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# UNITARY CORRELATES OF LINGUO-PHARYNGEAL EVENTS IN A DYSKINETIC RAT MODEL

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**Abstract**—1. The hypoglossal nucleus unitary correlates of ketamine- and electrically induced tongue contractions and swallowing events were recorded and compared in stereotaxically mounted rats.

2. Very few of the units recorded could be identified as motoneurons by antidromic invasion through electrical stimulation of the hypoglossal nerve.

3. The sample consists of 109 units, $\frac{2}{3}$ of which were located in the retrusor pool and $\frac{1}{3}$ in the protrusor region.

4. Linguo-pharyngeal events were considered to be electrically induced if they followed consistently, and with a relatively fixed latency, a single electrical pulse delivered to the superior laryngeal nerve, and ketamine-induced if no such time-locked sequence existed or if they occurred in the absence of electrical stimulation.

5. No differences could be discovered between the two types of linguo-pharyngeal events whether they were compared at the polygraphic or the unitary level.

6. These findings suggest that pharmacologically (ketamine)-induced dyskinetic activity does not differ from comparable motor patterns induced by classical electrophysiological means.

## INTRODUCTION

In a series of studies we have investigated the dyskinetic potential of ketamine hydrochloride in anesthetic doses of 100 mg/kg and related drugs in rats and the properties of this effect by means of polygraphic monitors of linguo-pharyngeal events (LPE) consisting of tongue protrusions and retrusions and swallowing events (Aldes *et al.*, 1988; Marco *et al.*, 1989a).

Some of the questions posed by the results described in these reports were: (a) can a unitary correlate of the dyskinetic activity be demonstrated?; (b) where is the pacesetter of such activity?; and (c) are there clearly recognizable differences at the gross polygraphic or unitary level between the ketamine-induced dyskinetic events and those elicited by standard electrophysiological techniques?

To answer these questions unitary recordings by means of microelectrodes were obtained from the hypoglossal nucleus (N-XII), obviously the first and easiest structure to look for unitary correlations. This was done during ketamine anesthesia to take advantage of the profusion of LPE occurring under the effects of ketamine. Such unitary patterns would thus be compared with those triggered by electrical stimulation of the superior laryngeal nerve (SLN) which is capable of eliciting a whole complex of LPE consisting of swallows tightly followed by tongue contractions of protrusive and retrusive character.

## METHODS

The results herein reported were obtained from 32 Sprague-Dawley rats weighing 125–225 g–used for these experiments. All animals were anesthetized with one injection of ketamine hydrochloride (ketaset Bristol Laboratories) 100 mg/kg i.m. One half of this dose was repeated every hour during the surgery and the recording experiments. Within 10 min of the initial dose, the surgery could proceed first with a tracheostomy and cannulation of the trachea for unimpeded respiration; next the superior laryngeal nerve (SLN) was exposed on both sides according to techniques described elsewhere (Kessler and Jean, 1985). Stimulation of the SLN was conducted by single pulses at a rate of 1 every 3 sec or by means of trains of 0.5 V, 0.3 msec; 30 Hz every 6–7 sec as described by Kessler and Jean (1985) if single pulses were not consistently effective in eliciting swallowing responses. The medial and lateral branches of the hypoglossal nerve were also dissected away from the surrounding tissues (Marco *et al.*, 1988) in order to stimulate them later during the recording experiment so that protrusor and retrusor motoneurons could be identified antidromically.

The animal was then mounted on a Kopf stereotaxic instrument when the above described surgery stages had been completed. The tip of the tongue was transfixed with a loop of 4.0 silk and tied to the cantilever of a force displacement transducer (FDT) to monitor tongue protrusions (P) and retrusions (R) through an extension arm of the cantilever small enough to fit between upper and lower teeth with the mouth slightly open (Marco *et al.*, 1988). A tiny balloon at the end of a polyethylene tubing was inserted through the side of the mouth to reach the soft palate where it was to rest for the duration of the experiment and at the exit of the tubing from the mouth it was taped to the palate piece of the stereotaxic for immobilization. The other end of the tubing was connected to a pressure transducer (PT) for monitoring swallowing events (Marco *et al.*, 1988).

