Neural mechanisms of sneeze

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Batsel, H. L., AND A. J. Lines. Neural mechanisms of sneeze. Am. J. Physiol. 229(3):770-776. 1975.—Sneezes were induced in anesthetized cats by repetitive stimulation of the ethmoidal nerve. Activity of bulbar respiratory neurons during sneezing was recorded extracellularly through tungsten microelectrodes. Most expiratory neurons could be locked onto the stimulus pulses so that they responded either throughout inspiration as well as expiration or so that they began responding at some time during inspiration. As inspiration approached termination, multiple spiking occurred, finally to result in high-frequency bursts which just preceded active expiration. A fraction of expiratory neurons were activated only in bursts. Latent expiratory neurons were recruited in sneezing. Inspiratory neurons near nucleus ambiguous and most of those near fasciculus solitarius displayed similar response patterns consisting of silent periods followed by delayed smooth activations. Temporal characteristics of the silent periods, “inhibitory gaps,” suggested that they resulted from inhibition whose source was the expiratory neurons which were driven throughout inspiration. Some inspiratory neurons in the area of fasciculus solitarius failed to exhibit inhibitory gaps.

SNEEZES IN THE CAT (18, 22) like those in man (8), are characterized by deep preparatory inspirations, each of which is followed by forceful, active expiration. Previous studies in this laboratory (18) have shown that bulbar inspiratory and expiratory neurons are strongly accelerated in sneezes provoked by mechanical stimulation of the anterior nasal mucosa. In addition, a number of silent, or latent, expiratory neurons located in the caudal medulla are recruited into activity just before the expiratory thrust. The mobilization of bulbar respiratory neurons in sneezing resembles the process occurring in cough (15).

The primary objective of the present investigation was to determine the temporal events operative in the activation of bulbar respiratory neurons in sneezing. Mechanical stimulation of the nasal mucosa does not reveal much about the temporal processes. For this reason, we have made use of Sandmann’s method (21), which employs repetitive stimulation of the ethmoidal nerve to induce sneezing.

METHODS

Successful experiments were conducted on 43 adult cats, weighing from 2.5 to 4.5 kg. About 30 other animals could not be made to sneeze either as a result of mechanical stimulation of nasal mucosa or as a result of electrical stimulation of the ethmoidal nerve. All animals were anesthetized with 30 mg/kg sodium pentobarbital intraperitoneally. When anesthesia became too shallow, 5–10 mg of ethyl isobuturate hydrochloride (Diquel) was given intravenously to prevent neck movements.

Surgical procedures included placement of tracheal cannula, intravenous cannula, and diaphragmatic EMG electrodes. The ethmoidal nerve was exposed according to the directions of Sandmann (21). In the final stages of the exposure, the eyeball and periosteum of the orbital plate were retracted laterally to expose the ethmoidal nerve and artery as they entered the ethmoidal foramen. The periosteum and supporting tissues were peeled laterally to expose the nerve for a length of about 5 mm. The eyeball was maintained in its retracted position by a short length of dental roll, which was wedged between eyeball and orbital plate. The cavity was filled with warm mineral oil.

An intrapleural pressure cannula was placed at mid-thoracic level near the costovertebral junction. Pneumothorax, when it occurred at all, was corrected immediately. A vertebral clamp was placed on the spinous process of one of the lower cervical vertebrae. This clamp was connected to the stereotaxic apparatus in order to limit neck movements.

Microelectrodes were fabricated from 2 inch lengths of 0.0075-inch-diam tungsten wire. These were electropolished to tip diameter of about 1 μm and then insulated with acrylic resin.

The dorsal aspect of the medulla was exposed by procedures reported previously (1, 2). The microelectrode was clamped in the microdrive of the stereotaxic apparatus. Lateral and anterior-posterior coordinates of the obex served as reference for each microelectrode penetration. Respiratory neurons were located in two areas of the medulla using the stereotaxic positions suggested by von Baumgarten and colleagues (4, 5) for one area and the maps of Nelson (17) and our own experience (1, 2) for the more laterally placed area. Since these two areas have distinct and characteristic stereotaxic coordinates, we have dispensed with histologic confirmation of microelectrode position.

The activity of respiratory units was amplified by a conventional resistor-condenser- (RC) coupled amplifier with cathode follower input. Differential input was employed, with the microelectrode versus an indifferent electrode. Output of the amplifier fed the vertical amplifier of a recording cathode-ray oscilloscope (CRO), a monitor CRO, and an audiomonitor. Intrapleural pressure was recorded by methods reported earlier (18).

