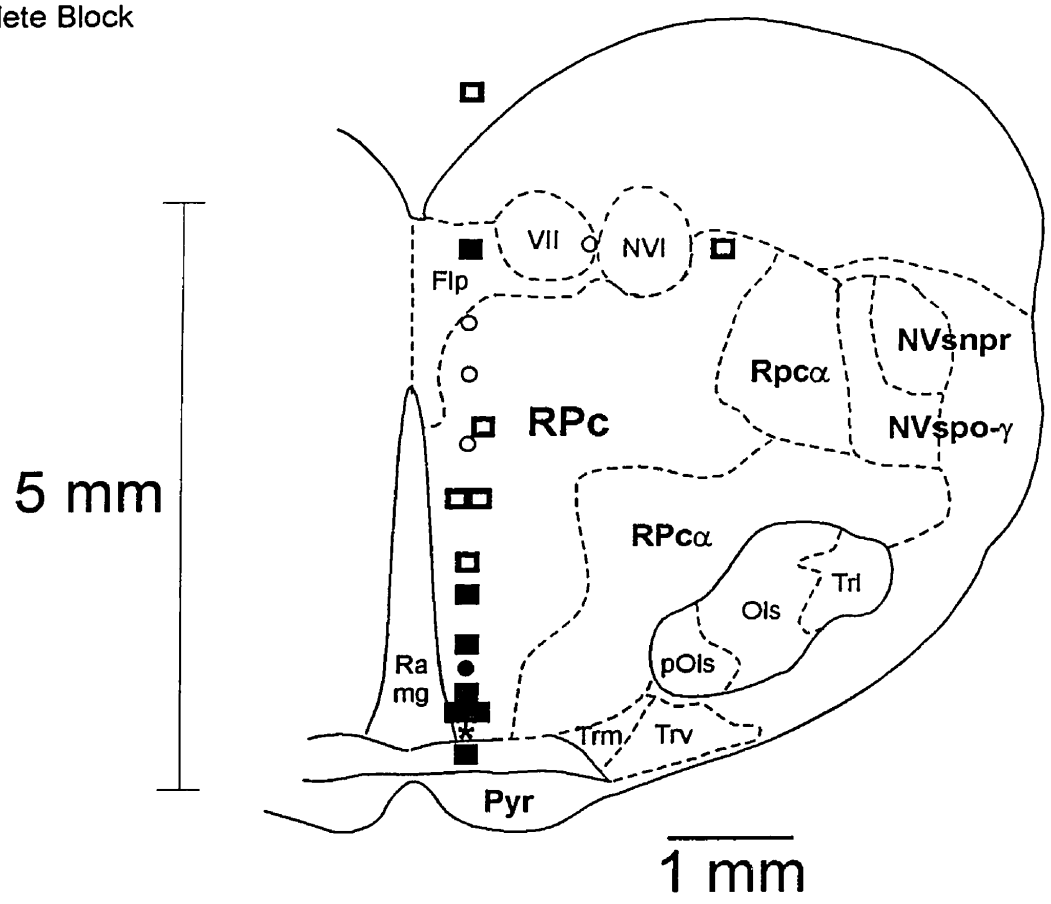
Flp - fasciculus longitudinalis posterior
NVspoγ - subnucleus tractus spinalis trigemini oralis gamma
NVsnpr - nucleus trigemini sensibilis principalis
NVI - nucleus nervi abducentis
Ols - nucleus olivaris superior
pOls - nucleus paraolivaris superior
Pyr - tractus pyramidalis
Ramg - nucleus raphes magnus
RPc - nucleus reticularis pontis caudalis
RPcα - pars alpha nuclei reticualris pontis caudalis
Rpcα - pars alpha nuclei reticularis parvocellularis
Trl - subnucleus trapezoides lateralis
Trm - subnucleus trapezoides medialis
Trv - subnucleus trapezoides ventralis
VII - genu nervi facialis

Figure 18. **Neurons with Multiple Peripheral Inputs**

A diagram showing the eleven neurons that received multiple peripheral inputs including IAn inputs (filled squares).

