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

PROGRESS REPORT

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

Abstract

The effects of x-rays on the olfactory system were examined in two different experiments. In one, x-rays, delivered to the head in anesthetized, tracheotomized rats, were employed as an olfactory stimulus. The response to x-ray of single neurons in the olfactory bulb were studied as a function of the oxygen concentration of gas used to perfuse the nasal cavities. The experiment is still in progress, but the results obtained thus far support the hypothesis that the stimulation of the olfactory system by x-ray is an indirect effect within the olfactory mucosa, one which probably is potentiated by the presence of oxygen. These conclusions are based on the following data: (a) Response of olfactory bulb units is essentially unchanged as a consequence of altering the oxygen concentration over the range from 1 to 100 percent. (b) When pure nitrogen (0 percent oxygen) is the perfusion gas the response is clearly reduced. (c) Irradiation of gas entering the nasal cavities does not produce responses.

In the other experiment the effects of high-dose x-irradiation of the rostral head region on olfactory response to odor was examined in anesthetized rabbits. The results unequivocally support the following statements: (a) The large sinusoidal potentials recorded from the surface of the olfactory bulb ("induced waves") as a result of strong odor stimulation are completely abolished by x-ray doses in the 50-70 KR range. (b) The responses of single olfactory bulb units to odor may persist for some time after doses just sufficient to abolish the induced waves, but they too are abolished acutely by small additional doses of x-ray. (c) The failure of olfactory bulb response to odor is primarily a result of damage within the bulb itself rather than to an effect on the receptor apparatus, since the gross electrical response of the olfactory mucosa (electro-olfactogram) and single receptor responses to odor can still be obtained after x-ray doses as great as 95 KR.

General Introduction

Previous research has shown that some neurons in the olfactory bulb respond when rats, cats, dogs, and rabbits are exposed to x-rays (Cooper and Kimeldorf, 1966, 1967).

Evidence that this effect results from an action of the radiation exerted on the olfactory receptor epithelium was presented in the previous Progress Report under this AEC contract. Specifically, it was shown in one experiment that the response to x-rays can be described quantitatively by functions typical of many normal sensory stimulus-response relationships. In another experiment beta rays, which had not been previously employed in this type of research, were shown to be effective olfactory stimuli only if the beta source was focused directly on the olfactory epithelium. This material was presented at the annual meeting of the Radiation Research Society in April, 1968 (Cooper, 1968a). Since the results of the two experiments were clearly complementary, they were incorporated into one paper, which is now in press (Cooper, 1968b). Reprints are not yet available, therefore the manuscript is included here as Appendix I and may be referred to for further details.

The data discussed above implicate an effect of ionizing radiation on olfactory receptors. But the questions remained of precisely what the radiation-produced stimulus actually is and where it is produced. In one experiment described in this Progress Report an initial attack on these problems was made. The purpose of the experiment was to determine whether the concentration of oxygen in gas flowing through the nasal cavities influences the strength of response of olfactory bulb neurons to x-rays, and whether intense irradiation of air entering the nasal cavities is capable of producing a response. This experiment is nearing completion and a final manuscript will be ready within three months.

Since there was no experimental data available concerning the effects of high-dose irradiation on the olfactory response to odors, a new line of investigation was begun this year which was aimed at obtaining reliable information of this nature. The details of the first experiment in this series are given below and a manuscript for publication is now in preparation.

Response of Olfactory Bulb Neurons to X-Ray as a Function of the Oxygen Concentration of Gas Within the Nasal Cavities

Most olfactory bulb neurons do not respond to ionizing radiation, even though they do respond to weak odor stimulation (Cooper and Kimeldorf, 1966). This tends to indicate that x-rays do not have a direct action on the olfactory system,

for if some indiscriminate, generalized, direct action on receptors or nerve fibers occurred one would expect many more of the olfactory bulb neurons to respond. In fact, however, there appears to be considerable specificity in the response to radiation, which led us to suppose that an indirect effect on the olfactory system is involved -- such as the production of an intermediary substance like ozone or hydrogen peroxide which then stimulates the olfactory receptors (Cooper et al., 1966). Support for this line of reasoning is given by the finding that ozone was reported to be capable of specifically masking the ability of rats to detect x-rays, suggesting that the animals may respond to ozone or an ozone-like substance produced by radiation (Gasteiger and Helling, 1966). In any case, if an intermediate radiation-produced substance is involved in the response of the olfactory system to x-ray, it is most probable that oxygen is of primary importance in the reaction. And if oxygen is indeed involved then one should be able to demonstrate a dependence of the response magnitude (firing rate of olfactory bulb neurons) on the concentration of oxygen in gas present within the nasal cavities. The primary purpose of this experiment, then, was to study the responses of olfactory bulb neurons to x-irradiation during the perfusion through the nasal cavities of gas containing different concentrations of oxygen.

