An Excitatory Component of the Jaw Opening Reflex in the Temporal and Masseter Muscles of Cats and Monkeys

It has recently been reported that mechanical stimulation of periodontal receptors and electrical stimulation of the gingiva evokes a short latency excitatory reflex response in the masseter muscles of man[1]. However, in a similar study it was concluded that the excitatory masseteric reflex evoked by mechanical stimulation of the teeth was not the result of periodontal receptor excitation but was due to vibration induced activation of muscle spindles in the jaw closing muscles[2]. Since there had been no previous reports of a short latency excitatory reflex evoked in the jaw closing muscles of man by stimulation of receptors in the mucosa of the oral cavity, the usual response being one of inhibition of activity of these muscles[3–5], it was important to determine whether a similar excitatory reflex could be obtained in animals.

Methods. The experiments were performed on 4 adult cats and 2 adult monkeys (*Macaca mulatta*). The animals were lightly anesthetized; sodium methohexital (Brevital) in the cats and phenylcyclidine hydrochloride (Sernylan) in the monkeys. Bipolar recording electrodes were inserted into the anterior fibers of the temporal muscle, the anterior belly of the digastric muscle, and the masseter muscle, using a technique described by SAUERLAND and MITCHELL[6]. The recording electrodes consisted of 2 enamel coated stainless steel wires, 0.16 mm in diameter, with 1 mm of insulation removed from the tips and the bare ends kept approximately 3 mm apart in the muscle. Stimulation of intra-oral mucosal afferents was obtained in the monkeys by single electrical pulses delivered through bipolar electrodes placed on the gingiva, and in the cat by single shocks delivered to the cut central end of the lingual nerve just before it entered the tongue. The cats were placed in a stereotaxic apparatus and the monkeys lay in a supine position with the head supported by a pillow. The electrical activity of the muscles was recorded on tape and subsequently played back into an oscilloscope and photographed. A more complete description of the stimulation and recording system has been presented elsewhere[1].

Results and discussion. In all cats stimulation of the ipsilateral or contralateral lingual nerve evoked the jaw opening (linguomandibular) reflex. This reflex has been characterized by excitation of digastric motoneurons accompanied by inhibition of activity in masseteric motoneurons[7]. The lingual nerve stimulus also evoked an electrical response in the ipsilateral digastric muscle beginning approximately 7 msec after the shock artifact (Figure 1). The digastric activity was usually preceded by a short latency response (5–7 msec) in the temporal and masseter muscles (Figure 2A).

In some instances, however, this short latency response was not present. In the experiment illustrated in Figure 1, lingual nerve stimulation consistently evoked digastric muscle excitation and jaw opening, but the only consistent response in the ipsilateral temporal muscle appeared after a latency of 16–18 msec (Figure 1A). This result was obtained with the jaw at rest. Under Brevital anesthesia the jaw elevator muscles did not have sufficient tone to hold the jaw closed. The separation between the incisal edges of the upper and lower anterior teeth was approximately 18 mm. The lower jaw was then further opened approximately 5 mm by downward pressure on the lower anterior teeth and the jaw held in that position. The effect of lingual nerve stimulation with the jaw in the open position is shown in Figure 1B. The digastric muscle response was slightly modified. However, a marked change was seen in the response of the temporal muscle. The stimulus now consistently evoked a short latency (5 msec) response which clearly preceded the digastric activation (Figure 1B). Furthermore, the late response seen with the jaw at rest (Figure 1A), was abolished (Figure 1B). The late temporal muscle response could also be blocked without reduction of the short latency response by fixing the lower jaw in the maximal elevated position with the teeth in occlusion. In this position the lingual nerve stimulus produced digastric muscle excitation but no jaw opening movement was permitted.

