AROUSAL REACTION OF THE OLFACTOR Y BULB

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It has been well recognized that the mesencephalic reticular formation (RF) exerts the important regulatory influence upon electrical activity at various levels of the central nervous system such as the neocortex, the limbic cortex, the diencephalon, the sensory relay nuclei and so on.

The problem as to whether the olfactory bulb receives a regulatory influence from the RF has attracted the particular interest of investigators. The first to deal with this problem were ARDUINI and MORUZZI3) but they failed to find any noticeable changes in electrical activity of the olfactory bulb when the RF received high frequency stimulation. Recently HERNÁNDEZ-PEÓN et al.5) observed in chronic experiments that when the RF was stimulated directly or indirectly through any of its principle inputs, intermittent bursts of rhythmic activity occurred in the olfactory bulb.

The experiment reported here was aimed at re-examining the effects of RF stimulation upon electrical activity in the olfactory bulb of curarized rabbits. The results obtained were not in agreement with those of ARDUINI and MORUZZI and of HERNÁNDEZ-PEÓN et al. In our experiment, direct or indirect stimulation of the RF was found to suppress the electrical activity occurring in the bulb spontaneously or evoked by olfactory stimulation. The identical results were also obtained in the chronic experiment. A preliminary account has been published elsewhere.12)

METHOD

Acute experiment. Adult rabbits weighing from 3 to 3.5 Kg were used. After an intravenous injection of 0.1 mg/Kg atropine, they were anesthetized with ether and the tracheotomy was performed. Ether was discontinued and d-tubocurarine (0.8 mg/Kg) was administered intravenously, additional doses being given as required. Animals were maintained on artificial respiration.

Bipolar silver electrodes were usually used for recording from the surface of the neocortex and of the olfactory bulb. The monopolar recording was made to pick up bulb potentials evoked by electrical stimulation of the olfactory epithelium. In this case the indifferent electrode was placed on the edge of the scalp. For stimulation of the deep structure of the brain a concentric needle electrode was inserted stereotaxically consulting the map of SAWYER et al.10) Stimulus parameters were usually 100 cps in frequency, 6 to 12 volts in intensity and 0.2 msec in pulse duration.

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The stimulation was repeated at intervals of 3 min or longer. The stimulated point was electrolyzed at the end of the experiment and determined in a hematoxylin-stained serial section. For olfactory stimulation, a polyethylene cannula was inserted into the postnasal cavity through the oral end of the transected trachea and connected to the inlet pipe of an artificial respirator which permitted room air to be drawn periodically through the nostril. CR-coupled amplifiers were used in connection with an oscilloscope. Ink-writer tracings were obtained with an EEG-machine of San'ei. Occasional measurements of blood pressure were made from the femoral artery by means of a pressure transducer of Nihon-kohden (type MP-3).

Chronic experiment. Under pentobarbital anesthesia, recording electrodes were implanted aseptically in the rabbits. The cortical electrodes were a pair of stainless steel screws inserted into the bone to rest on the dura mater. For the olfactory bulb a pair of fine steel wires, insulated except for the tips, were implanted. Ordinarily one of these wires was placed on the surface and the other was thrust deeply (about 1 mm) into the bulb. One or two days after the operation the animal was brought into a dark box and responses of the olfactory bulb to arousal stimuli were observed in parallel with the EEG of the neocortex. No observation was continued beyond 1 week after the operation.

RESULTS

Acute Experiment

1. The intrinsic wave. In the unanesthetized rabbits, spontaneous activity of the olfactory bulb is characterized by the rhythmic oscillation which has been referred to as the intrinsic wave. As seen in Fig. 1, B-1, the main component of the intrinsic wave is regular waves of a frequency of about 100 cps, being intermingled with irregular small oscillations. Fig. 1 shows the effects of RF stimulation on the cortical EEG and on the intrinsic wave of the olfactory bulb. In a well-known manner, high frequency stimulation of the RF brought about an arousal reaction in the EEG, i.e., a sleeping EEG composed of slow waves and spindle bursts was replaced with low voltage fast activity (Fig. 1 A). From

![Fig. 1. Effects of RF stimulation on the intrinsic wave. A, cortical EEG and B, the intrinsic wave. Each record in B was obtained during the underlined period with corresponding numeral in A. With initiation of RF stimulation (dotted line) EEG altered from sleeping to arousal pattern (A) and rhythmic activity in the induced wave disappeared during and for several sec after the stimulation (B, 2 & 3).](image-url)
a long continuous record of the intrinsic wave, four short sample records were picked up and arranged in B. The regular rhythmic oscillation observed in the control (B-1) was suppressed immediately after the initiation of RF stimulation (B-2) and almost recovered within several sec after turning off the stimulation (B-4). Usually the recovery of the intrinsic wave preceded the reappearance of the sleeping pattern in the cortical EEG. In records B-2 and -3, it is noticeable that the irregular small oscillation survived during RF stimulation. In some experiments in which the irregular wave was a main constituent of the intrinsic wave, the suppression of the intrinsic wave was not explicit.

