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STUDIES ON THE MECHANISM OF THE EFFECT OF
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STUDIES ON THE MECHANISM OF THE EFFECT OF IONIZING RADIATIONS ON THE OLFATORY SYSTEM

Abstract

In the experiments described below the response of single olfactory bulb neurons to x-irradiation and beta irradiation has been studied, using anesthetized rats and rabbits. In one experiment with rats the response of olfactory bulb neurons was studied in relation to the intensity and duration of x-irradiation. In this study it has been shown that (a) the frequency of firing of an olfactory bulb neuron is a function of the logarithm of the dose rate, (b) there is an inverse relation between the intensity and the duration of x-irradiation required to bring a particular olfactory bulb neuron to a threshold state, (c) the threshold dose for the most responsive units is about 5 milliroentgens, (d) the threshold dose rate for the most responsive units is about 10 milliroentgens per second, (e) the minimum response latency is no less than approximately 125 milliseconds.

In work carried out on anesthetized rabbits the olfactory epithelium was exposed and the response of single olfactory bulb neurons to both x-irradiation and beta-irradiation was studied. This work has demonstrated that beta-irradiation of the olfactory epithelium does evoke responses of olfactory bulb neurons whereas beta-irradiation of the olfactory bulb itself does not. Furthermore, the population of cells which responds to beta rays appears to be identical to that responsive to x-rays.

In other work in which tracheotomized rats are being used, dry air or air saturated with water vapor is being perfused through the nasal cavities of rats. Cells which respond to irradiation of the head thus far have failed to respond to irradiation of the air used to perfuse the nasal cavities.

Quantitative Relationships Between the Dose Rate and Duration of X-Irradiation and the Response of Olfactory Bulb Neurons

Introduction

Previous work on the response of olfactory bulb neurons to x-irradiation has been directed primarily toward an anatomical delineation of the site or sites which are sensitive to x-rays (Cooper and Kimeldorf, 1966; Cooper, *et al.*, 1966). In most of this work no attempt was made to systematically study the effects on the response of varying the x-ray strength or duration. But since an effect of x-rays on olfactory receptors was indicated, we felt that such studies should be undertaken to determine whether or not the resulting stimulus-response relationships would be typical of other sensory stimulus-response functions. Therefore, the present experiment was designed to characterize quantitatively the relationship between the responses of olfactory bulb neurons and such parameters as the intensity and duration of x-irradiation.

Procedures

Adult Wistar rats were used. The dorsal aspect of the olfactory bulb was surgically exposed and each animal was tracheotomized in order to eliminate the cyclic variations in olfactory unit activity associated with respiration. Each rat was placed in a stereotaxic instrument located within an electrically-shielded enclosure. Olfactory bulb unit activity was recorded with stainless steel microelectrodes and monitored with an oscilloscope and audio system. Permanent records, when desired, were made with a Honeywell Visicorder oscillograph. A Westinghouse x-ray machine operated at 250 KVP (15 ma; 1 mm Al and $\frac{1}{2}$ mm Cu filtration) was used to make all exposures. A specially constructed shutter which was attached to the x-ray machine allowed the presentation of x-rays for periods as brief as 40 milliseconds. A light mounted between the x-ray tube and the shutter activated a phototube when the shutter opened, thereby providing an accurate indication of the duration of exposure. The diameter of the shutter opening was 1.5 cm, sufficiently large for irradiation of that part of the rat's head containing the olfactory bulb and olfactory epithelium. The dose rate was varied by altering the distance between the x-ray tube and the rat and by manipulation of the tube milliamperage.

When searching for an olfactory bulb unit which was responsive to x-rays, long duration, high intensity exposures were employed (2 seconds; 1.5-2.0 R/second). After a responsive unit had been obtained, the duration of exposure was decreased stepwise, with recordings of unit activity made at each exposure. Then the distance between the animal and the x-ray tube was increased to provide a lower dose rate and the sequence of exposures of different duration repeated. The dose rates employed ranged from 0.005 R/second to 2.0 R/second and exposure durations from 40 milliseconds to 3 seconds.

Results and Discussion

Figure 1 shows the relationship between the firing rate of 5 olfactory bulb neurons (from 5 different rats) and the x-ray dose rate. For this figure all data are derived from exposures having durations of 2 to 3 seconds, which is sufficiently long for a unit to reach its maximum firing rate at any dose rate used. As can be seen in the figure, within limits the firing rate of these units is linearly related to the logarithm of the x-ray dose rate, a relation commonly encountered in sensory physiology. Garcia and coworkers (1963) previously showed that the percentage of rats which could be aroused from sleep by x-rays was a function of the dose rate, and they suggested that sensory stimulation might be involved. It is pertinent to mention this finding in the present context because subsequent research showed that the capacity of x-rays to arouse rats from sleep depends upon an intact olfactory system (Hull, et al., 1965; Cooper and Kimeldorf, 1965).