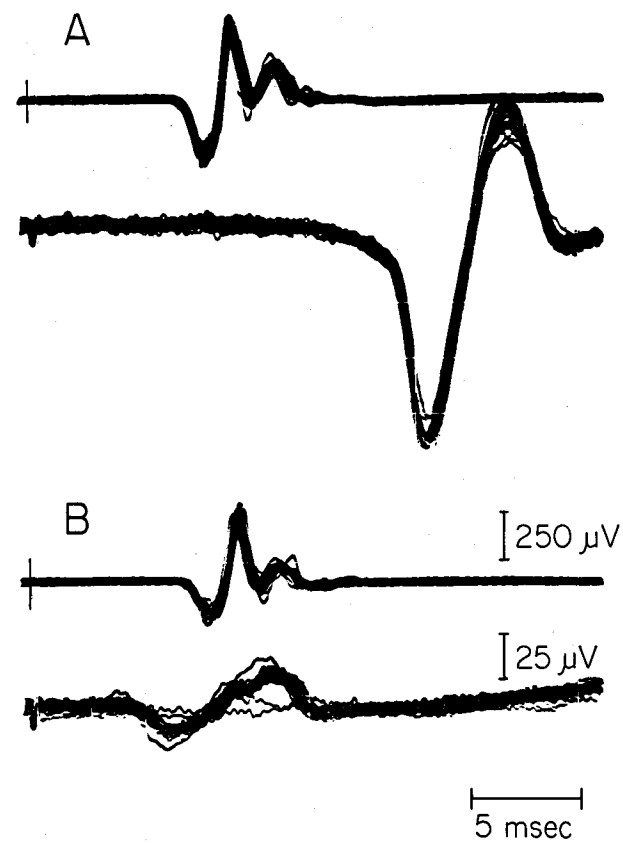

Fig. 1. The effect of lingual nerve stimulation on the electrical activity in the diagastric and temporal muscles of a cat. The upper trace in each record is from the diagastric, and the lower from the temporal muscle. Each trace is the photographic superimposition of 20 oscilloscope sweeps. In A) the stimuli were delivered with the jaw at rest. In B) the lower jaw was depressed by approximately 5 mm and held in that position during stimulation.

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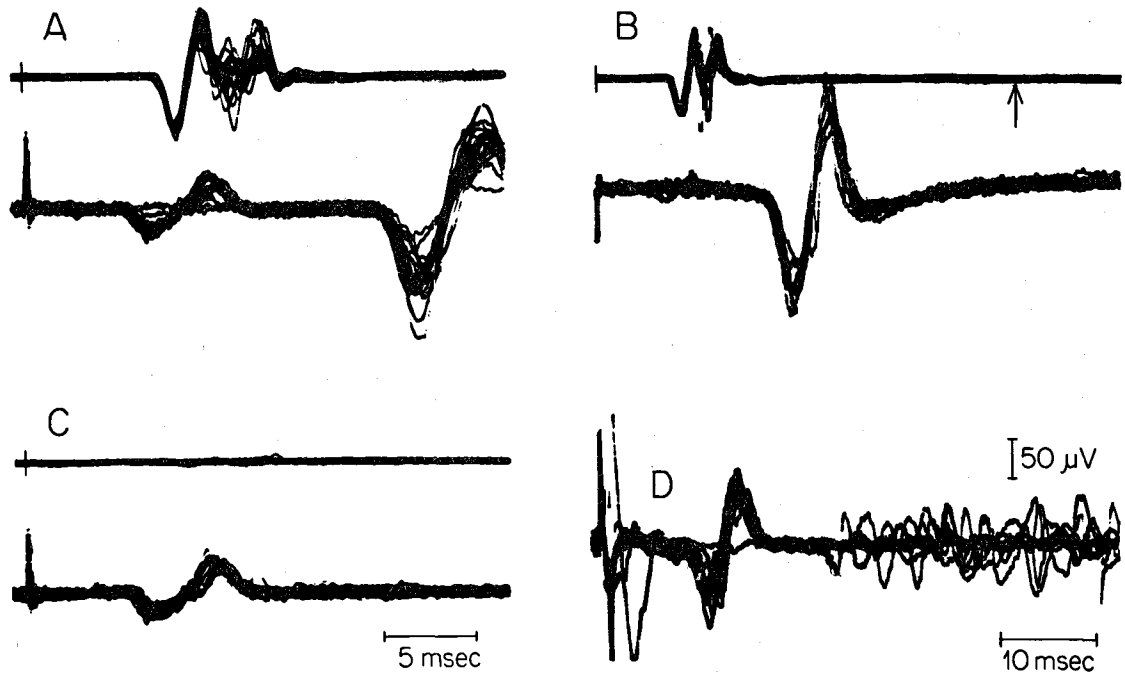