Procedures and Equipment

The activity of single olfactory bulb neurons was studied in adult anesthetized Wistar rats. The trachea of each rat was severed and both ends cannulated. The animal breathed room air through the caudal cannula and the rostral cannula was connected to a gas perfusion system. Rats were placed in a stereotaxic machine located within an electrically-shielded enclosure. Spike potentials of olfactory bulb units were picked up by tungsten microelectrodes, amplified, and led, in parallel, to an oscilloscope and to a high speed oscillograph recorder (Honeywell Visicorder). A Westinghouse x-ray machine operated at 250 KVP was used to make all exposures.

The gas perfusion system permitted the perfusion of either odorized or unodorized gas containing any desired concentration of oxygen. Pressurized tanks of nitrogen and oxygen were used, with the desired percentages of the two gases being obtained through a system of flowmeters and needle valves. For precise and continuous monitoring of the oxygen concentration an oxygen meter was included in the gas perfusion system. A gas flow rate of 250-300 ml/minute was used.

The experimental procedure consisted of, first, finding a unit which responded to x-irradiation at a dose rate of about 60 R/minute, with 10% oxygen flowing through the nasal cavities. Then the x-ray dose rate was decreased until the response magnitude (spike frequency) was approximately half that observed at the higher dose rate. Thereafter the dose rate was held constant during the testing of any one neuron. All exposures were three seconds long. In some cases the oxygen concentration was changed stepwise starting at 0% and progressing to 100%, in other cases the reverse procedure was followed; and in still other cases the oxygen concentration was selected randomly. At each oxygen concentration used, the response of the neuron to x-irradiation of the head was recorded. In each case the gas was allowed to flow for 5 minutes before the testing of x-ray response began.

Results and Discussion

Figure 1 shows the response to x-ray of four different olfactory bulb units as a function of the oxygen concentration of gas used to perfuse the nasal cavities. The results are representative of all data collected thus far. There are three salient features to be pointed out in this figure: (1) The firing rate of a unit in response to x-ray appears to be essentially the same for all oxygen concentrations in the 1-100 percent range although (2) it is possible that some depression of response may occur at 1 percent oxygen (units 5 and 9), and (3) the firing rate falls dramatically when oxygen is completely excluded from the perfusion gas. Similar results have been obtained with ten other units.

Since variation of oxygen concentration of the perfusion gas over a wide range does not appreciably alter the response, and since responses are still obtained during the perfusion of 100 percent nitrogen, it would appear that radiation effects on gaseous oxygen within the nasal cavities probably are not involved. However, because responses are evidently greatly depressed by 0 percent oxygen we cannot discount its importance. For instance, small amounts of oxygen dissolved in the olfactory mucosa may potentiate the radiation effect. In other words, the curves shown in figure 1 may be "oxygen effect" curves such as those which have been obtained in a variety of other experiments (Gray, et al., 1953; Deschner and Gray, 1959; Evans and Neary, 1959; Hutchinson, 1960). All of these curves have in common an almost flat segment over a large oxygen concentration range and a rapidly-falling segment at low oxygen concentrations. Unfortunately, interpretation of data from the present experiment is complicated by the fact that oxygen concentrations of 1 percent or less depress the normal resting activity and slightly depress the response to