Figure 2 shows the interrelationship between stimulus duration and stimulus intensity at threshold for two different olfactory bulb units. Each plotted point represents the threshold dose rate for that particular duration. Thus, the shorter the x-ray stimulus duration the greater must be the dose rate to reach threshold. The Bunsen-Roscoe relation, which states that stimulus duration multiplied by stimulus strength is a constant for a given response magnitude (threshold response in the present instance), is approximately valid for these data, as can be seen from the figure.

The minimum response latency observed was about 125 milliseconds, which is in agreement with the figure previously reported (Cooper and Kimeldorf, 1966). Since exposures of 40-50 milliseconds duration are capable of evoking a response it is apparent that a large amount of the time represented by the 125 milliseconds latency must be required by neural mechanisms within the olfactory system itself (conduction of action potentials from receptors to olfactory bulb, etc.).

FIGURE 1. THE FIRING RATE OF SINGLE OLFACTORY BULB NEURONS IN THE RAT AS A FUNCTION OF THE X-RAY EXPOSURE RATE. EACH PLOT REPRESENTS DATA OBTAINED FROM THE STUDY OF ONE NEURON.

FIGURE 2. THE MINIMUM DURATION OF X-RAY EXPOSURE REQUIRED TO PRODUCE A RESPONSE OF AN OLFACTORY BULB NEURON IS PLOTTED AGAINST THE ASSOCIATED X-RAY EXPOSURE RATE. DATA ARE FROM TWO UNITS STUDIED IN TWO DIFFERENT RATS.

Threshold dose rates ranged from 0.01 R/second to 0.5 R/second. Of 40 units tested thus far, 1 had a threshold of 0.01 R/second, 2 had thresholds of 0.025 R/second and the remainder had thresholds ranging from 0.05 to 0.5 R/second. The minimum dose rate of 0.01 R/second found in this study approaches the value (0.004 R/second) found by Morris (1966) in behavioral experiments. The minimum threshold dose found in the present experiment was approximately 5 milliroentgens, a value determined during the study of the one unit which responded at a dose rate of 0.01 R/second ($\frac{1}{2}$ -second exposure at a dose rate of 0.01 R/second).

The data presented above indicate that the response of olfactory bulb neurons to x-rays may be described by functions similar to those characteristic of other sensory responses. These data also provide further indirect support for the contention that responses of the olfactory system to x-irradiation is a consequence of an effect of the x-rays on the olfactory epithelium.

Responses of Olfactory Bulb Neurons to Beta Ray Exposure of the Olfactory Epithelium

Introduction

Thus far only x-rays have been employed in the investigation of the response of the olfactory system to ionizing radiation. Therefore, it was of interest, in and of itself, to determine whether or not beta rays would evoke responses of olfactory bulb neurons, although there were other substantial reasons for undertaking the experiment: Beta rays are easily collimated and the distance they penetrate tissue is quite limited and easily controllable. They therefore provide a means by which the olfactory epithelium or the olfactory bulb can be irradiated separately. Thus, it was reasoned, a response of olfactory bulb neurons to beta ray exposure of the olfactory epithelium, which did not occur when the olfactory epithelium was shielded or when the olfactory bulb alone was irradiated, would be almost conclusive evidence that irradiation produces responses of olfactory bulb neurons through an effect on the olfactory epithelium.

Procedures

Twenty adult New Zealand white rabbits have thus far been used. After anesthetization the trachea was cannulated, the dorsal aspect of the olfactory bulb surgically exposed, and the olfactory epithelium exposed on one side by removal of the nasal bone and underlying membrane. Usually the olfactory epithelium on the contralateral side was destroyed. Each rabbit was placed in a stereotaxic instrument located within an electrically-shielded cage. Microelectrode recordings of olfactory bulb unit activity were made before, during, and after both beta- and x-irradiation for each neuron tested. Sham exposures were made by placing a piece of sheet lead between the x-ray machine and the rabbit's head or, in the case of beta-irradiation, by interposing a heavy piece of cardboard between the beta source and the olfactory epithelium.

X-irradiation was carried out with a Westinghouse x-ray unit operated at 250 KVP (15 ma; 1 mm Al and $\frac{1}{2}$ mm Cu filtration). The dose rate was 1.5 R/second to the head only. The beta source was a strontium -yttrium medical applicator which delivered about 28 REP/second at the surface of the applicator. The active area of the applicator had a diameter of 5 mm. During exposures the active surface of the applicator was held 0.5 to 1 cm from the surface to be irradiated.

Results and Discussion

Thus far 153 neurons have been examined in 20 rabbits. Of these, 14 have responded both to x-irradiation and to beta irradiation of the olfactory epithelium. All units which responded to one type of irradiation also responded to the other type. Therefore, although the experiment is still in progress, it does appear that the same population of neurons is involved in the response to both beta- and x-irradiation and it is reasonable to assume that the same physical mechanisms operate to produce the responses to the two different types of radiation.