The posterior fossa was exposed by means of a midline incision extending from the interaural line to the upper neck. The neck muscles were separated at the midline and scrapped away to the side thus exposing the occipital bone which was then removed. The dura was slit longitudinally in the midline and the vermis of the cerebellum was aspirated to expose the floor of the fourth ventricle. The obex was used as a reference for mapping points of entry and for stereotaxic reconstruction of recording tracks within the N-XII.

Both swallows and tongue contractions were monitored polygraphically and by means of a cathode ray oscilloscope which also recorded unitary activity and from which pictures could be taken. All three different kinds of events (unit, swallows, tongue contractions) were also recorded in parallel in three different channels of a frequency modulated tape recorder. The bottom of each unitary tract was fulgurated with 20 $\mu$A applied to the tip, both polarities, for 20 sec.

Upon completion of each experiment, the animal was injected a lethal dose of Nembutal and then perfused with normal saline followed by fixation with 10% formaldehyde, the head was then severed and kept in an identical formaldehyde solution for two days and then replaced into the stereotaxic where a block containing the N-XII was cut in a plane parallel to those of stereotaxic atlas of Paxinos and Watson (1982). The tracks and points of unitary recordings were reconstructed from camera lucida drawings or from measurements made directly from the microscopic sections which were stained with Nissl stain. Depths of recordings from the 40 $\mu$m thick sections were compared with those obtained from the LED counter on a Burleigh Inchworm tracking system.

## RESULTS

### A. *General*

The first test to which every unit was subjected was to determine whether it was a motoneuron or an interneuron. It was concluded that the unit was a motoneuron if it could be invaded antidromically by electrical stimulation of either the lateral branch of the hypoglossal nerve (retrusor) or the medial branch (protrusor) or by means of collision tests between a spontaneous orthodromic spike and an antidromic spike (Wilson *et al.*, 1968). Very few motoneurons could thus be identified. Figure 1 illustrates some of