Burst Duration

- No effect
- Increase
- Decrease
- Complete Block



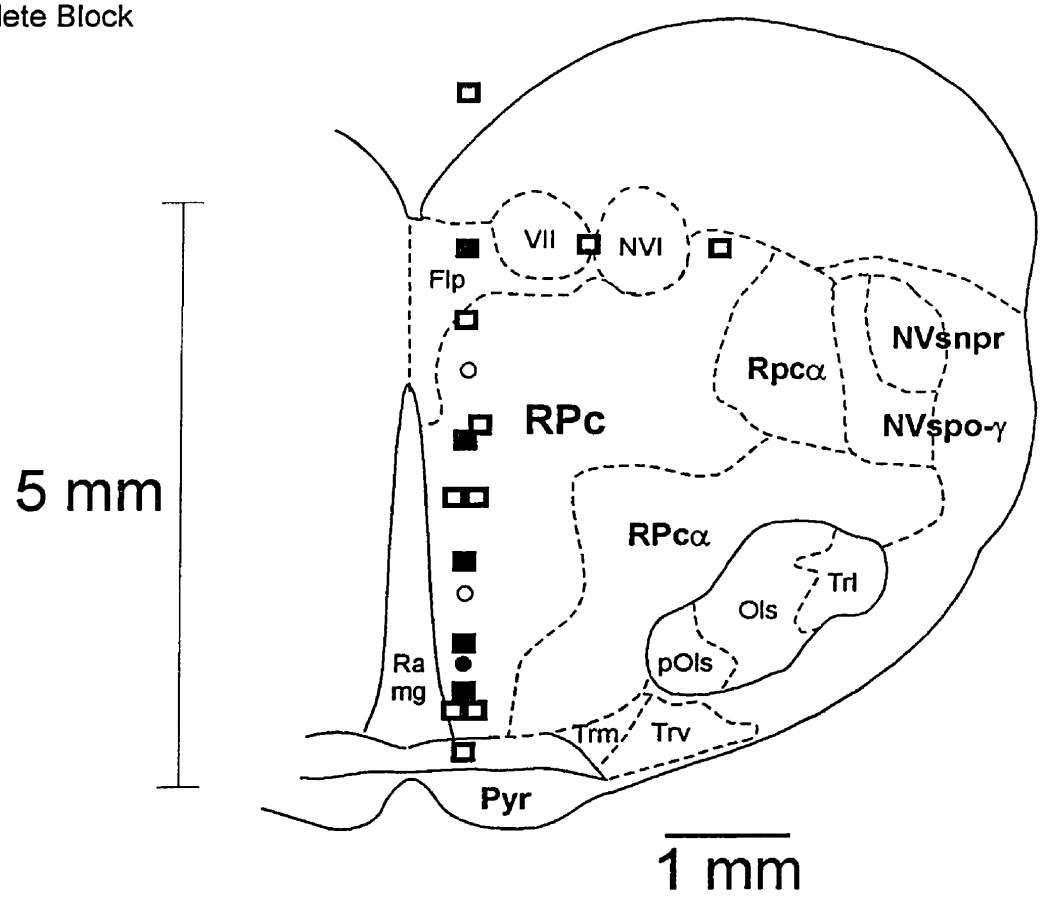
Flp - fasciculus longitudinalis posterior
NVspoγ - subnucleus tractus spinalis trigemini oralis gamma
NVsnpr - nucleus trigemini sensibilis principalis
NVI - nucleus nervi abducentis
Ols - nucleus olivaris superior
pOls - nucleus paraolivaris superior
Pyr - tractus pyramidalis
Ramg - nucleus raphes magnus
RPc - nucleus reticularis pontis caudalis
RPcα - pars alpha nuclei reticualris pontis caudalis
Rpcα - pars alpha nuclei reticularis parvocellularis
Trl - subnucleus trapezoides lateralis
Trm - subnucleus trapezoides medialis
Trv - subnucleus trapezoides ventralis
VII - genu nervi facialis

Figure 19. **Burst Duration Changes**

This is a diagram illustrating the effect of lidocaine injections on muscle burst duration. Lidocaine injections in the areas of 8 cells (53%; filled squares) resulted in a significant increase in muscle burst length. Six of these eight cells were located in the ventral part of nPC. Lidocaine injections in the areas of 2 cells (13%; open circles) resulted in a significant decrease in burst duration. Both cells were found in the dorsal part of nPC. Also, lidocaine injections in the areas of 3 cells (20%; open squares) found near the mid dorso-ventral area had no effect on burst length. Finally, 2 neurons, one in ventral and another in dorsal nPC, resulted in a complete block of mastication (filled circle)