Figure 3a shows the response of an olfactory bulb neuron to x-irradiation of the head and 3b to beta-irradiation of the olfactory epithelium. Notice that the activity of two units is shown in these recordings, only one of which responded to radiation. In figure 3c a small piece of cardboard (1 cm diameter) was placed between the olfactory epithelium and the beta source, and as can be seen no response was evoked. Similarly, beta irradiation of the surface of the olfactory bulb produced no response, as shown in figure 3d; in this case the olfactory epithelium was not

FIGURE 3. RESPONSES OF AN OLFACTORY BULB NEURON IN THE RABBIT TO IONIZING RADIATION. (A) X-IRRADIATION OF THE HEAD, (B) BETA-IRRADIATION OF THE OLFACTORY EPITHELIUM, (C) FAILURE OF RESPONSES TO OCCUR WHEN A PIECE OF CARDBOARD WAS PLACED BETWEEN THE BETA APPLICATOR AND THE OLFACTORY EPITHELIUM, AND (D) FAILURE TO EVOKE A RESPONSE BY BETA-IRRADIATION OF THE OLFACTORY BULB. SOLID LINES BENEATH TRACINGS INDICATE PERIODS OF IRRADIATION.

shielded. Indeed, during the course of this experiment it has become evident that for beta rays to produce a response the active surface of the beta applicator must be pointed directly at the olfactory epithelium.

This experiment has so far demonstrated that beta-irradiation can produce responses of olfactory bulb neurons and, further, that irradiation of the olfactory epithelium appears to be a necessary condition for the response. In an extension of this work, attempts have also been made to cover the responsive area of the olfactory epithelium with a very thin sheet of plastic material in order to determine whether responses to beta-irradiation persist when air no longer has access to the epithelium. Under these circumstances one would not expect the response to occur if the radiation-produced stimulus is generated in ambient air. However, the olfactory epithelium in the rabbit has not proved to be satisfactory for this experiment because of its highly convoluted structure. Therefore we plan to pursue the same line of investigation with frogs, which have a simple, unconvoluted olfactory epithelium. This experiment is described in the grant continuation application.

Concluding Remarks

In the application for the research grant covered by this progress report, three main objectives were proposed for investigation: (a) a description of the quantitative relationships between the dose rate and duration of an x-ray stimulus and the firing rate of olfactory bulb neurons, (b) the determination of whether or not beta ray exposure of the olfactory epithelium would evoke responses of olfactory bulb neurons and if so whether or not the same population of cells is involved as in the response to x-irradiation, and (c) the determination of whether the population of cells responsive to x-irradiation is also more responsive to one class of odors than to others. Work toward the first two objectives, although still in progress, has yielded significant results and is nearing completion. Work toward the third objective has been discontinued as a consequence of recent experimental developments, and replaced by what is believed will be a more productive approach. As stated briefly above, the original approach consisted essentially of comparing the response of

olfactory bulb neurons (those units which respond to radiation) to different classes of odors, with the expectation that those cells which respond to radiation would also be more responsive to one class of odors than to others, thus allowing one to make an inference regarding the approximate nature of the radiation-produced stimulus. However, it is now believed that the first step toward this objective has been taken in behavioral studies which have demonstrated that ozone in ambient air selectively masks the ability of rats to detect x-rays (Gasteiger and Helling, 1966). This clearly suggests that the olfactory stimulus produced by ionizing radiation is, if not ozone itself, similar to ozone.

In a previous experiment (Cooper and Kimeldorf, 1966) data were obtained which showed that, if the olfactory bulb and epithelium were shielded, x-irradiation of the body (this includes air in the lungs, some of which surely reached the olfactory epithelium during expiration) or the anterior part of the nose did not produce responses of olfactory bulb units. Nevertheless, in view of the results of the behavioral experiment mentioned above, it was deemed necessary specifically to test the hypothesis that irradiation of air flowing through the nasal cavities could cause a response of olfactory bulb units. To this end we are using anesthetized, tracheotomized rats in which the nasal cavities are perfused with air at a flow rate of about 100 ml/minute. After obtaining a cell responsive to irradiation of the whole animal, the rat is shielded with a sheet of lead and the air flowing through the perfusion system is irradiated at a dose rate of 2.5 R/second. In 20 units examined so far no responses of olfactory bulb units have been obtained as a result of irradiating the air used to perfuse the nasal cavities - regardless of whether dry air or air saturated with water vapor was used. These experiments are still in progress.

The next phase of our program, described in detail in the grant continuation application, will consist of experiments designed to study the effects of varied oxygen concentration on the response of olfactory bulb neurons to x-rays, with irradiation, in turn, of both the head of the animal and the gas used to perfuse the nasal cavities. These experiments will provide information concerning the importance of oxygen in the response and will serve as the final test of the hypothesis that irradiation of ambient gas produces the stimulus to the olfactory system.

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