The ethmoidal nerve was stimulated through a bipolar,
hook electrode with the centrally placed electrode made cathodal. Rectangular pulses of 10 \( \mu \)s duration were delivered from an electronic stimulator through an isolation transformer. Frequency and strength of stimulation required to produce sneeze were assessed by noting the effect on intrapleural pressure as displayed on an inkwriter monitor. As a rule, stimulation had to be continued for several seconds before sneezes began to appear. The main criterion for sneezing was generation of a positive pressure in the intrapleural recording. This event was always accompanied by visible contraction of the abdominal wall.

Respiratory unit activity and intrapleural pressure were displayed on separate unswept channels of a Tektronix 365 CRO. The aligned vertical excursions were photographed on bromide paper transported horizontally at 50 mm/s. Negative phases of neuronal spikes were intensified by \( z \)-axis modulation. Stimulus artifacts were kept to minimal amplitude by adjusting the location of the indifferent electrode.

Stimulation of the cervical vagus was utilized, in some cats, in an attempt to identify vagal motoneurons in the region of ipsilateral nucleus ambiguus. Presence of an antidromic response, characterized by fixed, short latency (<2 to 3 ms), elicited at relatively weak stimulus strength, and showing decomposition on high frequency stimulation (200–300 Hz), was accepted as evidence that the neuron in question was a vagal motoneuron. Absence of an antidromic response in other respiratory neurons in the same, or adjacent, penetrations was assumed to identify these neurons as nonvaginal. In such negative cases, the stimulus strength was increased to insure adequate strength. The latter procedure frequently resulted in coughing.

RESULTS

Effect of ethmoidal nerve stimulation on respiratory movements. Adequate stimulation of the ethmoidal nerve produced sneezes in most cats in which it was attempted. Development of sneezes in three different cats is illustrated in the intrapleural pressure recordings of Fig. 1. In these cases, sneezes were produced by stimulating at strength just adequate and at frequency of 17 Hz. These examples were selected to illustrate the marked similarity in sneezing patterns as well as the differences in the initial effects of the stimulation. The latter effect was some form of passive expiratory effect, either expiratory apnea as in Fig. 1A or diminution of inspiratory force as in Fig. 1B. These two patterns were encountered about equally in 90% of our cases, but there were individual variations in the initial effects. In some cats the apneic period lasted more than 10 s before a long preparatory inspiration developed. As Fig. 1 shows, expiratory thrusts became progressively more forceful as the stimulation period progressed.

In spite of differences in the early period of stimulation, the form and the rate of sneezing were similar in these three animals and in other animals of this series. In most of these animals, the inspiratory pressures showed relatively less increase in sneezes than did the expiratory pressures. In the same cats, mechanical stimulation of the nasal mucosa produced sneezes of similar form and rate. This form of individual sneezes agrees with the description given by Tomori and Widdicombe (22). The rate of sneezing exhibited wide individual variation, which seemed to depend more on the physiological status of the animal than it did on the parameters of stimulation. Animals in good condition sneezed at rates as high as 90/min and as low as 6/min under continuous ethmoidal nerve stimulation. Cats that exhibited slower sneezes were usually poor subjects, since sneezes finally failed to occur after a few stimulations. Such animals usually showed very long periods of apnea in the early stages of stimulation.

Our experiments have shown that sneezes could be produced by stimulating the ethmoidal nerve at 3–35 Hz. At lower rates, 3–9 Hz, the time required to produce full sneezes was very long and the inspiratory and expiratory pressures usually did not reach maximal. At 10–20 Hz, buildup to full sneezing was shortened and above 20 Hz buildup was still shorter. In most cats there was an upper limit, usually around 35 Hz, above which the stimulus became ineffective. (One cat sneezed at stimulus frequency above 45 Hz while another sneezed at frequencies up to 100 Hz.)

In about 15% of our animals, a priming stimulation of the ethmoidal nerve was sufficient to start sneezing which continued one or more cycles after the stimulation had been discontinued.

Initiation of stimulation during late inspiration was commonly responsible for premature termination of inspiration without any sign of active expiration. Stimulation started...
during expiration was also not immediately responsible for an active expiration. In short, active expirations occurred only after an inspiration of normal, or near normal, magnitude.

Sneezes were absent from the outset in a large number of additional cats. In still other cases, sneezes began to weaken after a few repetitions, finally disappearing altogether. In both situations, the stimulus still had a transitory effect on respiratory excursions in that one or two inspirations were depressed. Incidence of these failures was lessened by selecting cats that had been in the vivarium for several weeks.