Interburst Interval

- No effect
- Increase
- Decrease
- Complete Block



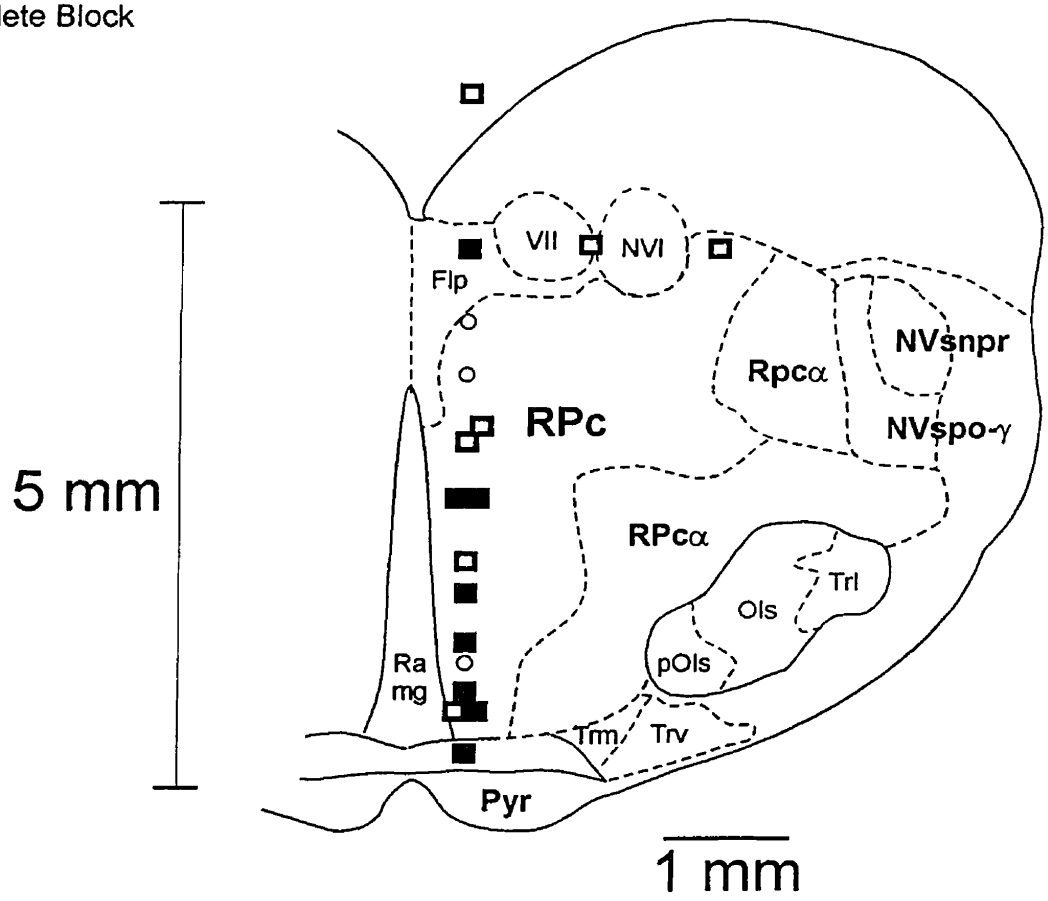
Flp - fasciculus longitudinalis posterior
NVspoγ - subnucleus tractus spinalis trigemini oralis gamma
NVsnpr - nucleus trigemini sensibilis principalis
NVI - nucleus nervi abducentis
Ols - nucleus olivaris superior
pOls - nucleus paraolivaris superior
Pyr - tractus pyramidalis
Ramg - nucleus raphes magnus
RPc - nucleus reticularis pontis caudalis
RPcα - pars alpha nuclei reticualris pontis caudalis
Rpcα - pars alpha nuclei reticularis parvocellularis
Trl - subnucleus trapezoides lateralis
Trm - subnucleus trapezoides medialis
Trv - subnucleus trapezoides ventralis
VII - genu nervi facialis

Figure 20. **Interburst Interval**

The effect of lidocaine injection on interburst interval is illustrated. Injections in the areas of 5 nPC neurons (33%; filled squares) resulted in a significant increase in interburst length. Also, lidocaine injection in the area of 1 neuron (7%) resulted in a significant decrease (open circles), while lidocaine injections in the areas of 6 neurons (40%) resulted in no significant change (open squares). No clusters of the three groups were discernable. Instead, the neurons appeared well distributed in either part of nPC.

