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Reticular Formation Neurons Related to Tongue Movement in the Behaving Cat

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We have found a number of cells related to tongue movement in the medial brain stem reticular formation of the unanesthetized cat. These cells constituted less than 2% of the cells tested in this region and were distributed throughout several nuclei in the medulla and pons including nucleus reticularis pontis caudalis, nucleus reticularis gigantocellularis, and the border between nucleus reticularis paramedianus and nucleus interfascicularis hypoglossi. All observed tongue movement cells *(N* = 6) fired maximally during protrusive tongue movements. One medullary cell discharged primarily during the protrusive tongue movement to the ipsilateral side, whereas no lateral preference was detected in the other cells. Gustatory and mechanosensory stimulation of the tongue was unnecessary for inducing discharge in these cells. Tongue movement-related cells shared several characteristics that differentiated them from adjacent reticular formation cells, including absence of response to startle-inducing auditory stimuli and low levels of spontaneous waking and sleep activity. In two pontine cells located near the trigeminal motor nucleus, spike-triggered averages of tongue EMG revealed a short-latency (5 ms) inhibitory effect on the ipsilateral genioglossus muscle by the units' discharge. We suggest that neurons of this type might be involved in tongue-jaw coordination during mastication, licking, and grooming.

INTRODUCTION

The mammalian tongue with its intricate mobility is involved in various vital functions such as mastication, lapping, suckling, deglutition, and

Abbreviations: EMG—electromyogram, REM—rapid eye movement.

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respiration. The motoneurons innervating the tongue musculature are situated in the hypoglossal (XII) nucleus. As reviewed recently by Lowe (7), a number of acute electrophysiological studies have been carried out to elucidate various peripheral and central pathways controlling the activity of hypoglossal motoneurons. Most of those studies were concerned with the reflexive effects of sensory inputs from the trigeminal (V), glossopharyngeal (IX), superior laryngeal, and hypoglossal nerves on the activities of hypo-glossal motoneurons or tongue muscles. The latency data indicate that these craniohypoglossal reflexes are polysynaptic, involving relay neurons whose locations within the brain stem are not well understood. Although anatomic evidence (4, 5) implicates the pontomedullary reticular formation as containing such relay neurons and probably other neurons influencing hypoglossal motoneurons, few data have been available on the activity of reticular formation neurons that might be involved in the regulation of reflexive and other tongue movements.

In a continuing study on the behavioral correlates of reticular formation neurons in the unrestrained cat, we found a small number of neurons that discharge in relation to tongue movement. We present here a detailed description of these tongue-related cells and suggest that some of these cells might act as inhibitory relay neurons involved in tongue-jaw coordination during mastication, licking, and grooming.

METHODS

Our techniques for single-unit recording and for the analysis of behavioral correlates of unit activity have been described elsewhere (15, 16). Under sodium pentobarbital anesthesia (35 mg/kg, i.p.) adult female cats were implanted with mechanical microdrives containing movable bundles of 32-μm, Formvar-coated, Nichrome microwires as well as macroelectrodes for recording pericruciate gyrus EEG, lateral geniculate activity, electrooculogram, and neck electromyogram (EMG). After a 1-week recovery period, these bioelectric activities were recorded polygraphically from the unrestrained cat placed in a recording chamber (58 X 60 X 85 cm). Extracellular unit discharge was monitored continuously on the oscilloscope. The activity of each isolated unit was observed systematically during various spontaneous movements and sensory stimulation in order to determine its sensorimotor correlates. Unit activity was also recorded during sleep-waking cycles including periods of waking, rapid eye movement (REM) sleep, and nonREM sleep as defined polygraphically. All cells were examined for rhythmic activity during lapping of water and chewing of a cat chow pellet, and during manipulation and stimulation of the tongue and oral cavity with a cotton swab.

When cells related to tongue movement were encountered, two bipolar EMG electrodes were inserted bilaterally into the genioglossal muscle (the major protruder of the tongue) through the skin between the mandibular symphysis and the hyoid bone. Each electrode consisted of a pair of 60-μ*m,* Formvar-coated, Nichrom wires deinsulated 1 mm from the tips. The two wires were inserted into a 27-gauge hypodermic needle and their tips were bent sharply to form hooks. After the intramuscular insertion of the needle-wire assembly, the needle alone was withdrawn, leaving the wires *in situ.* The same technique has been used for recording genioglossal EMG in humans (2, 11).