2. The induced wave. When odorous air or unpurified room air is introduced into the nose, there occurs in the olfactory bulb a train of synchronous sinusoidal oscillation which has been designated as the induced wave. The frequency of this wave is about 30 to 50 cps. The amplitude of the induced wave differed from experiment to experiment and even in a given experimental animal it changed appreciably within 3 to 5 hours after the experiment was started. In some experiments, successive induced waves varied widely in amplitude even if the same odorous stimulation was repeated.

As was observed in the intrinsic wave, the induced wave was also suppressed by high frequency stimulation in the RF. In Fig. 2, olfactory stimulation, repeated at a frequency of 0.2 to 0.3 cps, is indicated by dotted lines, and RF stimulation by a solid line. Fig. 2 A shows the effects of RF stimulation observed most frequently throughout the present study. In this case the induced wave was suppressed as a whole as long as RF stimulation was continued. Even after the stimulus was turned off, the suppression was observed to continue for several sec.

Records B and C show rather exceptional cases. In B, the control induced

Fig. 2. Types of suppression of the induced wave. Dotted lines indicate olfactory stimulation and solid lines RF stimulation. In A, the induced wave was suppressed as a whole. In B, initial burst of the induced wave was strongly suppressed while late one survived during RF stimulation. In C, augmentation was observed during post-stimulation period. C-1, control. RF was stimulated in C-2, C-3, 30 sec after C-2.
wave seemed to consist of the initial large burst lasting for about 0.3 sec and the late smaller one continuing for about 1 sec. The initial burst was suppressed markedly by RF stimulation while the late one remained almost unaffected. In C, the after-augmentation was observed following the suppression. Record C-1 is a control record. The RF was stimulated for about 6 sec (solid line) in record C-2. Record C-3 was obtained about 30 sec after record C-2. The induced wave which suffered a suppression by RF stimulation, tended to augment about 10 sec after the stimulation was ended. The augmentation reached a maximum in record C-3 and gradually disappeared within 2 min. Such augmentation of the induced waves during the post-stimulatory period was frequently found with variable extents in the course of the present experiment. As a matter of fact, however, the degree of post-stimulatory augmentation was unusually conspicuous in record C.

As is well known, for activating the cortical EEG, RF stimulation can be substituted by peripheral arousal stimulation. This is true of the olfactory activity. Fig. 3 A shows the induced wave of the olfactory bulb (upper trace) and the EEG of the neocortex (lower trace). As soon as a hind leg was pinched, the slow waves and the spindle bursts in the EEG were eliminated. Simultaneously with this, the induced wave was reduced in amplitude. After several sec the induced wave restored its amplitude to the control level while EEG arousal lasted for approximately 30 sec.

As a rule, an arousal stimulus required for modifying electrical activity of the bulb must be stronger than for activating the cortical EEG. In acute ex-

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**Fig. 3.** Effects of peripheral arousal stimuli. In A, pinch of hind leg (underlined) caused suppression in the induced wave (upper trace) and arousal reaction in EEG (lower trace). B came from the same animal about 1 hour later. In this time, pinch of hind leg no longer suppressed the induced wave though EEG arousal was brought about.
Experiments, the peripheral arousal stimulus was sometimes effective but sometimes ineffective in suppressing the activity of the bulb. This is probably because the peripheral arousal stimulus has a weak activating effect. In addition to this, we have obtained the impression that the peripheral stimulus, once found to be effective in causing the olfactory arousal, gradually loses its stimulation effect as the experimental animal deteriorates. This will be seen by comparing record A with record B, both being obtained from the same animal with an interval of about 1 hour. In record B the same stimulus as used in record A failed to cause any marked change in the induced wave though low voltage rapid activity took place in the cortical EEG. Even in this condition, RF stimulation was still capable of inhibiting activity in the bulb.

3. **Distribution of the excitable spot.** The site of the stimulation, which was effective in suppressing the induced wave as well as the intrinsic wave, was marked by passing a small D.C. current through the stimulating electrode and

![Fig. 4. Distribution of excitable spots. The points effective in suppressing the oscillatory bulb potential were projected on the median sagittal plane. They were distributed in the RF and the hypothalamus. AC, anterior commissure. CC, corpus callosum. MM, n. mammilaris medialis. OCH, optic chiasm. PC, posterior commissure.](image-url)
was identified histologically. In Fig. 4, the points are projected on the median sagittal plane. It is clearly seen that the effective points were distributed in the entire extent of the midbrain tegmentum with the reticular structure. Some of them were found in the hypothalamus.

