OLFACTORY BULB POTENTIALS TO ELECTRICAL STIMULATION OF THE Olfactory MUCOSA

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In the previous studies examining electrical activity of the olfactory bulb, an adequate olfactory stimulus such as blowing odorous air into the nose has been used as a routine method of activating the olfactory bulb1,2). With this method of stimulation, however, the olfactory bulb is activated only after the stimulus is converted into the impulse train of the olfactory nerve. Therefore, the method of adequate stimulation has an obvious disadvantage to study the properties of the olfactory bulb independent of the stimulus-impulse-conversion process in the receptor cells of the olfactory mucosa. It is considered that if electrical stimulation of the olfactory mucosa is used instead of adequate stimulation, one may obtain in this simplified condition more exact information concerning the mode of impulse transmission in the olfactory bulb. Recently this problem was dealt with in the frog by Ottoson, who observed that electrical stimulation of the nasal mucosa brought about in the olfactory bulb a negative response consisting of two successive waves3). Though he considered the entire response to be developed postsynaptically in the bulb, none of the explicit bases has been presented for this conjecture. The results of the present experiments, done on rabbits, show that the seat of the main components of the evoked potential resides in the distal portion of the dendrites of the secondary olfactory neurons. Further, some properties of the evoked potential were studied in relation to the unitary spike discharges.

METHOD

Being given 0.1 mg/Kg atropin intravenously, rabbits were anesthetized with ether. Necessary operations were performed under ether anesthesia. Following this, the animals were immobilized with d-tubocurarine and maintained on artificial respiration.

Recordings of electrical activity from the surface of the olfactory bulb were made with a monopolar silver electrode placed gently on it. The reference electrode was placed on the edge of the scalp. Stainless steel microelectrodes, insulated except at the tip, were employed for recording electrical activity from the deep layers of the bulb. The detection of the recording points was done using the technique devised by Green4). Unit discharges were picked up by using conventional KCl-filled glass
pipettes having resistance of 10~20 megohms. Amplification and registration of the electrical activities were done with the use of a CR-coupled amplifier of long time constant in conjunction with a dual beam oscilloscope.

For stimulation of the olfactory epithelium, the bones covering the nasal cavity were removed carefully so as not to impair the epithelium covering the roof of the nasal cavity and then a bipolar electrode with tip separation of 0.5~1 mm was placed on the exposed epithelium. For stimulation of the deep structures of the brain a bipolar concentric electrode insulated except at the tips was inserted with the aid of a stereotaxic instrument. The positions of the stimulating electrodes were marked by passing a small d.c. current and subsequently identified histologically on frozen sections stained with hematoxilin. Electrical stimulation was carried out with a square pulse generator. The parameters for nasal mucosa stimulation varied from 0.2 to 0.5 msec. in pulse width and from 1 to 20 volts in strength. For deep stimulation of the brain square pulses of 0.2 msec. were used exclusively.

Gamma aminobutyric acid was dissolved in physiological saline solution in strength of 1%. The drug was allowed to soak into a small cotton pad and applied topically on the bulb. In order to avoid the electrical shunt, the cotton pad was removed and the fluid around the recording electrode was wiped off before the records were taken.

RESULTS

An electrode resting lightly on the surface of the olfactory bulb recorded a monophasic negative wave lasting for about 20 msec. in response to threshold stimulation of the olfactory mucosa (Fig. 1, top record). At higher stimulus strengths and in favorable preparations, a second deflection was often observed on the declining phase of the first one (Fig. 1, traces 2, 3 and 4). Hereafter, each deflection will be designated as N1 and N2 according to the order of appearance. Increasing the stimulus strength, N1 grew larger holding N2 on its shoulder. At very high strengths of stimulus, however, the inflection between N1 and N2 became obscure because of the steep slope of the descending phase of N1 (trace 5). The latent period of the evoked potential also decreased from about 40 msec. in

![Fig. 1](image)

**FIG. 1.** Olfactory bulb potentials for varying stimulus intensities. Traces 1 and 5, threshold and supramaximal responses, respectively. In traces 3 and 4 two deflections (N1 and N2) are clearly distinguishable. Numerals on individual records indicate shock intensity in arbitrary unit.
trace 1 to about 35 msec. in trace 5. Occasionally a slow smaller negative wave appeared after the N1–N2 complex (Fig. 7, record 6). In contrast to the stability of N1, the magnitude and the contour of N2 varied widely in successive recordings and disappeared rapidly with deterioration of the experimental subjects.