To examine the effect of tongue cell discharge on genioglossal muscle activity, EMG signals triggered by action potentials were full-wave rectified and averaged by a computer. The sweep duration was 50 ms after the spike onset and 100 points (bin width = 0.5 ms) were sampled per sweep; 200 sweeps were averaged. The technique of spike-triggered averaging of EMG has been used successfully in recent studies on the cortical control of finger and wrist muscles (3), and the reticular control of limb (12) and jaw muscles (10).

After the experiment, the cats were deeply anesthetized with pentobarbital and perfused intracardially with saline and 10% Formalin. The brains were removed, sectioned sagittally and stained with cresyl violet or carbol-fuchsin red. The locus of each unit was calculated by measuring the distance of the recorded unit on the microdrive from the final (deepest) microdrive position, after allowance was made for brain shrinkage.

RESULTS

A total of 351 cells in 27 cats were tested for tongue movement relations. Six neurons in four cats were observed to fire specifically during tongue movement. Thus, these tongue-related cells constituted less than 2% of the tested reticular formation cells. The loci of these cells determined histologically are shown in Fig. 1; none was localized to the hypoglossal nucleus. Five (cells 2 through 6) of the six cells were located in the pontine reticular formation between stereotaxic coordinates P 3 and P 7, L 1.6 and L 3.0, in the nucleus reticularis pontis caudalis and nucleus reticularis gigantocellularis. The remaining cell (cell 1) was in the border between the nucleus reticularis paramedianus and nucleus interfascicularis hypoglossi at about P 12.5 and L 1.2, ventrally to the hypoglossal nucleus (21).

The tongue cells discharged bursts of action potentials during certain spontaneous behaviors involving tongue movements such as grooming, eating, and drinking. The tongue movements during those behaviors, as revealed by visual observation and tongue EMG recording, involved rhythmic

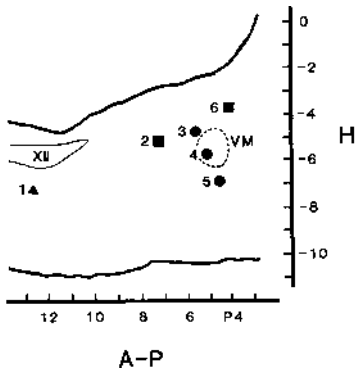

FIG. 1. Locations of six cells related to tongue movement, plotted on a sagittal section of the brain stem. Different symbols indicate millimeters lateral to midline: triangle, 1.2 mm; squares, 1.6-2.2 mm; circles, 2.2-3.0 mm. The hypoglossal nucleus (XII) is located between L 0.5 mm and L 1.5 mm, and the trigeminal motor nucleus (VM) between L 3.0 mm and L 4.5 mm.

(2 to 4 Hz) contractions of the genioglossal and other tongue muscles. Most spikes occurred at a rate as much as 100 Hz during an EMG burst lasting 100 to 250 ms (see Fig. 2).

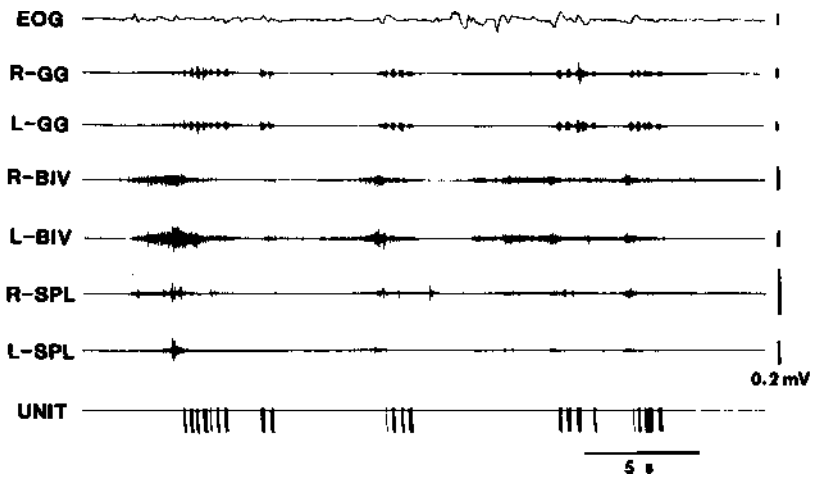

FIG. 2. Discharge of tongue movement cell (cell 5) during rhythmical licking movements induced by intraoral honey. Note that unit burst discharges were correlated with tongue EMG bursts but independent of the EOG or EMG from neck muscles. EOG—electrooculogram, R—right, L—left, GG—genioglossus, BIV—biventer cervicis, SPL—splenius, UNIT—pulse output of window discriminated unit discharge.