As was reported in the preliminary account, stimulation of the hypothalamus sometimes caused augmentation of the induced wave. This was true in some of the cases where the electrode tip was introduced into the extremely lateral part of the anterior hypothalamus or near the anterior commissure. The phenomenon of augmentation of the induced waves will be treated in a forthcoming paper.

4. The evoked potential to electrical stimulation of the olfactory mucosa. In response to a single shock to the olfactory mucosa, a negative wave of about 40 msec duration develops on the surface of the bulb. This evoked potential is considered to represent depolarization in the proximal portion of the dendrite of the secondary olfactory neuron. The experiment cited in Fig. 5 was made to see what changes occurred in the evoked potential when the suppression of the intrinsic wave was brought about by high frequency stimulation in the RF. In each set of Fig. 5, column 1 is the evoked potential and column 2 the intrinsic

![Fig. 5](image-url)

**Fig. 5.** RF stimulation and the evoked potential induced by a single shock to the olfactory mucosa. A, control, B, during and C, 10 sec after RF stimulation. In each set, column 1 shows the evoked potential and column 2, the intrinsic wave. Though the intrinsic wave was suppressed by RF stimulation (B), no marked change occurred in the evoked potential.
wave. During stimulation in RF, the intrinsic wave was markedly suppressed (B, 2), whereas the evoked potential remained unaltered (B, 1) though in some cases it seemed to increase slightly in duration.

No definite explanation of this finding is possible until the generating mechanism of the intrinsic wave and of the induced wave is clarified. If one presumes, however, that while the evoked potential is brought about monosynaptically the generation of the oscillatory potential of the bulb comprises an event occurring in a multisynaptic pathway such as the reverberating circuit of Herrick,\(^6\) then the results mentioned in this section are considered to mean that RF stimulation suppresses only impulse transmission mediated through the multisynaptic chain within the olfactory bulb.

5. Destruction of the olfactory epithelium. One may argue that RF stimulation suppresses the induced and the intrinsic wave of the olfactory bulb not directly but indirectly by modifying, neuronally or humorally, activity of the olfactory mucosa. This possibility was excluded by the experiment of Fig. 6 in which the effect of RF stimulation was studied after the olfactory mucosa was almost completely destroyed. Figs. 6-A and -B were obtained before and after lesion of the olfactory mucosa respectively. No noticeable difference was observed either in the control records (A-1 and B-1) or in the suppressing effect of RF stimulation (A-2 and B-2).

\[\text{Fig. 6. Destruction of the olfactory epithelium. A and B, before and after the destruction of the mucosa respectively. 1, before, 2, during and 3, after RF stimulation. Suppressing effect of RF was almost the same in A and B.}\]

6. Section of the anterior commissure and neuronal isolation of the bulb. In 1953, Allison\(^2\) reported the presence of centrifugal fibers entering the olfactory bulb through the anterior limb of the anterior commissure. Later, Kerr and Hagbarth\(^7\) found that repetitive stimulation of this tract suppressed the wave activity of the bulb and considered this suppression as due to activation of the centrifugal fibers contained in the anterior commissure. Previously, however, Cajal\(^4\) had observed another group of centrifugal fibers which originated presumably in the sphenoidal cortex and came to the bulb through a way other than the anterior commissure.
FIG. 7 shows to what extent the anterior commissure participates in mediating the effect of RF stimulation to the olfactory bulb. After RF stimulation was ascertained to be effective in suppressing the induced wave (FIG. 7, 1), the anterior commissure was sectioned electrolytically (see inset figure). Following this operation, record 2 was taken. In comparing records 1 and 2, one may conclude that the anterior commissure plays a scarce part, if any, in mediating the suppressing effect from the RF.

Between records 2 and 3, the olfactory bulb was isolated neuronally at the olfactory peduncle. This operation made the RF unable to modify activity of the olfactory bulb in response to high frequency stimulation. From this fact, the possibility is excluded that stimulation in the RF may suppress the wave potential of the bulb extraneuronally, for example, by raising the blood pressure or by constricting the blood vessels. So far as the blood pressure is concerned, it was revealed that changes of electrical activity of the olfactory bulb usually preceded a slow rise of the blood pressure, both occurring in response to RF stimulation.