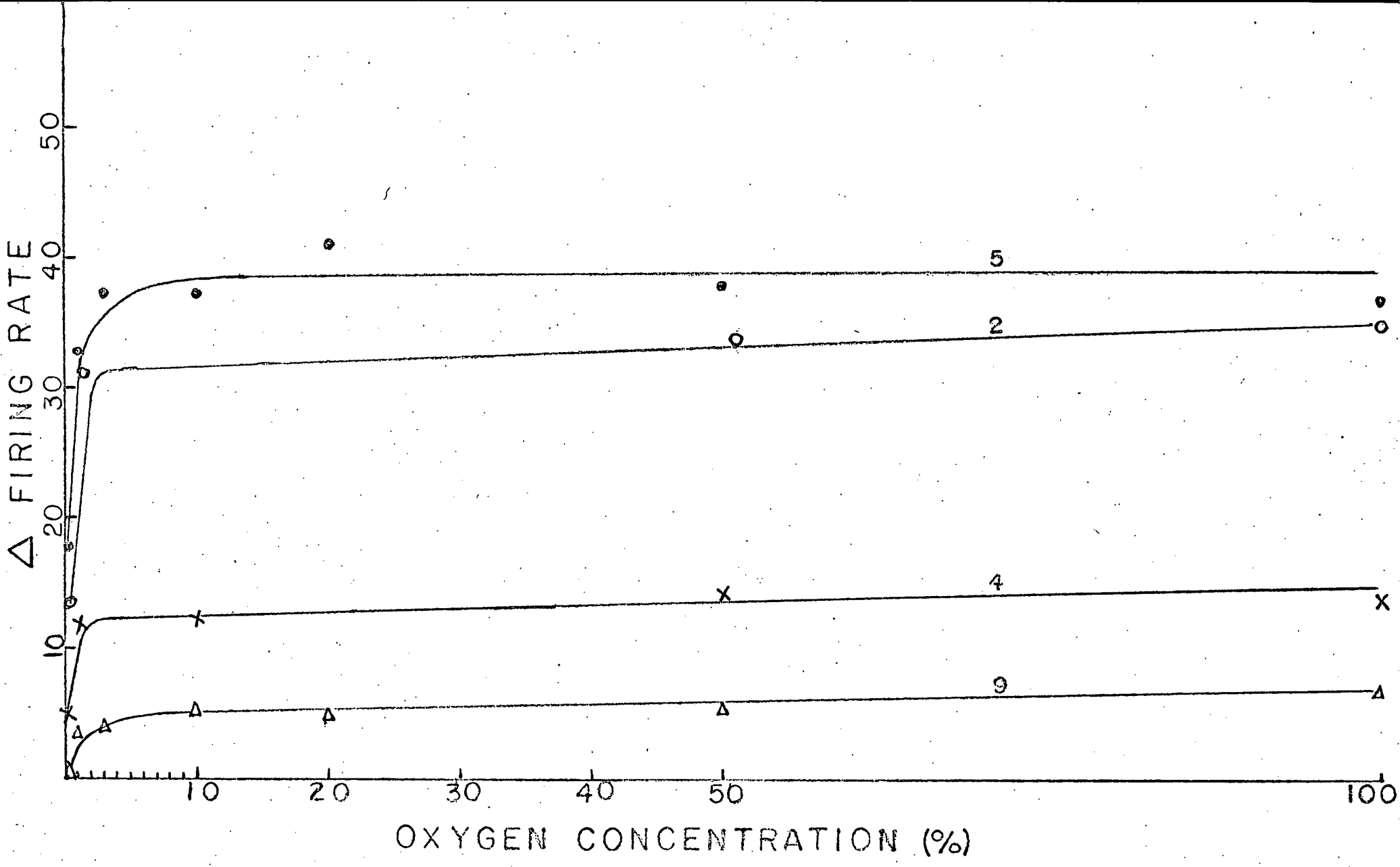

Figure 1. The change in firing rate of single olfactory bulb neurons in response to x-rays as a function of the oxygen concentration of gas perfused through the nasal cavities. The numbers 5, 2, 4, and 9 are identification numbers for these units. Each curve represents data from one unit and each point is the mean of 2 observations.

odor in the tortoise olfactory nerve (Tucker, 1963). Oxygen-free gas has also been observed to depress the resting activity of single olfactory bulb neurons in the rabbit (Cooper, et al., 1966). Therefore the depression of response to x-rays may be due to a decrease in receptor sensitivity rather than to a decreased participation of oxygen in the radiation reaction. All that can be said at present is that the control experiments which have been carried out thus far indicate that responses to odor are less affected by the absence of oxygen than the response to radiation, thus suggesting, at least, that a decrease in receptor sensitivity cannot account for the diminution of response to x-rays. However, additional work must be done before any final conclusion can be drawn.