Tongue movements could be induced by inserting various edible and nonedible objects into the cat's mouth. A small amount of water, honey, or peanut butter placed in the mouth induced rhythmic tongue movements similar to those observed during spontaneous eating and lapping. The discharge pattern of the tongue cells during induced tongue movements was also similar to that during spontaneous tongue movements. Whenever a cotton swab was inserted into the mouth, the cat attempted to expel it by moving its tongue. A burst of spikes was observed during each expulsive tongue movement in all tongue cells recorded.

The possibility that tongue cell discharge resulted from the activation of gustatory or mechanosensory receptors of the tongue appeared to be remote. When the cat's mouth was held open by the experimenter, all tongue cells consistently discharged during protrusive tongue movements "in the air." This observation also indicated that jaw displacement was not required for discharge in these cells. Passive movement of the jaw was also ineffectual in eliciting discharge. Three cells were tested with saturated water solutions of sodium chloride, glucose, or quinine placed on the tongue; there was no response to those stimuli.

We examined whether or not the discharge of each tongue cell had a preferred direction of correlated tongue movements. Although firing appeared to be related primarily to the onset or termination of protrusive movements of the tongue in all tongue cells, a lateral preference was detected in the one cell (cell 1) located near the left hypoglossal nucleus. When a cotton swab was placed in the left side of the mouth, the expulsive tongue movement directed to the swab was accompanied by burst discharge in this medullary cell. In contrast, when a cotton swab was placed in the right side, the expulsive tongue movement to the same side was not accompanied by the unit's discharge.

The activity of the tongue cells was also monitored during various spontaneous movements, sensory stimulation, and sleep-waking states. These cells did not fire during eye or pinna movement, or during active or passive head movement unless tongue movements occurred concurrently (see Fig. 2). None of the cells responded to somatosensory or auditory stimulation. Five tongue cells (one in the medulla and four in the pons) were completely silent during waking without tongue movement, nonREM sleep, or REM sleep, and therefore were classified as NSA (no spontaneous activity) or type I cells (13-15, 18). The remaining pontine cell (cell 3) was a type 3 cell, displaying a slow firing rate in both waking without tongue movement (0.15 spikes/s) and nonREM sleep (0.04 spikes/s), and a relatively increased rate in REM sleep (1.16 spikes/s). These data were based on a 10-min sample for each state. As tongue EMG was not recorded during sleep, it was not clear if the enhanced discharge during REM sleep was

associated with some activity in the tongue muscles during this state. It should be noted, however, that the spikes did not occur in rhythmic bursts as were typically observed during tongue movements in waking. Insofar as the reticular tongue cells had little or no spontaneous discharge during sleep or waking without tongue movement, they should not contribute to tonic background genioglossal EMG reported to be present during quiet waking and nonREM sleep (11).

Spike-triggered averages of genioglossal EMG were obtained from two pontine cells (cells 4 and 5) located medially to the trigeminal motor nucleus. Five averages, each based on 200 sweeps, were obtained from these cells during spontaneous and induced tongue movements. As shown in Fig. 3, the averages from one of the cells (cell 4) consistently contained a short-latency inhibition (trough) in the ipsilateral genioglossal EMG. The onset latency, onset-to-peak latency, and duration of this inhibition were 4.5 to 5.5 ms, 5.0 to 8.5 ms, and 11.5 to 14.0 ms, respectively. To quantitatively examine if the spike-triggered averages from a given muscle contained a significant deviation from the baseline EMG, we compared the ratios of baseline to maximum or minimum EMG amplitude from all simultaneously recorded, bilateral muscles (genioglossus, biventer cervicis, and splenius). In each average the mean EMG amplitude of the first six bins (3 ms) was taken as the baseline value, and the maximum and minimum values were derived from six *consecutive* bins (3 ms) showing the maximum and minimum mean EMG amplitudes, respectively. The minimum:baseline ratios from the ipsilateral genioglossus ranged from 96.0% to 96.9% with a mean of 96.5%, indicating a 4.5% reduction (=26 μV) of EMG amplitude in the spike-triggered average. The Mann-Whitney *U* test showed that these ratios were significantly *($P < 0.01$)* lower than those from any of the neck muscles (99.1 to 99.9%). In contrast, the minimum:baseline ratios from the contralateral genioglossus, biventer, and splenius were not different from each other. Furthermore, there was no significant peak (maximum:baseline) in the averages from any muscle. In summary, these findings indicated that the postspike inhibition of ipsilateral genioglossal activity was significant and selective to this muscle. The averages from the other cell (cell 5) also showed a postspike inhibition of smaller magnitude at the same latency, though this was not statistically significant.