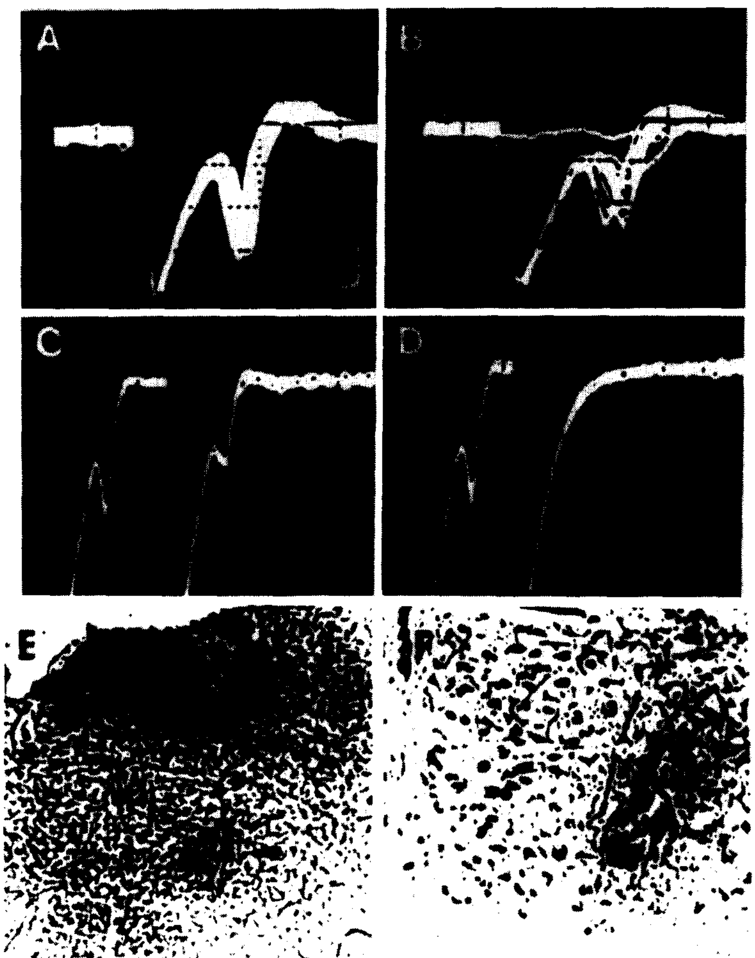


Fig. 1. Unit in the protrusor pool of N-XII identified as a protrusor motoneuron which was invaded antidromically by electrical stimulation of the medical branch of the hypoglossal nerve. A: 10 super imposed responses to stimulation at 50/sec. B: responses of same unit to 100/sec stimulation. C: responses to twin pulses at 2.5 msec pulse interval. D: responses to twin pulses at 2.25 msec interval. Note suggestion of relative refractory period in C by decrease of spike response amplitude to second pulse and absolute refractory period in D by failure of response to second pulse. Calibrations in A: horizontal bar 1 msec, vertical bar 2 mV. E: histological section through N-XII showing mark of tip of electrode fulgurated at point of optimal response (arrow head, depth of 1565 $\mu$m) by passing 20 $\mu$A of current of 20 sec at the lateral border of the protrusor pool on the right side (L 0.3 mm; 0.3 mm rostral to obex). Photograph magnified 10 ×. F: same, magnified 25 ×.

the successful tests in a unit identified as a motoneuron in the protrusor pool at a depth of 1565 $\mu$m which could be invaded antidromically. Most of the units described below could not be demonstrated to be motoneurons by antidromic invasion combined or not with a collision–cancellation effect with the orthodromic spike.

A total of 109 units were recorded from within the N-XII; 67% were located in the retrusor pool and 33% in the protrusor pool. Of those found in the retrusor compartment only 16% fired during retrusion and of those located in the protrusor region only 25% fired during protrusion. Some 58% firing during a swallow event were located in the protrusion pools and 42% were localized within the retrusion pool; some 8% of those firing in the retrusor pool were inhibited during swallows.

B. *Unitary correlations with retrusion*

Figure 2 C, D illustrates a unit in the retrusor pool (depth 887 $\mu$m) which started firing single spikes spontaneously and quite regularly without any correlation with retrusion (Fig. 2C); after a while the pattern of firing, although still at regular intervals, changed to doublets or triplets rather than singlets and then a clear correlation with retrusion became apparent (Fig. 2D). Fig. 3A illustrates another unit in the midst of the retrusor pool (depth 610 $\mu$m) firing in bursts of about 150 msec duration during the retrusion that followed an electrical pulse applied to the SLN and which was effective in inducing a swallow. Figure 3B shows the same unit still being picked up some 25 $\mu$m deeper and firing synchronously with the rising phase of a similar retrusion but this time induced by ketamine rather than by electrical stimulation of the SLN.

C. *Unitary correlations with protrusion*

Figure 4A illustrates a unit in the lateral portion of the protrusor pool firing in bursts of about 90 msec during the protrusion that follows a SLN-induced swallow. In Fig. 4B, the same unit is shown firing during a ketamine-induced protrusion that was also preceded by a ketamine-induced swallow. The configurations and durations of the unitary bursts or the swallow–tongue contraction complexes can not be distinguished. Figure 5 demonstrates the variability in onset of tongue contractions despite constancy of swallow responses to stimulation of the SLN (Fig. 5B, D). The composite of frames in this Fig. 5 identifies the unit as a protrusion-related element rather than as a swallow unit. It is noteworthy that in Fig. 5C, there is no swallow yet the unit fires in correlation with a marked ketamine-induced protrusion. The unit was located at a depth of 1850 $\mu$m within the protrusor pool.