Fig. 2. The effect of a conditioning contralateral lingual nerve stimulus on the activity evoked in the ipsilateral temporal and digastric muscles by a test stimulus delivered to the ipsilateral lingual nerve. A) is the response of the right digastric (upper trace) and temporal (lower trace) muscles to right lingual nerve stimulation; B) is the response to left lingual stimulation. In C) the response to right lingual nerve stimulation is shown on a delayed sweep when delivered at arrow in B), 43 msec after the conditioning left lingual stimulus. Each trace is the photographic superimposition of 20 sweeps. The time base for A) is the same as C); and B) is the same as D). Voltage calibration for digastric and temporal muscle records are the same as in Figure 1. D) is the activity recorded in the masseter muscle of a monkey after electrical stimulation of the labial gingiva of the contralateral upper canine. See text for details.

Figure 2 illustrates the independance of the short latency temporal muscle response on both digastric muscle excitation and jaw opening. Figure 2A shows the response of both the digastric and temporal muscles to ipsilateral lingual nerve stimulation and Figure 2B shows the response of the same muscles to contralateral lingual stimulation. When the contralateral lingual shock was used as a conditioning stimulus and the ipsilateral lingual shock as the test stimulus the results shown in Figure 2C were obtained. At a conditioning-test interval of 43 msec (test stimulus delivered at arrow in Figure 2B) the digastric muscle response and the jaw opening reflex were inhibited, the short latency reflex in the temporal muscle remained the same or was slightly enhanced, and the late temporal response was abolished (Figure 2C).

These findings are remarkably similar to the results of THEXTON[8] who described short and long latency excitatory reflex responses in the temporal muscle of decerebrate and hypothalamic cats to electrical stimulation of the ipsilateral infraorbital nerve. Thexton also reported that the short latency temporal response was enhanced, and the later response reduced, when evoked with the jaw held wide open or fixed in an isometric position.

The presence of the short latency temporal muscle response does not appear to be dependent on jaw movement or digastric muscle activity since it can be obtained in their absence. However, this is not the case for the late temporal response. It could be postulated that the late response is due to stretch of muscle spindles in the jaw elevator muscles as a consequence of the jaw opening. Activation of these muscle spindles results in excitation of jaw closing muscles[9]. This view is supported by the evidence which shows that if jaw opening is prevented the second response does not appear.

In Figure 2D the response of the masseter muscle in a monkey to electrical stimulation of the labial gingiva of the contralateral upper canine tooth is shown. A short latency reflex could be observed in the masseter muscle when a wooden block, 18 mm thick, was placed between the molar teeth on the right side. This jaw opening procedure elicited a continuous barage of spontaneous activity in the masseter muscle. The latency of the reflex was 9 msec and is similar to the latencies of the response observed in the masseter muscles of man to periodontal and gingival receptor stimulation[1]. Note the brief period of inhibition of the spontaneous activity which follows the excitatory reflex (Figure 2D). This silent period was also present in the human subject studies. In both man and monkeys the excitatory reflex could only be evoked when the jaw closing muscles were in isometric contraction.

To summarize, it has been shown here that electrical stimulation of the lingual nerve in cats, and the gingiva in monkeys, evokes a short latency excitatory reflex response in the jaw closing muscles. These findings, coupled with similar results in man[1], strongly indicate that there exists a short latency excitatory reflex involving intra-oral, non-muscular, trigeminal afferents and the jaw closing temporal and masseter muscles. A discussion of possible central pathways subserving this reflex has been presented elsewhere[1].

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Résumé. Après stimulation électrique de la gencive chez le singe et du nerf lingual chez le chat, une réponse réflexe de courte latence à été enregistrée au niveau du muscle temporal et du masseter. La latence de cette réponse est plus rapide que celle de la réponse digastrique induite par le même stimulus. La réponse temporale de courte latence peut également être observée en l'absence tant d'une réponse digastrique que du réflexe d'ouverture de la gueule.