With 8 of the units studied thus far the gas perfusion system was disconnected and a suction pump connected to the tracheal cannula to draw air into the nares and through the nasal passages at a flow rate of about 300 ml/min. The olfactory bulb and mucosa were shielded with a lead block, leaving the rostral 8-10 mm of the rat's nose unshielded. The tip of the nose and ambient air were irradiated at 2 R/sec. No response whatever has been observed under these conditions. Therefore, irradiation of ambient air or air within the nasal passages apparently is not responsible for the olfactory response to radiation.

In summary, the response of the olfactory system to x-rays does not appear to be a consequence of an effect of radiation on gaseous oxygen since the response is little affected by large changes in oxygen concentration, and because responses may still be obtained during the nasal perfusion of oxygen-free gas. Secondly, it is unlikely that an effect of radiation on any component of air is important, whether before or after it enters the nasal passages, because irradiation of ambient air produces no response. Thirdly, since response magnitude is relatively stable over large changes in oxygen concentration but drops precipitously at a concentration of 1 percent or less, it is probable that oxygen facilitates the response.

Effects of High-Dose X-Irradiation on Olfactory Function

There have been no previous experimental studies devoted to the effects of high doses of ionizing radiation on olfactory response to odors. A few reports are available based upon exposures of humans (Kimeldorf and Hunt, 1965) but the information is scanty and imprecise, and provides no basis for drawing reasonably sound conclusions concerning the

deleterious effects of ionizing radiation on olfaction. Since it is now known that the olfactory system is extremely sensitive to low doses of ionizing radiation, it was deemed desirable to have some information concerning the effects of high doses on the system. Because there was no information available on the subject, the experiments reported below were exploratory in nature and were designed to answer two questions: (1) What dose of x-rays is required to abolish olfactory bulb response to strong odor stimulation under acute conditions, and (2) What is the primary site of damage; in other words, is response failure due primarily to damage within the olfactory bulb or in the olfactory mucosa.

Procedures and Equipment

Twenty male New Zealand rabbits weighing 1.9-2.6 kg were used. All animals were anesthetized with ethyl carbamate (Urethan). Small holes were drilled through the bone overlying the olfactory bulb, and copper or silver ball electrodes were secured in place on the dorsal surface of the bulb with dental acrylic.

The head of the animal was placed in a head holder during irradiation. The head caudal to the olfactory bulb and the body were shielded with a 5 mm-thick lead sheet. X-rays were generated by a Westinghouse machine operated at 250 KVP and 15 ma, with 1 mm Al and 0.5 mm Cu filtration. Dose rates, measured in air at the dorsal surface of the head, ranged from 450 to 500 R/min.

The electrodes implanted on the surface of the olfactory bulb were used to record the "induced waves" -- large sinusoidal potential oscillations having a frequency of 30-40 cycles/sec in the anesthetized rabbit. They are evoked maximally by strong olfactory stimuli such as cigarette smoke, which was used in the present experiment. Irradiation was carried out in 5 KR steps, and the induced wave response to a puff of cigarette smoke was recorded immediately after each increment in dose. Irradiation was continued at least until the induced wave was abolished.

Since the induced waves are only a gross indicator of olfactory bulb response, in many experiments tungsten microelectrodes were used to explore the olfactory bulb after the induced wave was abolished. As was to be expected, responses of single neurons to odor persisted after x-ray doses sufficient to abolish the induced waves. Therefore, in some experiments irradiation in excess of that required to abolish the induced waves was given in order to be certain that unit responses were also abolished.

In an effort to localize the site of action of x-rays in producing olfactory failure, recordings were made from the olfactory mucosa in 5 animals which had received x-rays in doses sufficient to completely abolish all unit and induced wave response to odor. Recordings from the mucosa were of two kinds: the large slow surface potential of the mucosa (electro-olfactogram or EOG) was recorded with saline-soaked cotton wick electrodes or saline-filled capillary electrodes having a tip diameter of about 100 microns. Recordings were also made from single receptors or nerve fibers in the mucosa using tungsten microelectrodes or capillary microelectrodes filled with an indium alloy. While recording from the mucosa, odor stimuli consisted of small puffs of cigarette smoke or puffs of air from a wash bottle containing amyl acetate.

Results and Discussion

Figure 2 shows the induced wave response to cigarette smoke recorded during a typical experiment. Below a dose of 40 KR no effects were apparent in any of the experiments. After doses of 40 to 50 KR a reduction in induced wave amplitude was commonly observed, as shown in Figure 2-D. Then the rhythmic nature of the response was lost, leaving it small and irregular as shown in Figure 2-E. Finally, at doses between 50 and 70 KR, the induced wave response was completely abolished.