D. *Unitary correlations with swallows*

Units were encountered in the dorsal portion of the protrusor pool which fired in bursts of duration about 200 msec in correlation with swallows (Fig. 6A, B). These bursts had an onset which preceded the beginning of the swallow by about 75–80 msec and overlapped with the entire plateau of the swallow. No signs of tongue protrusion could be demonstrated, however, even at the highest

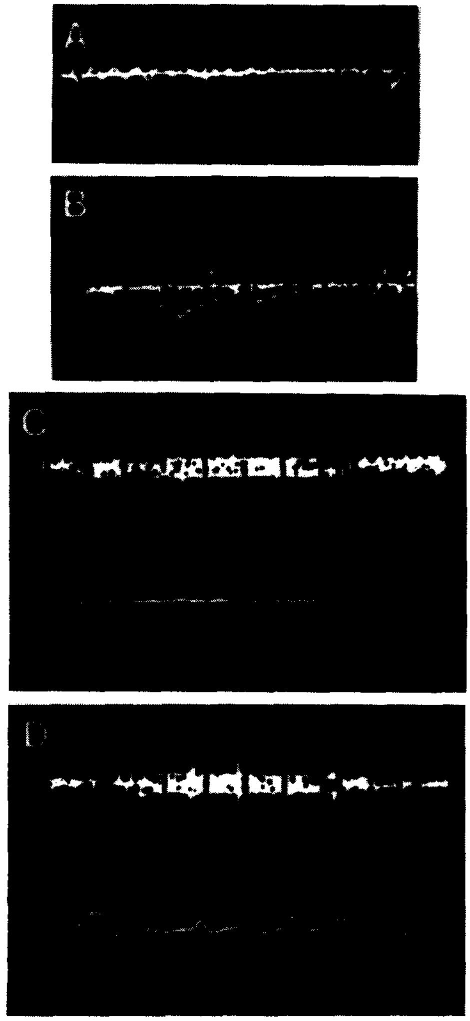


Fig. 2. A and B: samples of ketamine-induced clonic protrusive–retrusive activity recurring at a rate of 10–15/sec from two different experiments. For this and subsequent force displacement transducer records, retrusion is represented as an upward deflection and protrusion as downward. These clonic P–R sequences appeared under the microscope as continuous vermiculation of the surface of the tongue. C: upper trace represents spontaneously firing retrusor neuron (depth 877 mm) without tongue contractile activity (lower trace). D: moments later the same unit (upper trace) undergoes change in firing pattern (doublets, triplets) which coincides with retrusive activity of the tongue (lower trace). For this and subsequent sweeps of the cathode ray oscilloscope, total duration of sweep is about one second, unless otherwise specified. Retrusion is represented as an upward deflected and protrusion as downward.

amplification. This correlation could be demonstrated with either electrically induced swallows by stimulation of the SLN (Fig. 6A, B) or with ketamine-induced swallows (Fig. 7A, B; Fig. 8A, B). Figure 8 further illustrates the similarities between ketamine-induced swallows (Fig. 8A) and SLN-induced swallows (Fig. 8B). The only apparent difference between the two records is that in (A) the unit is firing spontaneously before the swallow begins, the firing rate accelerates about 60 msec before the onset of the swallow event, whereas in (B) the unit is silent but begins its burst, again, just about