Stimulation of the ethmoidal nerve at sneeze strength frequently induced time-locked twitches of the laryngeal region. These quick movements resemble “elementary reflexes,” which are associated with repetitive stimulation of the superior laryngeal nerve (14).

**Effect of ethmoid nerve stimulation on bulbar respiratory neurons.** After some practice it was a simple matter to set parameters of stimulation which would be effective in producing sneezes. These parameters were easily judged by the effect of the stimulus on expiratory neurons when the ipsilateral ethmoidal nerve was stimulated. Critical strength for sneeze was usually the stimulus strength at which expiratory neurons began to “lock in” on the stimulus.

A majority of the expiratory neurons exhibited some form of time-locked response to the stimulus pulses. For descriptive purposes, we classified expiratory neurons in three types based on their responsiveness during inspiration.

**Type I** expiratory units were discharged with every stimulus pulse. As a result they responded throughout inspiration as well as in expiration. In most cases the latencies were relatively stable and shorter (4.5–12 ms) than in other types of expiratory neurons. Type I responses were encountered in 14 of 62 expiratory neurons. An example of this type is illustrated in Fig. 2, A and B.

**Type II** expiratory neurons consistently failed to fire during early inspiration but began to fire sometime later during inspiration. In a given unit the onset of firing was fairly stable from one inspiration to the next. The range of latencies to individual stimulus pulses was 10–25 ms. Responses of this type were exhibited by 39 of the 62 neurons. A typical case is shown in Fig. 2, C and D, which was recorded from a different site in the same cat as the unit of Fig. 2, A and B. This large amplitude recruited unit began firing somewhat irregularly with onset of stimulation (at arrow, Fig. 2C), but responses finally occurred regularly in the second third of inspiration. The lower amplitude unit which was spontaneously active (Fig. 2C) was also discharged in type II fashion.

A small number of expiratory neurons, 9 of 62, exhibited stimulus which was delayed until near end inspiration. The latencies were long, 25–35 ms, and extremely variable. Such type III neurons were most common in animals that had rather sluggish sneezes.

We have not noticed any characteristics of expiratory neurons which would predict which of the three types of responses would result. Late, early, and latent expiratory neurons had about equal distribution in the three types. There is evidence as well that motoneurons of laryngeal adductors exhibited similar response types.

Qualitatively, the same types of responses were present on both sides of the medulla. Comparison of the effect of contralateral stimulation to that of ipsilateral stimulation frequently revealed delays in the onset of discharge with prolongation of latencies. Figure 3 illustrates a case in which the same neuron exhibited strikingly different responses to each of the two nerves. Stimulation of the ipsilateral ethmoidal nerve (Fig. 3, A and B) resulted in a type I response, whereas stimulation of the contralateral nerve (Fig. 3, C and D) induced a type III response. Despite the fact that these changes were quite common, exceptions were encountered. Nine expiratory neurons exhibiting type I responses to ipsilateral nerve stimulation were tested with

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**FIG. 2.** Responses of expiratory neurons to repetitive stimulation of ethmoidal nerve. Upper channel = ip pressure; inspiration upward. Lower channel = unit activity. A and B: continuous records, with stimulation of ipsilateral ethmoidal nerve at 20 Hz, 10 μs, and 1.0 V, starting at downward arrow in B. Expiratory neuron at site responded to each stimulus pulse, type I response, during both inspiration and expiration. In this and subsequent figures, monitor CR0 indicated that stimulus artifact was minimal or absent. C and D: continuous records, at another expiratory site in same animal. Stimulation of ipsilateral ethmoidal nerve at 17 Hz, 10 μs, 1.0 V, beginning at arrow in C provoked discharge of a latent expiratory neuron whose response period began in first half of inspiration, type II response. Time mark = 1 s.
contralateral nerve stimulation. Three of these were found to display similar type I patterns and similar short latencies (6–8 ms) when either nerve was stimulated.

As Figs. 2 and 3 indicate, expiratory neurons generated high-frequency bursting, which preceded the expiratory thrusts. Essentially all expiratory neurons were capable of generating bursts in which the instantaneous frequency of discharge was much higher than the resting frequency. Analysis of high-speed recordings of nine expiratory neurons in sneeze bursts showed that instantaneous frequencies exceeded 400/s. In one case the unit attained an instantaneous rate of 800/s! Before stimulation, none of these units exhibited discharges above 50/s. Motion of the brain and irritation by the electrode were not major factors in these cases, for stimulation of the ethmoidal nerve was capable of provoking similar bursts in cats paralyzed with succinylcholine.