EMG

- No effect
- Increase
- Decrease
- Complete Block



Flp - fasciculus longitudinalis posterior
NVspoγ - subnucleus tractus spinalis trigemini oralis gamma
NVsnpr - nucleus trigemini sensibilis principalis
NVI - nucleus nervi abducentis
Ols - nucleus olivaris superior
pOls - nucleus paraolivaris superior
Pyr - tractus pyramidalis
Ramg - nucleus raphes magnus
RPc - nucleus reticularis pontis caudalis
RPcα - pars alpha nuclei reticualris pontis caudalis
Rpcα - pars alpha nuclei reticularis parvocellularis
Trl - subnucleus trapezoides lateralis
Trm - subnucleus trapezoides medialis
Trv - subnucleus trapezoides ventralis
VII - genu nervi facialis

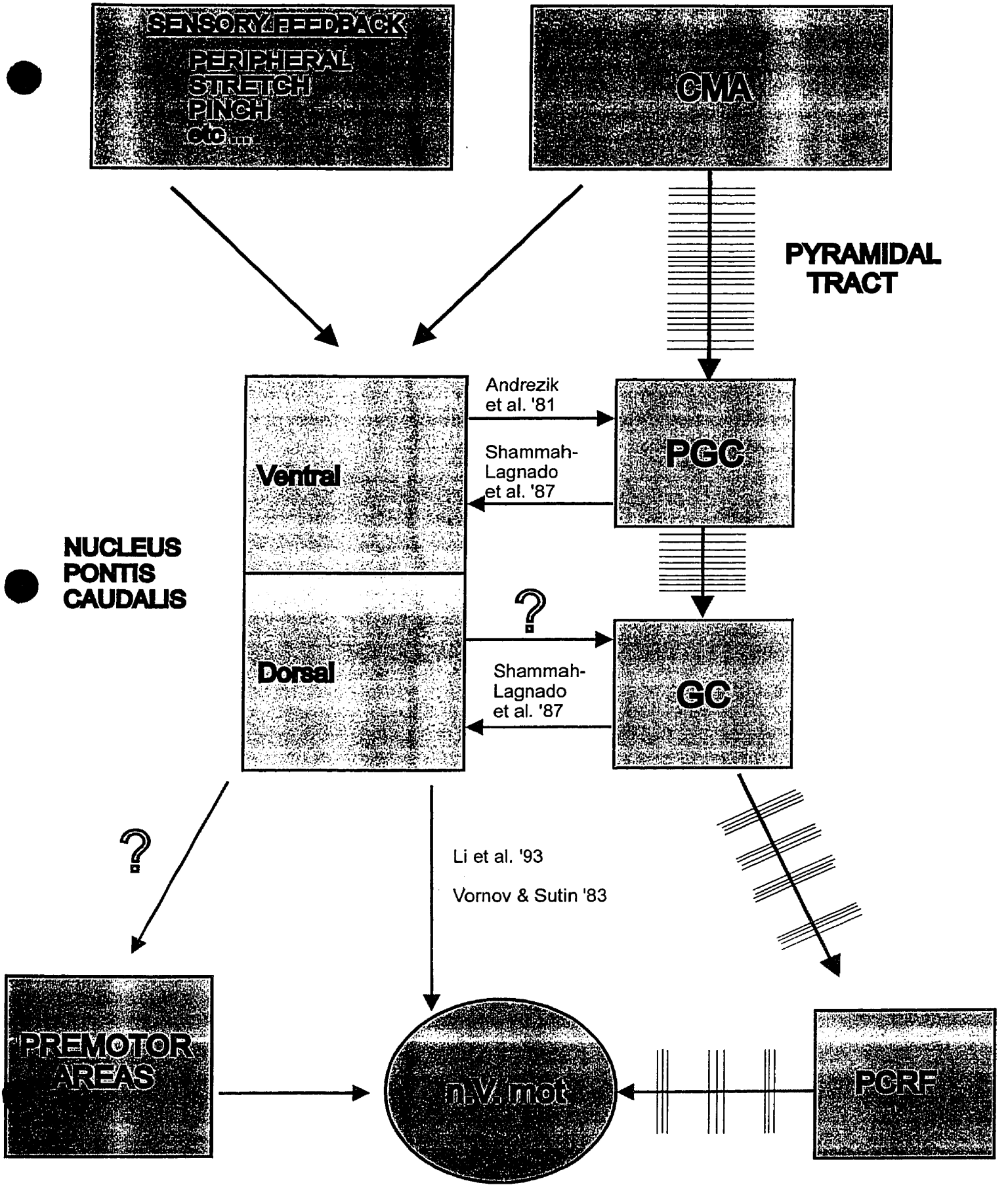
Figure 21. **EMG Area**

Lidocaine injections in the areas of 7 cells (47%; filled squares) resulted in a significant increase in EMG area. All seven were located near the mid dorso-ventral line or deeper in the ventral part of nPC. Injections in the area of one cell (open circles) in the dorsal part of nPC resulted in a decrease in EMG area. Also, injections in the areas of 4 cells (27%; 2 in ventral nPC, 2 in dorsal nPC; open squares) resulted in no significant change in EMG area. Lidocaine injections in the areas of 2 cells (filled circles), one in the ventral nPC and the other in the dorsal nPC, resulted in a total abolition of mastication.