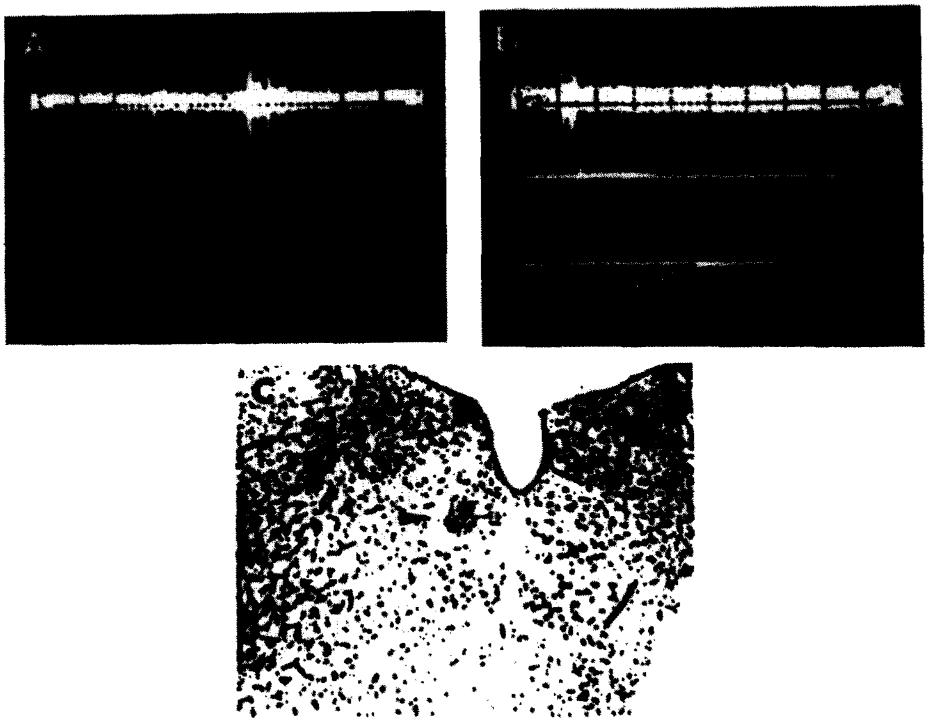

Fig. 3. A: unit in the retrusor pool firing in bursts during the retrusion that follows an electrical pulse applied to the superior laryngeal nerve (SLN) effective in inducing a swallow. Top tracing is unit monitor, middle is pressure transducer monitor of swallow, bottom is force displacement transducer monitor of tongue contractions. Stimulus to SLN is seen as a vertical break in the baseline. B: same unit firing synchronously with the rising phase of a similar retrusion induced by ketamine this time rather than by electrical stimulation of the SLN. C: histological section showing the fulgurated tip of the microelectrode in the midst of the retrusor pool at a depth of 610 $\mu$m (arrowhead); amplification 13 ×.

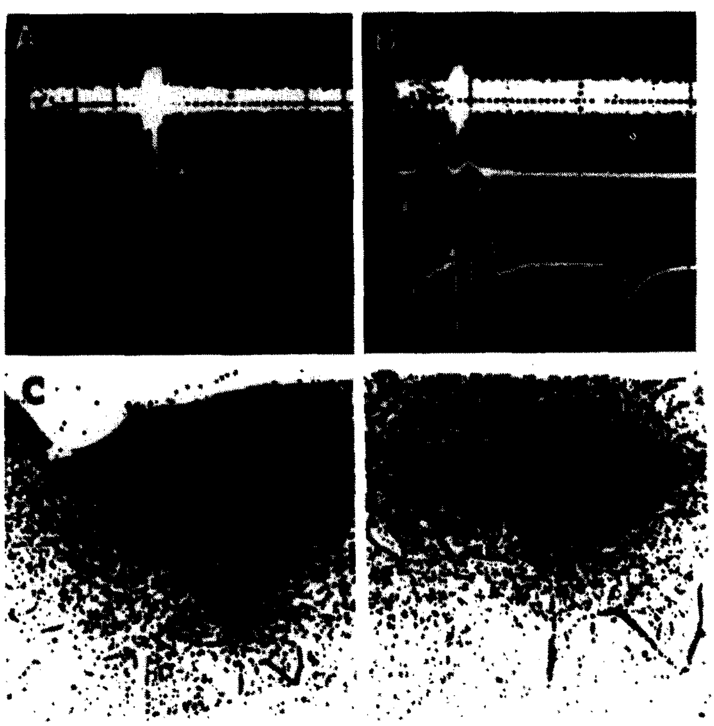

Fig. 4. A: unit in lateral portion of protrusor pool firing in burst fashion during the protrusion that follows a SLN-induced swallow. B: same unit firing during a ketamine-induced protrusion that was also preceded by a ketamine-induced swallow. Note the similarity of electrically induced and ketamine-induced events. Total sweep duration about 1.6 sec. C: section through N-XII showing track and fulguration point (arrowhead) on edge of protrusor pool; amplification 6 ×. D: same magnified 16 ×.