As the olfactory receptor cells have been proved to be electrically inexcitable, it is likely that the responsive element to the electrical current applied to the mucosa is the olfactory nerves arising from it.

The olfactory nerve fibers originating in the epithelium run centrally and collect to form nerve bundles before they pass through the cribriform plate. If too strong a stimulus was applied to the olfactory mucosa at a short distance from the bulb, the form of the evoked potential was sometimes not so simple as was described above. This might be attributed to the spread of the stimulus current to many nerve bundles. Therefore, in this experiment the stimulating electrode was usually placed on the mucosa at a distance of 2.5–3 cm forward of the bulb.

If the stimulating electrode was thrust deeply into the nasal cavity, the response recorded from the surface of the olfactory bulb was sometimes a positively going potential wave. In this case the needle electrode inserted in the bulb recorded the negative response from the undersurface of the bulb. Consequently this positive wave might be explained as follows; The stimulated nerve bundle terminated on the undersurface of the bulb and an electrical dipole developed there producing a potential field making the upper surface of the bulb positive.

Effects of anterior commissure stimulation. The anterior limb of the anterior commissure contains the centrifugal fibers through which the olfactory bulb presumably receives the central regulation from the higher centers. High frequency stimulation of the anterior commissure was reported by Kerr and Hagbarth to suppress electrical activity of the bulb occurring spontaneously (intrinsic wave) and that caused by olfactory stimulation (induced wave).

A single shock to the anterior commissure suppressed N2 selectively. In Fig. 2, record F is a control response to nasal mucosa stimulation. Being preceded by anterior commissure stimulation which produced some potential wave in the

![Fig. 2. Suppression of N2 by a conditioning shock to the anterior commissure. F, control evoked potential to olfactory mucosa stimulation. AC, wave potential evoked by anterior commissure stimulation. F+AC, olfactory mucosa stimulation was preceded by anterior commissure stimulation at various intervals. N2 decreased in amplitude at intervals shorter than 80 msec. White dots indicate anterior commissure stimulation.](image-url)
olfactory bulb, N₂ was reduced in amplitude at stimulus intervals shorter than 80 msec. while N₁ remained without any remarkable changes (records F+AC).

Repetitive stimulation of the anterior commissure at a frequency of 100/sec. suppressed almost completely N₁ as well as N₂. In the experiment cited in Fig. 3, the intrinsic wave was monitored in parallel with the evoked potential. In confirmation of Kerr and Hagbarth, the ongoing intrinsic wave was suppressed during anterior commissure stimulation (Fig. 3, 2). Further it was often observed that the suppressed intrinsic activity tended to augment within 0.5 sec. after turning off the stimulation (record 3). The responses to the olfactory mucosa stimulation disappeared during anterior commissure stimulation (record 2), and recovered rapidly after the cessation of stimulation (record 3). This result forces the conclusion that the evoked potential due to olfactory mucosa stimulation takes place postsynaptically in the olfactory bulb.