DISCUSSION

The close temporal conjunction of unit discharge with tongue movement suggests that these cells are involved in the regulation of tongue motility. Our studies on the reticular formation cells related to pinna, eye, neck, and other movements (15-17) indicated that most of these cells had a preferred,

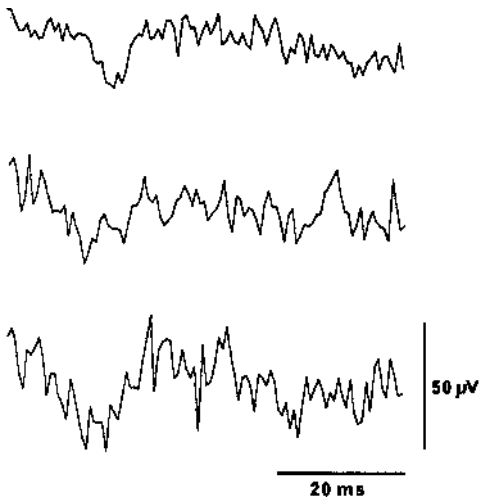

FIG. 3. Three spike-triggered averages of the ipsilateral genioglossal EMGs, showing a short-latency inhibition (cell 4). Each average was based on 200 sweeps.

usually ipsilateral direction of discharge-related movements. In contrast, the directional specificity of unit discharge was undetectable in all but one of the tongue cells. This may be related to the fact that most or perhaps all tongue movements require an intricate bilateral coordination of the tongue muscles, though it is entirely possible that more refined observational techniques might allow us to detect subtle directional specificity in reticular tongue cells. It was recently demonstrated with the HRP technique (1) that the motoneurons innervating the genioglossal muscle in one side are situated in both sides of the hypoglossal nucleus in the dog. However, it is not known if there exist motoneurons in the hypoglossal nucleus that project bilaterally to the genioglossal muscle.

Although the tongue muscles of subprimate species were reported to lack spindles, these muscles have been known to contain other types of proprioceptors (7). Available evidence does not allow us to decide whether or not the afferent activity of these proprioceptors contributes to tongue cell discharge. However, at least the discharge of one tongue cell (which generated a significant spike-triggered inhibition of genioglossal EMG) could not have been triggered by proprioceptive inputs in that the discharge preceded, rather than followed, a change in tongue muscle activity.

The spike-triggered average data also indicate that this type of neuron may inhibit certain genioglossal motoneurons mono- or oligosynaptically. The proximity of the tongue inhibitory cell to the trigeminal motor nucleus

suggests that neurons of this type may be involved in jaw-tongue coordination during such activities as mastication, licking, and grooming. It is known that the cell bodies of masseter muscle afferent fibers are situated in the mesencephalic trigeminal nucleus and that the central collaterals of these neurons project to the trigeminal motor nucleus (6, 20) and also to the hypoglossal nucleus (8). The existence of descending fibers from around the trigeminal motor nucleus to the hypoglossal nucleus has been reported (4). Morimoto *et al.* (9) recorded inhibitory postsynaptic potentials (IPSPs) in hypoglossal motoneurons evoked by electrical stimulation of muscle afferent fibers in the masseter nerve of the anesthetized cat. The mean onset latency of the masseter-evoked IPSPs was 10.7 ms (range, 8.5 to 12.2 ms), indicating that the masseter-hypoglossal pathway is polysynaptic. Sumino and Naka-mura (19) also observed masseter-evoked IPSPs in hypoglossal motoneurons with a slightly shorter onset latency (mean, 6.8 ms; range, 4.3 to 11.0 ms). The onset latency (4.5 to 5.5 ms) of the spike-triggered inhibition of genioglossal EMG in the present study is compatible with the hypothesis that the tongue inhibitory neuron in the reticular region adjacent to the trigeminal motor nucleus mediates the inhibition of genioglossal motoneurons by proprioceptive afferent fibers in the masseter and other jaw closer muscles. Functionally, this inhibition may represent a mechanism which prevents tongue biting by suppressing protrusive tongue movements during the jaw closing action of the masseter and other jaw muscles. These considerations should not be taken to preclude the possibility that the reticular tongue cells act as relay or modulatory neurons for other tongue-related activities.

Note added in proof. Since this paper was sent to press, we have received a paper by Takada *et al. (Neurosci. Lett.* 52, 141-146, 1984). Using the HRP technique, they demonstrated a major projection to the hypoglossal nucleus from the reticular regions caudomedial to the motor trigeminal nucleus. The cells we identified behaviorally as tongue-movement related, and which produced short latency inhibition of genioglossus activity, may correspond to the cells identified anatomically by Takada *et al.*

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