In 4 animals microelectrode recordings of unit acitivity in the olfactory bulb was begun as soon as possible after delivering an x-ray dose just sufficient to abolish the induced waves. Many units were still capable of responding to odor, as shown in Figure 3. It was clear, however, that the unit activity of the bulb was depressed both in terms of the resting activity encountered as well as in the number of units which responded to odor. With two of these animals (3 and 6) a period of 2 to 3 hours was allowed to elapse and then the unit activity was re-examined. No responses to odor were obtained; therefore doses of x-rays large enough to abolish induced waves will also abolish all unit responses within a few hours.

Five animals were given doses of x-rays large enough to abolish the induced waves and all unit responses of the olfactory bulb within the period of irradiation. Then the EOG and mucosal unit potentials were examined. Records obtained in one animal are presented in Figure 4. The induced wave was abolished at a dose of 60 KR. An additional 25 KR was given, after which a microelectrode examination of olfactory bulb unit response was attempted. No response to odor was obtained. However, records taken from the olfactory mucosa show clearly that the receptors were still functional. Figure 4-E and 4-F show, respectively, the EOG response and

the response of a unit (probably a nerve fiber or nerve bundle) to amyl acetate stimulation. Figure 5 again shows that the EOG and mucosal unit potentials are still present after abolition of the induced wave and olfactory bulb unit response. In this instance 70 KR was required to abolish the induced wave, after which an additional 5 KR was given. The EOG and mucosal unit potentials survived the largest dose given in this experiment, 95 KR.

Table I summarizes all the data except that from control animals, which will be discussed separately. The first 6 animals listed are those in which only the induced wave was studied. In column B "dose" is the accumulated dose required to abolish the induced wave. "Time" in the table always refers to elapsed time starting at the beginning of irradiation. In column C are listed the times that the first olfactory bulb unit response was obtained following a dose of x-rays just sufficient to abolish the induced wave, for the four animals studied in this way. Animal 12 was then given an additional 20 KR dose, bringing the total dose to 85 KR (see column D). Beginning at 430 minutes attempts to obtain olfactory bulb unit responses to odor were unsuccessful. At approximately 480 minutes both the EOG and mucosal unit responses were obtained (column E). For animals 11, 13, and 17 the radiation dose and associated time required to abolish the induced wave was noted and entered in column B, but irradiation was continued until the dose entered in column D had been accumulated. No olfactory bulb unit responses to odor could be obtained, but in all cases the EOG and mucosal unit responses persisted. In animal 20 the induced wave was abolished by 65 KR but the mucosal unit response and EOG were still observed after giving the animal an additional 30 KR.

As can be seen from the table, the dose required to abolish the induced wave ranged from 50 to 70 KR. In only one case did the response fail at 50 KR, however.

In 5 unirradiated control animals the induced wave remained essentially unchanged during periods of time equal to those used with irradiated animals.

Since the olfactory system responds to extremely small doses of ionizing radiation, it might have been expected that deleterious effects on the system would be observed at relatively low doses. The data of this experiment clearly refute such an idea. The dose required to abolish odor response in these experiments is approximately 10^7 to 10^8 times the dose necessary for a response of the system to x-ray (Morris, 1966; Cooper, 1968 B).

TABLE I

X-RAY DOSES REQUIRED TO ABOLISH INDUCED WAVES AND OLFACTORY BULB UNIT RESPONSES

A	B		C	D		E	
	Abolition of induced wave		Olfactory bulb unit response present	Abolition of olfactory bulb unit response		EOG and mucosal unit response present	
Animal Number	Dose (KR)	Time (min)	Time (min)	Dose (KR)	Time (min)	Dose (KR)	Time (min)
1	55	130					
5	70	240					
8	60	165					
15	65	230					
16	50	165					
18	65	200					
21	70	190					
2	55	130	240				
3	60	190	260				
6	65	140	200				
12	65	210	315	85	430	85	480
11	60	210		70	300	70	360
13	60	200		85	360	85	420
17	70	240		75	300	75	360
20	65	150				95	360

It should be remembered, however, that in this experiment only the acute effects of high-dose radiation were examined, and only intense olfactory stimulation was employed. Studies of the chronic effects of lower doses as well as studies of possible alterations in receptor threshold must be undertaken if our understanding of the harmful effects of ionizing radiation on the olfactory system is to be enlarged.