Laminar distribution. The potential wave was recorded from the deep layers of the bulb with a stainless steel microelectrode. On the surface of the bulb, the wave form obtained with this electrode was the same as that observed with the gross surface electrode. Inserting the microelectrode gradually, the evoked potential was recorded at measured depths. As shown in Fig. 4 A, no marked change occurred in the evoked potential until a level of 500 μ was reached, though the base line became noisy because of small multiple spikes. At a depth of 700 μ the response abruptly decreased in amplitude and with a slight penetration of the electrode it reversed in polarity. Progressing further there was observed at a level of 1000 μ a positive wave corresponding exactly with the surface response in the contour and the time course. Then, the electrode was withdrawn up to the site of the phase reversal and a current of 5~10 μA was passed for about 10 sec. through the electrode in order to mark the position of electrode tip by a small iron deposit. This point was found in this instance in about the middle of the external plexiform layer (Fig. 4, B). Throughout the several experiments, most of the reversal points were detected in about the same level though some of them fell in somewhat superficial or deeper parts of the external plexiform layer.
OLFACTORY BULB POTENTIALS

It is well known that the secondary olfactory neurons consist of the mitral cells and the tufted cells. While the mitral cells situate in the layer of mitral cells the tufted cells are found in the external plexiform layer throughout. The dendrite shafts of these secondary olfactory neurons extend from within outwards through the external plexiform layer and make the synapses with the olfactory nerves in the glomerulus. The results mentioned in this section show that in response to olfactory mucosa stimulation, an electric dipole develops in the olfactory bulb with the negative pole near the external surface. This finding may easily be explained by considering that the localized depolarization in the distal portion of the dendrites generates the negative wave on the surface and the superficial layers, acting as the "sink" of the current flow against the "source" in the proximal dendrite shafts and the cell bodies. When only the mitral cells are thrown into activity, the resulted dipole field will be simple in configuration, because the cell bodies of this type of neuron are arranged almost in line on a single layer (layer of mitral cells) with the dendrites extended to reach the glomerular layer. The same is true of the tufted cells having their cell bodies close to the layer of mitral cells. However, the existence of the tufted cells scattered in the superficial part of the external plexiform layer will introduce some complication into the dipole field produced by the mitral cells and the deeply situated tufted cells. Such complication is supposedly reflected in the potential configuration as observed at the depths of 800 and 900 μ in the experiment of Fig. 4. At these
depths the recorded potentials were not in agreement in time course with those from the surface and the extremely deep layer.

**Effects of gamma aminobutyric acid (GABA).** The action of GABA has been reported to inhibit activity of the dendrites in the stretch receptor neuron of the crayfish\(^8\) and also in the pyramidal cell of the cerebral cortex\(^9,10\). If, as was inferred in the previous section, the evoked potential studied in the present experiment is ascribable to activity of the dendrites of the secondary olfactory neurons, it is expected to be suppressed by GABA. However, Sigg and Grundfest\(^11\) observed in the frog that GABA was entirely without effect upon the bulb potential evoked by electrical stimulation of the olfactory mucosa. In the present study the action of GABA was reexamined and it was established that this drug was an effective depressant to the bulb potential. This is shown in the series of records presented in Fig. 5. The olfactory bulb negative potential was completely suppressed 7 minutes after the application of GABA (record 2) and restored, though incompletely, following the removal of the drug applied (record 3). It must be pointed out, however, that in comparison with the experiment in the cerebral cortex, a longer time had to be allowed for the suppressing effect to appear and in some cases the suppression remained incomplete even after a prolonged application of GABA. The difference in the results between the present experiment and that of Sigg and Grundfest may be accounted for by diversity of species used.

![Fig. 5. Suppressing effect of GABA. 1, control. 2, 7 min. after topical application of the drug. Evoked potential was completely depressed. 3, recovery after washing off GABA.](image)

**Effects of barbiturate anesthesia.** As was shown in Fig. 2, N\(_2\) was readily suppressed by anterior commissure stimulation. Moreover, N\(_2\) was observed being more susceptible to barbiturate anesthesia than N\(_1\). Record 1 of Fig. 6 demonstrates the control evoked potential. Records 2 and 3 were obtained

![Fig. 6. Effects of barbiturate anesthesia. 1, control. 2, after first injection of barbiturate (3 mg/kg, intravenously). 3, after second injection (6 mg/kg).](image)
after an intravenous injection of pentobarbital sodium in two steps with a dose of 3~6 mg/Kg successively. N₂ was suppressed almost completely in record 2, while N₁ kept the same height as that of the control.