Figure 22. **A Model for the Role of nPC in Mastication**

In the proposed model, nPC integrates 1) sensory information including information coming from periodontal, pinch, and jaw stretch receptors, 2) sensory information relayed by the IAn, and 3) cortical information from the CMA. In addition to its role in the integration of sensory and cortical information, nPC may also be involved in the modulation of masticatory output. The way that nPC actually helps modulate cortical input remains unexplored. One possibility may be related to its bi-directional connection with PGC (Andrezik et al., 1981; Shammah-Lagnado et al., 1987) and its input from GC (Shammah-Lagnado et al., 1987), as seen in this figure.

What output system does nPC use to modify the motor program appropriately ? Several possibilities exist : Firstly, nPC does have an output to PGC but this connection is not very extensive (Shammah-Lagnado et al., 1987). Secondly, nPC may have an output to GC but this area remains unexplored. Thirdly, anatomical studies have shown nPC output to n.V.mot. (Vornov and Sutin, 1983). The modulation of basic masticatory rhythm might occur by influencing GC or premotor areas, not the digastric motoneurons directly.


SENSORY FEEDBACK
PERIPHERAL
STRETCH
PINCH
etc ...
CMA
PYRAMIDAL TRACT
Andrezik et al. '81
Shammah-Lagnado et al. '87
Ventral
PGC
NUCLEUS PONTIS CAUDALIS
Dorsal
Shammah-Lagnado et al. '87
GC
Li et al. '93
Vornov & Sutin '83
PREMOTOR AREAS
n.V. mot
PCRF

REFERENCES

Aigouy L, Pajot J, Raboisson P, Vassel A, and Woda A. "Localization of the first order neurone of the jaw opening reflex elicited by periodontal stimulation". *Brain Res.* 473:380 (1988).

Amano N, Iwasaki T. "Response characteristics of primary periodontal mechanoreceptive neurons in the trigeminal mesencephalic nucleus to trapezoidal mechanical stimulation of a single tooth in the rat". *Brain Res*, 237:309 (1982).

Andrezik J, Chan-Palay V, and Palay S. "The Nucleus paragigantocellularis lateralis in the rat". *Anat. Embryol.* 161:373 (1981).

Appenteng K, Lund JP, and Seguin JJ. " Intraoral mechanoreceptor activity during jaw movement in the anesthetized rabbit". *J. Neurophysiol.* 48:27 (1982).

Appenteng K, Lund JP, and Seguin JJ. "Behavior of cutaneous mechano-receptors recorded in mandibular division of Gasserian ganglion of the rabbit during movements in lower jaw". *J. Neurophysiol.* 47:151 (1982).

Bazett HC and Penfield WG. "A study of the Sherrington decerebrate aniaml in the chronic as well as acute condition". *Brain.* 45:185 (1922).

Bremer F. "Physiologie nerveuse de la mastication chez le chat et le lapin. Reflexes de mastication.". *Acrh Int Physiol.* 21:308 (1923).

Byers MR, O'Connor TA, Martin RF, and Dong WK. "Mesencephalic trigeminal sensory neurons of cat : axon pathways and structure of mechano-receptive endings in periodontal ligament". *J. Com. Neurol.* 250:181 (1986).

Chandler SH and Tal M. "The effects of brain stem transections on the neuronal networks responsible for rhythmical jaw movements in the guinea pig". *J. Neurosci.* 6:1831 (1986).

Chandler SH, Turman JJr, Salem L, and Goldberg LJ. "The effects of nanoliter ejections of lidocaine into the pontomedullary reticular formation on cortically induced rhythmical jaw movements in the guinea pig" Brain Res. 526:54-64 (1990)

Collier TG and Lund JP. " The effects of sectioning the trigeminal sensory root on reflexes elicited by tapping the teeth". *J. Dent. Res.* 66:1533 (1987).

Crist CF, Yamasaki DS, Komatsu H, and Wurtz RH. "A grid system and a microsyringe for single cell recording". *J. Neurosci. Methods.* 26(2):117-22 (1988).

Dellow PG and Lund JP. "Evidence for central timing of rhythmical mastication". *J. Physiol.* 215:1 (1971).

Donga R and Lund JP. "Discharge patterns of trigeminal commissural last-order interneurons during fictive mastication in the rabbit". *J. Neurophysiol.* 66:1564 (1991).