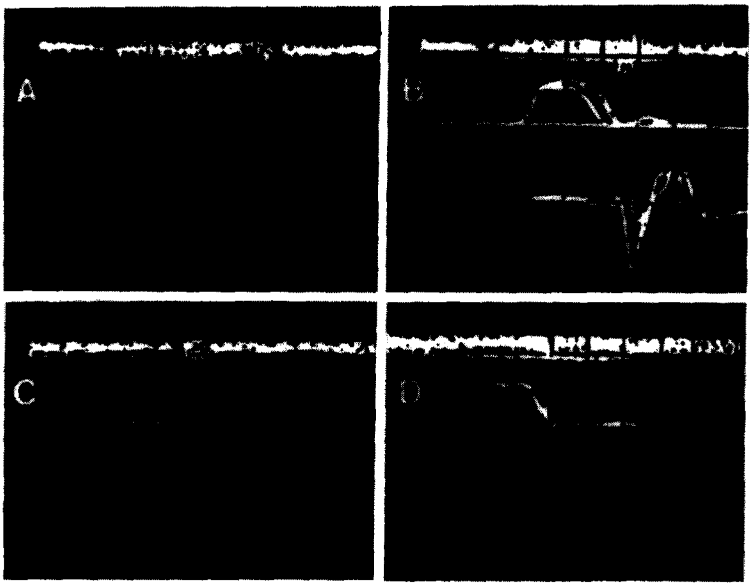


Fig. 5. Variability in onset of tongue contractile activity despite constancy of swallow responses to stimuli to the SLN and identification of correlated unit as a protrusion element rather than swallow unit. A: single sweep with unit on top trace, swallow in middle, protrusion–retrusion at bottom. B: same with 10 superposed sweeps to demonstrate greater tongue response variability than swallow response variability. C: ketamine-induced tongue contractions without stimulus to SLN showing unit correlated to KI tongue protrusion. D: similar to A and B with more dramatic variability in onset of tongue contractility. Depth of unit 1850 $\mu$m within the protrusor compartment.

60 msec before the onset of swallowing. Few records were obtained in which dual unit recordings demonstrated that while one unit in the bottom fringe of the protrusor pool was silenced during SLN-induced swallows and beyond (Fig. 9A, larger unit) another smaller unit was not arrested during the swallow event.

## DISCUSSION

The two most interesting aspects of these findings are that (a) they represent to our knowledge the first unitary correlates of dyskinesia in animal models, and (b) there seem to be no clear-cut distinguishing features between ketamine-induced and electrically SLN-induced LPE. To the extent that ketamine can be conceptualized as a dyskinetogenic agent, this finding (b) is significant in that it suggests that dyskinesia, a movement disorder by definition, may owe its abnormality to the inordinately high rate of recurrence of an elementary movement rather than to the quality or different pattern of the movement itself. There has been disagreement in the literature as to what constitutes dyskinesia, whether too much motor activity of an otherwise normal pattern of elementary movements (Rupniak *et al.*, 1985, 1986) or elementary patterns of motor activity which are not observed in normal individuals (Levy *et al.*, 1987).

That few units firing during the swallowing event were encountered within the retrusor or protrusor pools is not surprising and has also been reported repeatedly in numerous studies (Sumi 1969; Hockman *et al.*, 1979; Jean, 1984; Kessler and Jean, 1985; Car and Amri, 1987; Amri and Car, 1988).

This suggests that there is some degree of intermingling of functional pools or that such neurons do not function as motoneurons but rather as excitatory or inhibitory interneurons converging onto the final common path of motoneuron (Cooper, 1981). Since few of these units could be identified as motoneurons

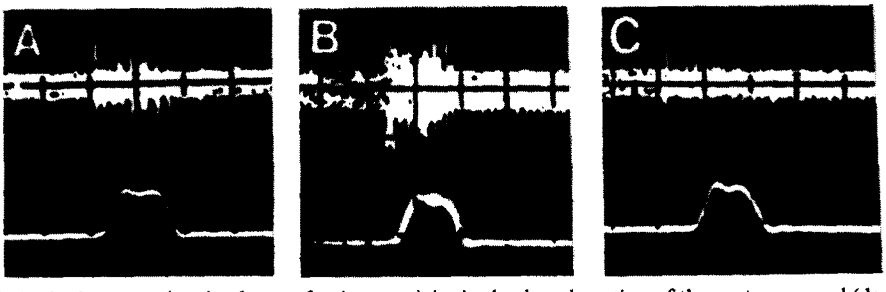


Fig. 6. A: single sweep showing burst of unitary activity in the dorsal portion of the protrusor pool (depth 1509 $\mu$m) during swallow induced by stimulation of the SLN. B: five superposed sweeps showing the same pattern. C: single sweep response showing the swallow, but the synchronous unit has been eliminated after fulguration of the recording point. Sweep duration 1.2 sec.