**Observation of the unitary activity.** The secondary olfactory neurons in the olfactory bulb of the rabbit have been divided into two groups, the mitral cell and the tufted cell. According to ALLISON, the mitral cells send their axon fibers through the lateral olfactory tract. On the other hand, the axons of the tufted cells enter the higher centers by way of the anterior limb of the anterior commissure. This anatomical finding suggests to us that, when activity of the single nerve cell is picked up by a microelectrode, the classification of cell will be possible by determining which pathway, the lateral olfactory tract or the anterior commissure, is capable of causing an antidromic firing upon single shock stimulation.

In order to stimulate the lateral olfactory tract, the bone covering the prepyriform lobe was removed and a bipolar stimulating electrode was placed on the caudal end of the lateral olfactory tract. The distance from the stimulated site to the surface of the bulb was about 2 cm. To estimate the conduction time of the antidromic impulses, the surface response to lateral olfactory tract stimulation was recorded by a bipolar gross electrode placed on the bulb. In a favorable arrangement of the electrode, a slow wave was observed to be preceded by a sharp wave which presumably represented the arrival of the antidromic impulses.

![FIG. 7. Response patterns of unitary discharges to electrical stimulation of the olfactory mucosa. 1; Unitary discharge of a mitral cell responding to antidromic stimulation of the lateral olfactory tract with a short latent period of about 2 msec. Neurons studied in records, 2, 3, 5 and 6 were confirmed to be mitral cells by the method of antidromic stimulation. 2; Spikes occurring twice at the two positive peaks of slow wave. 3; Discharges in the ascending phase of N₁. Spikes were retouched. 4; High frequency repetitive discharges. Note deep slow wave was recorded as a negative one. 5; Repetitive discharges sustaining for about 200 msec. 6; Interruption of spontaneous firing during the excursion of main component of the slow wave. About 10 traces were superimposed. Upper traces in records 3, 4 and 6 show the surface response recorded with a gross electrode. Time scale, 100/sec. in all records.](image-url)
The latent period of this sharp wave was about 1.5–2 msec. The unit which responded to a shock to the same tract with latency shorter than 2 msec. was identified as the mitral cell. A representative record of the invasion of antidromic impulse in the mitral cell is shown in Fig. 7, 1.

The anterior commissure was stimulated by a concentric bipolar electrode oriented stereotaxically. In contrast to lateral olfactory tract stimulation, no spike discharge with a short and invariable latency could be obtained following a single shock to the anterior commissure. The failure to encounter the neurons responding to antidromic activation of the anterior commissure has remained unexplained.

Records 2, 3, 5 and 6 of Fig. 7 show the effects of olfactory mucosa stimulation upon the cells which could be identified as the mitral cells. The cell of record 2 discharged at the two separate positive peaks of the slow wave which corresponded to N1 and N2 respectively. But such a pattern of spike discharge was rather exceptional. A most usual pattern is that the firing of the mitral cells occurs, as is seen in record 3, on the ascending phase of the slow wave or on the peak of N1. On rare occasions, the repetitive firing started at the foot of the evoked potential and was sustained for about 200 msec. (record 5). Another example of the response pattern of the mitral cell is interruption of the spontaneous discharges. This is exemplified by record 6 which was formed by superposition of about 10 traces. The inhibition of the spontaneous firing can be seen during the excursion of the main component of the evoked potential. This type was found not infrequently.

Apart from the sustained discharge as exemplified by record 5, the number of spike discharges of the mitral cell in response to a single shock to the olfactory nerve was usually one or two and did not exceed four as far as the stimulus strength was limited within an extent as employed in the present study. Occasionally, there were encountered the neurons which responded with several spikes (record 4), but none of the neurons exhibiting this response pattern could be identified as the mitral cells. Though the possibility that these cells might be the mitral cells in which the antidromic impulse was blocked before invading the cell soma could not completely be excluded, it was my impression that they were not the mitral cells, because they were mostly found in the superficial layers where the evoked potential was recorded as a negative wave. In such layers the mitral cells were very rarely found histologically.