The force of active expiration appeared to be graded by variation of discharge frequency as well as by duration of the burst train. Those latent expiratory neurons (n = 19) which were recruited came into action at least by the first sneeze, thus additional recruitment did not contribute to the increments of active expirations which always followed. Burst durations of 30–350 ms were commonly associated with active expirations. Progressive extension of burst duration with continued stimulation was a probable contribution to additional force of active expiration.

In susceptible cats, stimulation of the ethmoidal nerve at low frequency (3–5 Hz) showed that formation of the bursts need not have constant time relation to the driving pulses. Figure 4 illustrates the fact that bursts were generated out of phase with the ethmoidal pulses. In this same figure, bursts also continue to be formed after the nerve stimulation had been discontinued.

The activities of more than 100 inspiratory neurons were studied in sneezes. Such neurons were sampled in approximately equal numbers in the two anatomical sites of concentration. Inspiratory neurons in both areas exhibited biphasic response patterns in which each stimulus pulse was followed by a brief, early inhibition and a later excitation. A typical example of this form of response is shown in Fig. 5. During preparatory inspirations (Fig. 5B), the "inhibitory gaps" and the later periods of accelerated firing were clearly separable. Despite the presence of the inhibitory gaps, the net effect of the stimulation was an increase of spike output. Response patterns of this type were recorded in 90% of the inspiratory neurons in the area of nucleus ambiguus. The remainder of the inspiratory neurons were completely inactivated by the stimulation. The same type of response occurred whether ipsilateral or contralateral ethmoidal nerve was stimulated.

Inspiratory neurons located ventrolateral to fasciculus solitarius exhibited a wider variety of response patterns than did those inspiratory neurons in the area of nucleus ambiguus. Although the majority of the neurons (70%) exhibited inhibitory gaps like the example of Fig. 5, the remainder of the neurons showed only traces of gaps or, in a few cases, no gaps at all. An inspiratory neuron in which inhibitory gaps were irregular is illustrated in Fig. 6. Comparison of the discharge frequency in the resting condition (Fig. 6A) to the frequency attained in sneezes (Fig. 6B) shows the characteristic acceleration which occurred in sneezing. Our results indicated that such two extremely different patterns (Fig. 5 and Fig. 6) could coexist in the same area of the medulla. The absence of inhibitory gaps was more frequently encountered in cats that had vigorous and rapidly rising preparatory inspirations.

Inspiratory neurons were accelerated up to maxima of 300–400/s. This factor alone appeared sufficient to account for deep preparatory inspirations. Increase of frequency was especially striking in the cases of late inspiratory neurons. Recruitment of inspiratory neurons, although present, was relatively infrequent.

Only a few inspiratory neurons exhibited time-locked
firing. The latencies were commonly in the range of 6–8 ms, but unlike the cases of expiratory neurons, these responses were not persistent.

Effect of ethmoidal nerve stimulation on laryngeal motoneurons. Seven respiratory neurons, located in the area of nucleus ambiguous from 3 mm rostral to 1 mm caudal to the obex, were identified as laryngeal motoneurons. Weak stimulation of the ipsilateral cervical vagus caused these neurons to discharge at fixed latencies (less than 2.0–3 ms) and at frequencies above 200 Hz. On the basis of these features, these units were classified as laryngeal motoneurons. In the same animals, 33 other respiratory neurons in the same general area, gave no trace of antidromic responses.

Four laryngeal motoneurons which had spontaneous inspiratory rhythms were recorded during ethmoidal nerve stimulations which produced sneezes. All of these neurons exhibited responses that could not be distinguished from those of other inspiratory neurons. Typical inhibitory gaps and frank accelerations were present in each case.

Three expiratory neurons located rostral to the obex gave definite antidromic responses to relatively weak stimulation of the vagus. One of these neurons was inactive during spontaneous breathing but with ethmoidal stimulation was activated at short latency (8 ms) throughout inspiration and exhibited pronounced bursts in sneezes. The response of this unit could not be distinguished from other expiratory neurons of type I. The two other expiratory laryngeal motoneurons exhibited type II responses when the ethmoidal nerve was stimulated.

The yield of antidromic responses in the region of nucleus ambiguous was surprisingly low, but other investigators have reported similar findings (7 and unpublished data).