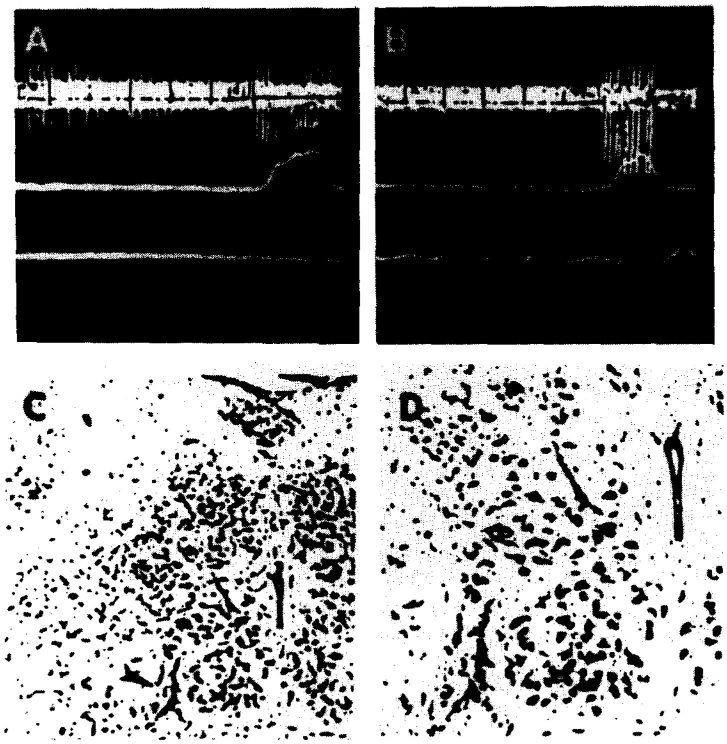


Fig. 7. A and B: bursts of unitary activity in the protrusor pool (depth 1900 $\mu$m) during a ketamine-induced swallow. Note absence of protrusive activity and minimal retrusive activity on the FDT tracing. Total sweep duration 1.6 sec. c: Histological section shows lesion at recording point on latero-ventral fringe of protrusor pool (at arrow head), amplification 13 × D: same at amplification of 25 ×.

by antidromic invasion and collision, we are not justified in reaching a conclusion as to their motoneuronal identity.

Obviously, the findings of bursts of unitary activity correlated with either protrusions, retrusions, or swallows in the N-XII cannot be construed as meaning that the N-XII is the exclusive target site for the action of ketamine or that the N-XII is the pacesetter for ketamine-induced LPE since these LPE may be following commanding bursts or volleys from higher centers. We have already shown that even the cortex mediating tongue movements reflects the tongue contractions elicited by ketamine (Marco *et al.*, 1989b), despite lack of evidence of direct cortical connections to hypoglossal nuclei in rats (Valverde, 1962). Finally, it appears that