DISCUSSION

There has been a noticeable discrepancy of opinion as to whether the wave potential generated in the dendrite of the nerve cell is conducted along the dendrite shaft. Chang¹²) considered that in the cerebral cortex the action
potential about 15 msec. in duration was conducted slowly along the shaft of the apical dendrite of the pyramidal cells. On the contrary, Grundfest\textsuperscript{13} and Eccles\textsuperscript{1-4} considered that such type of potential was a non-propagating postsynaptic potential elicited in the apical dendrite. In the preceding sections it was shown that the negative evoked potential resided in the distal portion of the dendrite of the secondary olfactory neuron and reversed in polarity at the deeper layers of the olfactory bulb. These findings indicate that the main component of the evoked potential represents the depolarizing postsynaptic potential generated in the dendrite, and moreover suggest that these slow waves do not propagate but spread electrotonically along the dendrite shafts, thereby initiating the all-or-none impulse of the cell bodies. In this respect, the secondary olfactory neuron is considered to behave in a similar manner to that of the stretch receptor neuron of the crayfish\textsuperscript{15}.

It has been shown in favorable conditions that on the declining phase of the main deflection there appeared the second wave which has hitherto been referred to as N\textsubscript{2}. N\textsubscript{2} was readily suppressed by the centrifugal volleys originating in the anterior commissure and also by barbiturate anesthesia. These features may be accounted for by considering that N\textsubscript{2} is produced after the olfactory nerve impulses are transmitted through the polysynaptic chain. An alternative explanation of the origin of N\textsubscript{2} is possible if one assumes that the active depolarization may occur in the dendrite of the secondary olfactory neuron following the postsynaptic depolarizing potential which is represented by N\textsubscript{1}. According to this notion, N\textsubscript{2} is the local partially conducted response in the distal portion of the dendrites.

Concerning the olfactory bulb of the frog, the potential evoked by electrical stimulation of the mucosa has been found to be composed of the two components\textsuperscript{3}). But the contour and the duration of the response are different from those found in the rabbit. The divergence between the two species in regard to the natures of the bulb potential was also detected in the experiment testing the effect of GABA. Before discussing the origin of the potential evoked in the frog in comparison with the data obtained from the rabbit, studies are required in frog to see the laminar distribution of the potentials and the effects of centrifugal fiber stimulation.

Studying unit activity, it was observed that spontaneous firing of some of the mitral cells was inhibited during the excursion of the evoked potential. If the presence of the inhibitory synapses is admitted between the olfactory nerves and the dendrite of the mitral cell, this finding can be easily understood. But no information is presented as yet concerning such synapses. Another possible explanation is to postulate some inhibitory interaction between the secondary olfactory neurons through a complex neuronal network within the bulb. The anatomical pathways responsible for this mutual interaction between the second-
ary olfactory neurons have been postulated by histological investigation\textsuperscript{5,16}).

**SUMMARY**

The properties of the olfactory bulb potential evoked by electrical stimulation of the olfactory mucosa were studied in rabbits immobilized with \textit{d}-tubocurarine. The evoked potential was a slow negative wave when recorded from the surface of the bulb. In favorable conditions, the falling phase of the main deflection carried the second negative wave which was readily suppressed by barbiturate anesthesia and by the centrifugal impulses. The evoked potential reversed in polarity when the recording microelectrode was inserted in the deep layer. During high frequency stimulation of the anterior commissure the evoked potential was completely inhibited. The potential was suppressed by local application of gamma aminobutyric acid. Studying the unitary activity, it was observed that olfactory mucosa stimulation caused not only excitation but also inhibition of the single neuron discharge. These findings were discussed in relation to the histological structure of the olfactory bulb.

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**REFERENCES**