Duncan GH, Bushnell MC, Oliveras JL, Bastrash N, and Tremblay N. "Thalamic VPM nucleus in the behaving monkey. III Effects of reversible inactivation by lidocaine on the thermal and mechanical discrimination". *J. Neurophysiol.* 70(5):2086-96 (1993).

Goodwin GM and Luschei EM. "Effects of destroying spindle afferents from jaw muscles on mastication in mokeys". *J. Neurophysiol.*, 37:967 (1974).

Ferrier D. "The function of the brain". 2nd edition, Smith & Elder & Co., London (1886).

Goodwin GM and Luschei ES. "Discharge of spindle afferents from jaw-closing muscles during chewing in alert monkeys". *J. Neurophysiol.* 38:560 (1975).

Graybiel AM. "Direct and indirect preoculomotor pathways of the brainstem: an autoradiographic study of the pontine reticular formation in the cat". *J. Comp. Neurol.* 175, 37-78 (1977).

Gurahian SM, Chandler SH, and Goldberg LJ. "Intracellular analysis of trigeminal motoneuron rhythmical activity during stimulation of pontomedullary reticulat formation in anesthetized guinea pig". *Journal of Neurophysiology.* 62:1225 (1989).
Hannam A.G. "The response of periodontal mechanoreceptors in the dog to controlled loading of the teeth". *Arch. Oral Biol.* 14:781 (1969).

Hannam A.G. "The innervation of the periodontal ligament". The periodontal ligament in health and disease. Pergamon Press, Oxford. 173 (1982).

Hashimoto N, Katayama T, Ishiwata Y, and Nakamura Y. "Induction of rhythmic jaw movements by stimulation of the mesencephalic reticular formation in the guinea pig". *J. Neurosci.* 9(8): 2887-2901 (1989).

Hikosaka O and Wurtz RH. "Saccadic eye movements following injection of lidocaine into the superior colliculus". *Science.* 166:1410-1412 (1969).

Inoue T, Kato T, Masuda Y, Nakamura T, Kawamura Y, and Moritomo T. "Modifications of masticatory behavior after trigeminal deafferentiation in the rabbit". *Exp. Brain Res.* 74:579 (1989).

Juch PJW, Van Willigen JD, Broekhuijsen ML and Ballintijn CM. "Peri-pheral influences on the central pattern-rhythm generator for tongue movements in the rat". *Arch. Oral Biol.* 30:415 (1985).

Kalivas PW, Jennes L, Nemeroff CB, and Sprange AJJr. "Neurotensin: Topographical distribution of brain sites involved in hypothermia and antinociception". *J. Comp. Neurol.* 210:225-238 (1982).

Kawamura Y and Tsukamoto S. "Neural descending pathways from cortical jaw motor area and amygdaloid nucleus to jaw muscle". *Jpn. J. Physiol.* 10:489 (1960).

Kogo M, Funk GD, and Chandler SH. "Rhythmical oral-motor activity recorded in an in vitro brainstem preparation" *Somatosen. Motor Res.* 13(1):39-48 (1996).

Landgren, Olsson KA, Westberg KG. "Bulbar neurons with axonal projections to the trigeminal motor neurons in the cat". *Exp. Brain Res.* 65:98 (1986).

Li YQ, Masahiko T, and Noboru M. "Identification of premotor interneurons which project bilaterally to the trigeminal motor, facial, and hypoglossal : a fluorescent retrograde double-labeling study in the rat". *Brain Res.*, 611:160 (figure 2 :85, 92) (1993)

Lovick TA and Wolstencroft JH. "Response of medial reticular neurons to tooth pulp stimulation: evidence for a monosynaptic input". *J. Physiol.*, London 292:40 (1979).

Lund JP and Lamarre Y. "The importance of positive feedback from periodontal pressoreceptors for voluntary isometric contraction of jaw-closing muscles in man". *J. Biol. Bucc.*, 1:345-351 (1973)

Lund JP, Smith AM, Sessle BJ, and Murakami T. "Activity of alpha and gamma motoneurons abd muscle afferents during performance of a biting task". *J. Neurophysiol.* 42:710 (1979).

Lund JP, Rossignol S, and Murakami T. "Interactions between the jaw-opening reflex and mastication". *Can. J. Phys. Pharm.* 59: 683 (1981).

Lund JP, Appenteng K, and Seguin JJ. "Analogies and common features in the speech and masticatory control systems". Pergamon press, London. 231 (1982).

Lund JP and Olsson KA. "The importance of reflexes and their control during jaw movement". *TINS*, 6, 458, 1983 page 4

Lund JP, Sasamoto K, Murakami T, and Olsson KA. "Analysis of rhythmical jaw movements produced by electrical stimulation of motor-sensory cortex of rabbits". *J. Neurophysiol.* 52:1014 (1984).