Effect of ethmoidal nerve stimulation on nonrespiratory bulbar inspiratory neurons. The ventromedial border of the descending trigeminal nucleus was the site of large numbers of neurons which responded with very short latency and with characteristic pattern when the ipsilateral ethmoidal nerve was stimulated (23). Some of these neurons responded to contra-
lateral as well as to ipsilateral ethmoidal stimulation (3). These neurons were situated a few hundred microns dorsal to the respiratory neurons which cluster about nucleus ambiguus.

A second group of neurons with properties similar to those described above were encountered just ventral to the inspiratory neurons located near fasciculus solitarius. Only eight neurons were encountered in this site.

These neurons displayed several characteristics which may help to explain the behavior of bulbar respiratory neurons in sneezing. The latencies showed minima of 2.0–2.5 ms. At sneeze strength, neurons in both sites responded with high-frequency multiple-spike trains which lasted as much as 25–30 ms. Some of these units also began to exhibit afterdischarge when repetitive stimulation was continued for a few seconds.

**DISCUSSION**

Sneezes produced by ethmoidal nerve stimulation are similar in form and in rate to those provoked, in the same cat, by mechanical stimulation of the anterior nasal mucosa. The form of the intrapleural pressure changes and the ratio of maximal inspiratory and maximal expiratory pressures compare favorably with those recorded by Tunori and Widdicombe (22).

Both the preparatory inspiration and the expiratory thrust are essential components of sneeze. Our observations indicate that there are processes by which the magnitudes of the two components are interlinked. Burkart (9) and Burkart and Bucher (10) implicated vagal feedback (pulmonary stretch receptors) as a potential mechanism for matching the two forces. It is possible that other feedback is delivered to the respiratory centers via the spinal cord, but nothing is known about this source.

It is clear from these results that the expiratory center receives numerous direct inputs concerned with sneezing. The existence of short-latency responses indicates that only a few synapses are interposed between trigeminal and expiratory neurons. Wall and Taub (23) reported that "respiratory" neurons responded to single-shock stimulation of nasal mucosa at latencies ranging from 5 to 10 ms. Such values would fit the latencies of the majority of the expiratory neurons, provided measurements were made during expiration.

Although there is indication of abundant inputs to the expiratory center, it is doubtful that the resultant activation is adequate by itself to explain the production of active expiration. That act requires that expiratory neurons generate the high frequency bursts. According to our findings, a foregoing inspiration favors the production of bursting discharge. Experiments utilizing low-frequency stimulation have shown that these bursts are not necessarily time locked to the stimulus pulses, but that they occur on a longer time base. The neural basis for production of the bursts is not known. It is possible that afterdischarge in the trigeminal and reticular neurons may produce the bursts. It is possible too that bursts are triggered by neurons of the pneumotaxic center. The inspiratory-expiratory, phase-spanning neurons of the pneumotaxic center (11 13) seem to have a firing pattern which would coincide with the beginning of the bursts. Studies by Bertrand and Hugelin (6) have shown that stimulation in the region of the pneumotaxic center is capable of activating expiratory neurons. Finally, vagal feedback may play a part in triggering the bursts (9, 10).

Expiratory neurons and expiratory laryngeal moto-neurons have many features in common. Since our sample of laryngeal motoneurons is so small, it is not possible to fully characterize these neurons. Other investigators have indicated that laryngeal adductors are activated in the expiratory phase of sneeze (16, 20). Romiti (unpublished data) found that some motor units of adductors are regular participants in the elementary reflex while other motor units are discharged at various later times. Earlier studies by Rijlant (19) had shown that "expiratory" fibers in the recurrent laryngeal nerves were discharged at short latency by electrical stimulation of nasal mucosa. Collectively, these facts imply that expiratory laryngeal motoneurons and expiratory neurons have common activators in sneezing.

The magnitude of the preparatory inspiration appears to be explained by the obvious acceleration of inspiratory neurons. Such acceleration occurs throughout the inspiratory neuron population, regardless of anatomical site. Although the present results indicate that inspiratory neurons are activated to marked degree, they do not indicate how the activation takes place. There are few inspiratory neurons which exhibit time-locked firing in response to ethmoidal nerve stimulation. Those few which respond with short latency do so transitorily; therefore, their activity may be unrelated to sneeze. Those few which respond with longer latency, i.e., after the inhibitory gap, are stable and persistent in their responses. Except for these two small groups, inspiratory neurons are accelerated without evidence of time-locked characteristics.

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