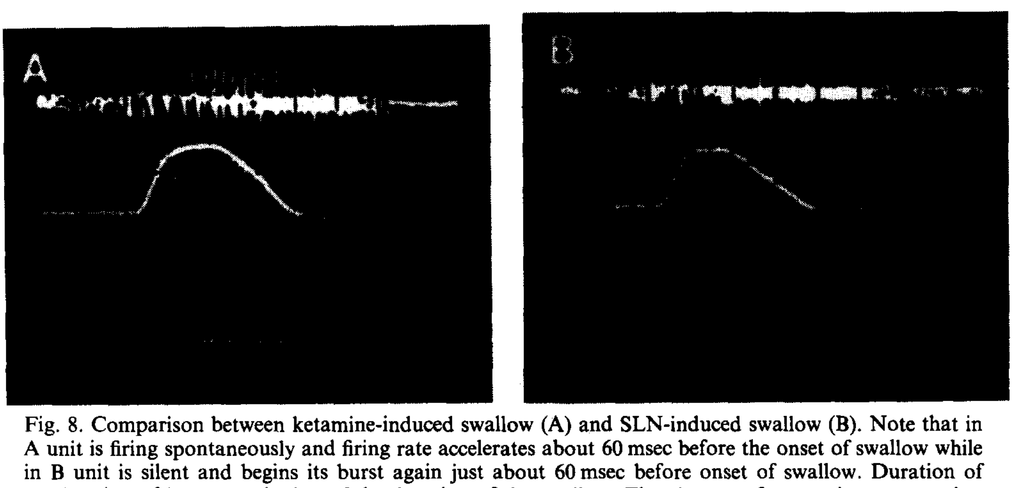


Fig. 8. Comparison between ketamine-induced swallow (A) and SLN-induced swallow (B). Note that in A unit is firing spontaneously and firing rate accelerates about 60 msec before the onset of swallow while in B unit is silent and begins its burst again just about 60 msec before onset of swallow. Duration of acceleration of burst equals that of the duration of the swallow. The absence of protrusion or retrusion (bottom trace) in both records could indicate that this neuron innervates at intrinsic tongue muscle and stimulation, therefore, would not produce protrusion or retrusion. Unit recorded at the bottom of the ventromedial compartment of the hypoglossal nucleus at a depth of 2013 $\mu$m. Total sweep duration 1 sec.

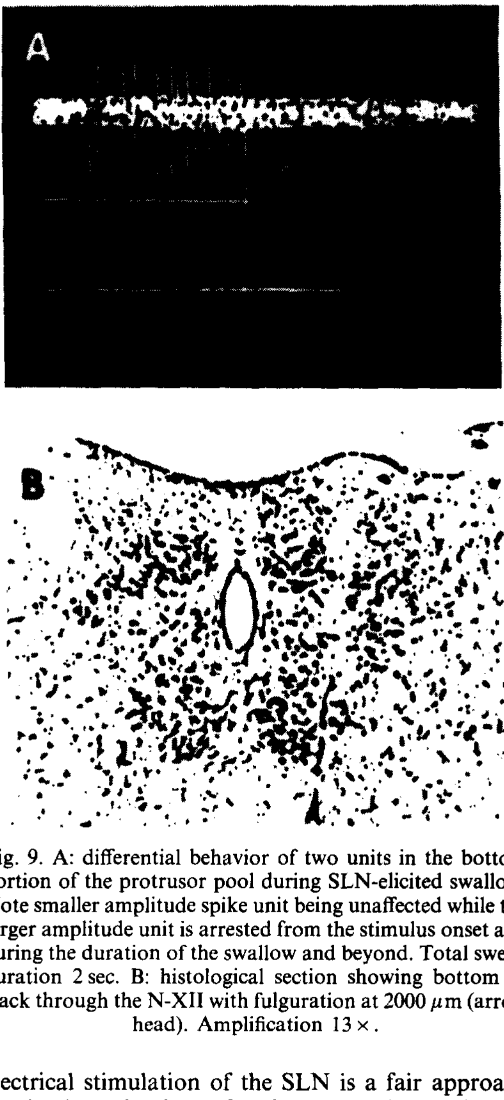

Fig. 9. A: differential behavior of two units in the bottom portion of the protrusor pool during SLN-elicited swallow. Note smaller amplitude spike unit being unaffected while the larger amplitude unit is arrested from the stimulus onset and during the duration of the swallow and beyond. Total sweep duration 2 sec. B: histological section showing bottom of track through the N-XII with fulguration at 2000 $\mu$m (arrow head). Amplification 13 ×.

electrical stimulation of the SLN is a fair approach to the investigation of unitary correlates of LPE such as P, R, and S, since P–R sequences are teleologically tightly linked to, and follow, swallowing events, the swallowing synergy or network (Jean, 1984). As pointed out by Hockman *et al.* (1979), muscle synergies under the control of this bulbar network are invariably involved in dyskinesias induced by drugs whether acute or tardive. Furthermore, by this approach one can gain some insight into the significance of other populations of non-motoneuronal unitary elements which contribute to protrusion or retrusion.

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