Lund JP and Enomoto S. "The generation of mastication by the mammalian CNS". John Wiley and Sons, New York 1988.

Lund JP " Mastication and its control by the brain stem". *Crit. Rev. Oral Biol. Med.*, 2(1): 33 (1991).

Luschei ES, and Goodwin GM "Patterns of mandibular movement and jaw muscle activity during mastication in the monkey". *J. Neurophysiol.* 37: 954 (1974).

Mantyh PW "Connections of midbrain periaqueductal gray in the monkey. II. Descending efferent projections" *J. Neurophysiol.* 49(3), 582-594 (1983).

Matsunami K and Kubota K "Muscle afferents of trigeminal mesencephalic tract nucleus and mastication in chronic monkeys". *Jpn J. Physiol.* 22:545 (1972).

Mei N, Hartmann F, and Aubert M. "Periodontal mechanoreceptors involved in pain". *Pain in the Trigeminal Region. Elsevier/North-Holland Biomedical Press.* p103 (1977).

Messen H and Olszewski J. "Cytoarchitektonischer Atlas des Rautenhirns des Kaninchens". Basel:Karger (1949).

Miserendino MJD and Davis M "NMDA and non-NMDA antagonists infused into the nucleus reticularis pontis caudalis depress the acoustic startle reflex". *Brain Res.* 623:215 (1993).

Miller FR. "The cortical paths of mastication and deglutition". *J. Physiol.* 53:473 (1920).

Nakamura Y, Takatori M, Kubo Y, Nozaki S, and Enomoto S. "Possible roles of lower brain stem reticular formation in orbital cortically induced masticatory rhythm in cats". Jaw position and Jaw movement, Veb Verlag Volk und Gesundheit, Berlin, 309 (1980)

Nakamura Y. "Localization and functional organization of masticatory rhythm generator in the lower brain stem reticular formation". *Neurosci. Lett.* 20:S3 (1985).

Ness AR. "The mechanoreceptors of the rabbit incisor". *J. Physiol.* 126:475 (1954).

Nozaki S, Iriki A, Nakamura Y. "Localization of the central rhythm generator involved in cortically induced rhythmical masticatory jaw-opening movement in the guinea pig". *J. Neurophysiol.* 55:806 (1986)

Nozaki S, Iriki A, and Nakamura Y. "Role of corticobular projection neurons in cortically induced rhythmical masticatory jaw opening movement in the guinea pig". *J. Neurophysiol.* 55:826 (1986).

Olsson KA and Westburg KG. "Antidromic activation from the digastric motoneurone subnucleus of premotor interneurons in the subnucleus-gamma of the oral nucleus of the trigeminal tract". *Proc Int U. Physiol.* Sci XVII 535 (1989).

Rioch JM. "The neural mechanism of mastication". *Am. J. Physiol.* 108:168 (1960).

Rossignol S and Dubuc R. "Spinal pattern generation". *Curr. Op. Neurobiol.* 4:894 (1994).

Schwartz G, Enomoto S, Valiquette C, and Lund JP. "Mastication in the rabbit : A description of movement and muscle activity". *J. Neurophysiol.*, Vol 62, No 1: 273, July 1989.

Shammah-Lagnado SJ, Negrao N, Silva A, and Ricardo JA. "Afferent connections of the nuclei reticularis pontis oralis and caudalis : A HRP study in the rat". *Neuroscience.* 20(3): 961 (1987).

Strutz J and schmidt CL. "Acoustic and vestibular efferent neurons in the chicken". *Acta Oto-Laryngologica.* 94(1-2):45-51 (1982).

Sumi T. " Some properties of cortically evoked swallowing and chewing rabbits". *Brain Res.* 15:107 (1969).

Tal M. "Neural basis for initiation of rhythmic digastric activity upon midbrain stimulation in the guinea pig". *Brain Res.* 411:58 (1987).

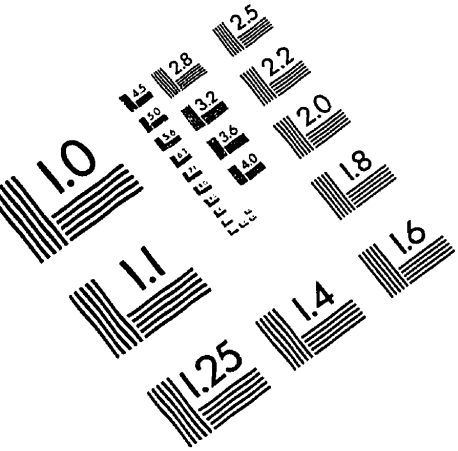
Thexton AJ, Hiiemae KM, and Crompton AW. "Food consistency and bite size as regulators of jaw movement during feeding in the cat". *J.Neurophysiol.*, 44:456 (1980).