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The Effect of *Lophozozymus pictor* Toxin on HeLa Cells

A crude extract of the coral reef crab, *Lophozozymus pictor*, has recently been reported to be toxic to rats and mice. Large doses rapidly depress the respiration and blood pressure and death follows within a few minutes; with low doses the action is more insidious and death only occurs after 20 or 30 h[1]. These findings suggested that the toxin may have a general cytotoxic action; HeLa cells have been used to study this possibility.

The toxic extract was prepared as described earlier[1]. 1 ml of extract contained 300 mouse units (MU)[2]. HeLa cells (Strain E, Commonwealth Serum Laboratories, Australia) were grown in monolayers in medium 199 (Burroughs Wellcome & Co.) containing 10% foetal calf serum (DIFCO), penicillin 100 units/ml and streptomycin 100 µg/ml in non-corrosive borosilicate culture tubes with non-toxic silicone rubber stoppers. The tubes were incubated in a Model T26 Rollertherm incubator at 37°C and rotated at $^1/_5$ g/min Trypsin 0.25% (Type III, Sigma Chemical Co.) was used for trypsinization of the cells and cell counts were made with a Neubauer haemocytometer.

The effect of the extract on HeLa cell attachment to glass was tested. Freshly introduced HeLa cells will normally attach themselves to the inside surface of the tube after about 2 h. Aliquots of approximately 1×10^5 cells in 1 ml medium were distributed in culture tubes. The extract (300 MU/ml) was added to the tubes to give concentrations from 0.006 to 60 MU/ml. Tubes with no toxin added were used as controls. All tubes were incubated for 48 h and observed with an inverted microscope at regular intervals for cell attachment to glass. The results show that at concentrations higher than 0.006 MU/ml, HeLa cells failed to adhere to glass (Table I).

Table I. Effect of *Lophozozymus pictor* toxin on HeLa cell attachment to glass

Toxin mouse units/ml	HeLa cell attachment to glass after 48 h incubation
0 (Control)	yes
0.006	yes
0.06	no
0.6	no
6.0	no
60.0	no

The effect of the toxin on cellular morphology was next investigated. Similar aliquots of HeLa cells as above were first distributed in tubes and allowed to adhere and grow for 48 h. The medium was then replaced with 1 ml aliquots of medium containing concentrations of toxin ranging from 0.003 to 3.0 MU/ml. Control tubes contained no toxin. The tubes were observed for cell toxicity over a 96 h period. Toxicity was correlated to the rounding of the polygonic cells, the appearance of granules and refractile bodies in the cytoplasm and cell lysis.

The cytoplasm of many of the cells stained less prominently with alcoholic eosin than the controls. The cells detached from the glass tubes within 20 h after cytotoxicity was first noted. The minimal concentration required to give a toxic effect was 0.006 MU/ml. The period before the appearance of cytotoxicity in 50% of HeLa cells varied with the dose of toxin and ranged from 10 min to 4 days (Table II). These results are of interest since in the whole animal the death times also varied from a few min to 30 h depending on the dose[1].

Table II. Effect of *Lophozozymus pictor* toxin concentration on the time of appearance of cytotoxicity in HeLa cells

Toxin mouse units/ml	Time at which cytotoxicity was observed in 50% of HeLa cells
0 (Control)	no toxicity after 4 days
0.003	no toxicity after 4 days
0.006	4 days
0.03	3–5 h
0.06	$1^1/_2$–2 h
0.3	35–60 min
0.6	25 min
3.0	10–15 min

The results of this study show that HeLa cells provide a more sensitive assay for the toxin than the mouse toxicity test. This cell culture method is therefore useful in following the purification of the toxin.

Zusammenfassung. Ein wässeriger Extrakt einer besonderen Krabbenart (*Lophozozymus pictor*) erweist sich als toxisch gegenüber HeLa-Zellen. Es kommt zu Umwandlungen der Zellform sowie des Cytoplasmas. Die Reaktion scheint leicht erfassbar und ist für einen Toxizitätstest besser geeignet als der bis jetzt verwendete Mäusetest.

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