7. Lesion of the RF. When the RF is destroyed in alert animals, the cortical EEG changes from low voltage fast activity to slow waves with spindle bursts. From this fact, it has been inferred that in the arousal state, the RF continuously discharges the ascending volleys to regulate activity of the neocortex. If RF exerts the tonic suppressing effect upon the olfactory bulb as well, electrical activity of the olfactory bulb is expected to augment when the function of the RF is surgically eliminated. According to the results of the several experiments,
however, this was not the case. Though the central core of the midbrain was destroyed at the intercollicular level electrolytically or cut with a knife blade so that the EEG altered from an arousal to a sleep pattern, changes observed in the wave potential of the bulb were within the spontaneous variation. These results are interpreted to mean that an arousal animal in the normal condition is put in an activation level in which the tonic suppressing effect of the RF upon the neocortex is very strong but that upon the olfactory bulb is insignificant, so that the release phenomenon due to elimination of the reticular function can be seen only in the neocortical activity, not in the olfactory activity. In other words it may be said that the regulatory influence of the RF exerted upon the olfactory bulb is not tonic but phasic in nature in the sense that the RF does not continuously send the suppressing impulses but does so only for a short while when it is activated.

Chronic Experiment

When the rabbits are allowed to breathe through the nose, electrical activity of the bulb must be influenced by changes in the pattern of respiration. This must be taken into consideration for evaluating the data of the chronic experiment. To avoid this complication the chronic experiment in the present study was carried out in such a way that the trachea was transected in the preliminary operation and the animals were made to breathe directly through the caudal cut end of the transected trachea. In our chronic experiment, therefore, observations were limited to modification of the intrinsic wave by arousal stimuli.

The findings of the acute experiment were confirmed in the chronic experiment. The lower trace of Fig. 8 reproduces the cortical EEG. When hand clapping was repeated (underlined part), the arousal reaction occurred in the EEG, and simultaneously the intrinsic wave decreased in amplitude for about 4 sec (upper trace). Not only auditory but also photic or painful stimuli caused similar suppression of the intrinsic wave.

Recently Hernández-Peón et al. insisted that in the chronic experiment arousal stimulation caused in the olfactory bulb the rhythmic burst activity, called "arousal discharge", which was considered by them to be different from the

![Fig. 8. Record from a chronic experiment. 1, the intrinsic wave and 2, cortical EEG. Hand clapping (underlined) caused suppression of the intrinsic wave simultaneously with EEG arousal.](image-url)
induced wave. In our experiment, similar burst activity was observed if the trachea was kept intact, but it could not be recorded after the transection of the trachea. Consequently it seems to us that their arousal discharges are not a newly produced activity but simply represent the pre-existing induced waves which are enhanced by augmented respiration due to general arousal reaction.

**DISCUSSION**

The findings of the present experiment that RF stimulation suppresses the intrinsic and the induced waves of the olfactory bulb are inconsistent with the results of the previous researchers. ARDUINI and MORUZZI,3) using the encéphale isolé cat, reported that RF stimulation could not modify electrical activity of the bulb. The difference between their results and ours might be attributed to the diversity of the species and/or that of the method of immobilization. The discrepancy between the findings of HERNÁNDEZ-PEÓN et al.5) and of our experiment has been discussed in relation to the results of the chronic experiment.

In 1955, KERR and HAGBARTH7) found that the intrinsic and the induced waves of the bulb were suppressed by high frequency stimulation of the anterior limb of the anterior commissure and became synchronous after sectioning the same tract. From these findings the anterior commissure was considered by these authors to be the tract carrying the tonic suppressing influence from the higher nervous centers to the olfactory bulb. The suppression observed during RF stimulation in the present experiment was of a phasic nature and mediated through a way other than the anterior commissure. In this respect, the suppressing system studied in this experiment is essentially different from that reported by KERR and HAGBARTH.

Recently the RF has been reported to be capable of modulating impulse transmission through the auditory, the visual and the somatosensory afferent pathways.9) Since it has been established that there is a definite modification of the induced waves by RF stimulation, it is evident that afferent conduction of the olfactory signals is also under control of the RF. Physiological significances of these findings remain to be clarified in future research.

**SUMMARY**

The functional relation between the olfactory bulb and the mesencephalic reticular formation (RF) was studied in curarized rabbits. The results obtained in the acute experiment were confirmed in the chronic experiment.

1. High frequency stimulation in the RF suppressed the intrinsic and the induced waves of the olfactory bulb. Peripheral arousal stimuli were sometimes effective in causing a similar changes in the olfactory activity.

2. The excitable spots suppressing the activity of the bulb were distributed
diffusely in the RF. They were also found to extend in the hypothalamus.

3. RF stimulation failed to modify the negative wave potential of about 40 msec duration evoked in the bulb by electrical stimulation of the olfactory mucosa.

4. Complete destruction of the olfactory mucosa and section of the anterior commissure were unable to influence the suppression of the olfactory activity due to RF stimulation. On the other hand, neuronal isolation of the bulb abolished the reticular influence.

5. In contrast to the neocortical EEG, the olfactory activity failed to show a release phenomenon upon acute destruction of the RF.

6. In the chronic experiment peripheral arousal stimuli were found to suppress the intrinsic activity of the olfactory bulb in the same manner as in the acute experiment.

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REFERENCES


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