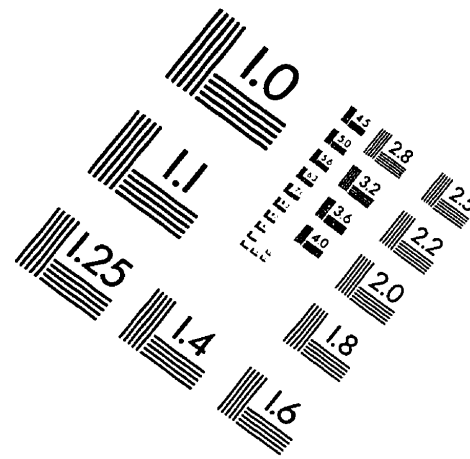
Van Willigen JD and Weijs-Boot J. "Phasic and rhythmic responses of the oral musculature to mechanical stimulation of the rat palate". *Arch Oral Biol.* 29:7 (1984).

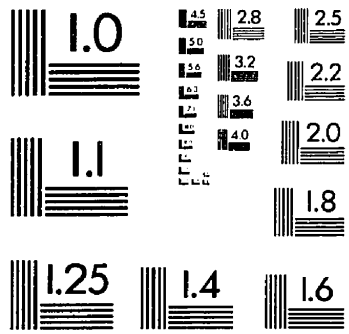
Vornov J and Sutin J. "Brain stem projections to the normal and noradrenergically hyperinnervated trigeminal motor nucleus". *J. Comp. Neurol.* 214:198 (1983).

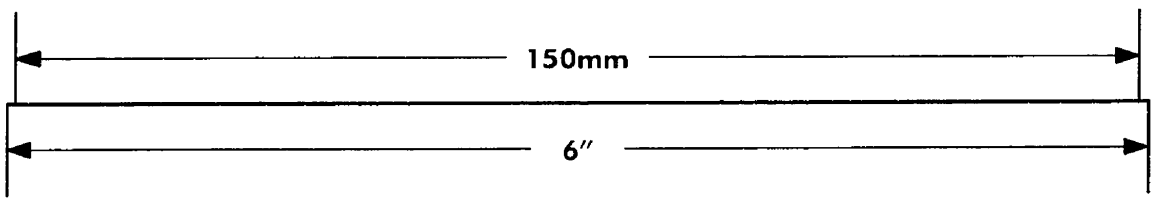
Westberg KG and Olsson KA. "Intergration in trigeminal premotor interneurons in the cat : Functional characteristics of neurons in subnucleus-g of the oral nucleus of the spinal trigeminal tract" *Exp. Brain Res.*, 84:102 (1991).

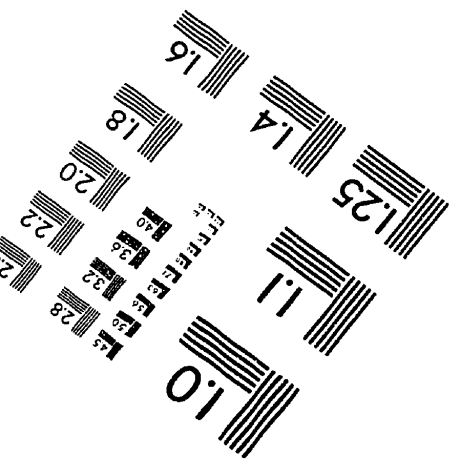
IMAGE EVALUATION
TEST TARGET (QA-3)

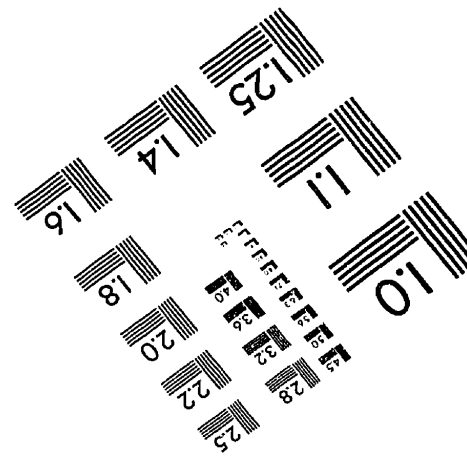












APPLIED IMAGE, Inc
1653 East Main Street
Rochester, NY 14609 USA
Phone: 716/482-0300
Fax: 716/288-5989