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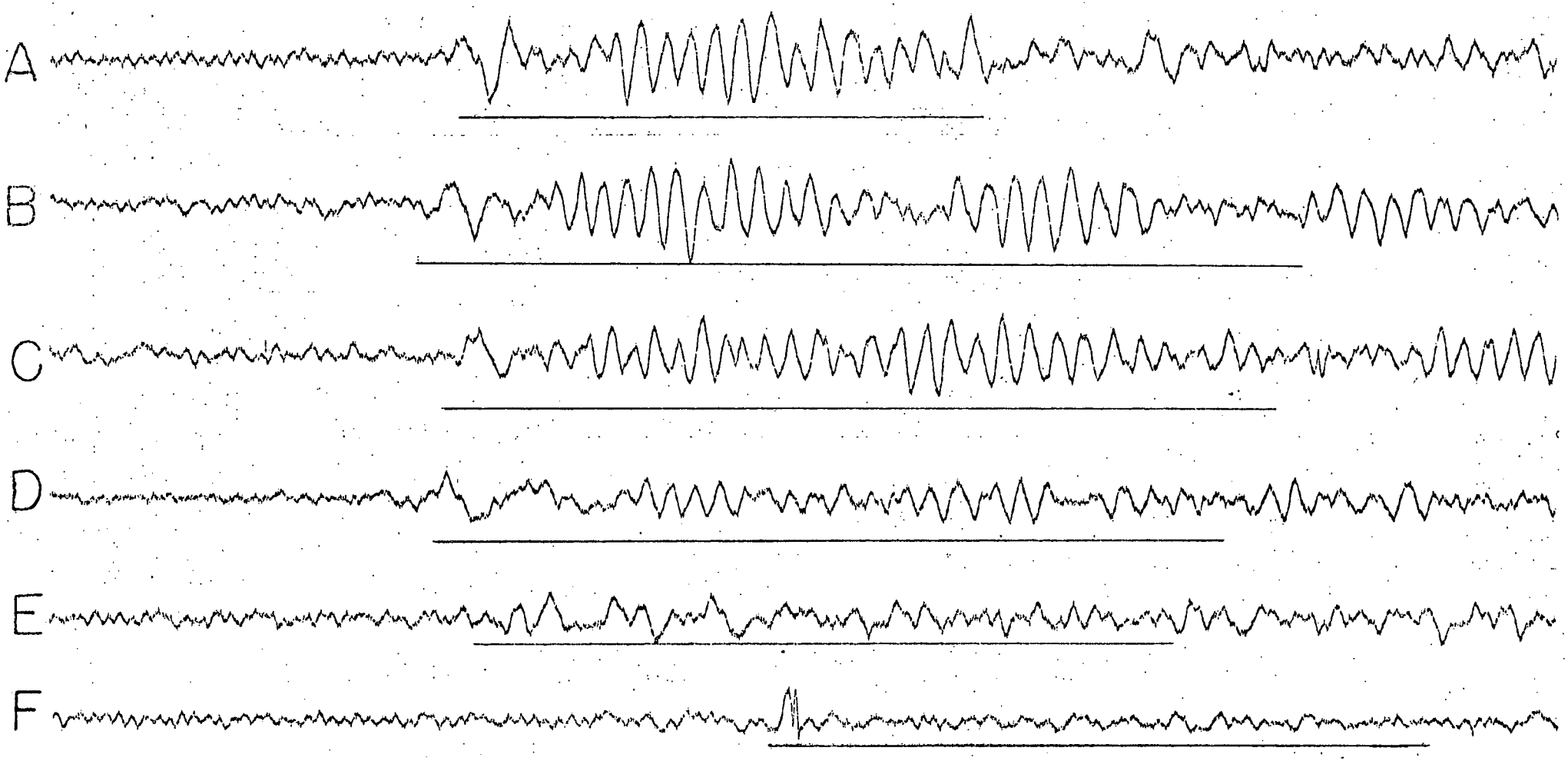

Figure 2. The induced wave response to cigarette smoke in rabbit 8. (A) control, (B) after 15 KR, (C) after 30 KR, (D) after 45 KR, (E) after 50 KR, and (F) after 60 KR. Straight lines under each record indicate odor stimulation in Figures 2-5.

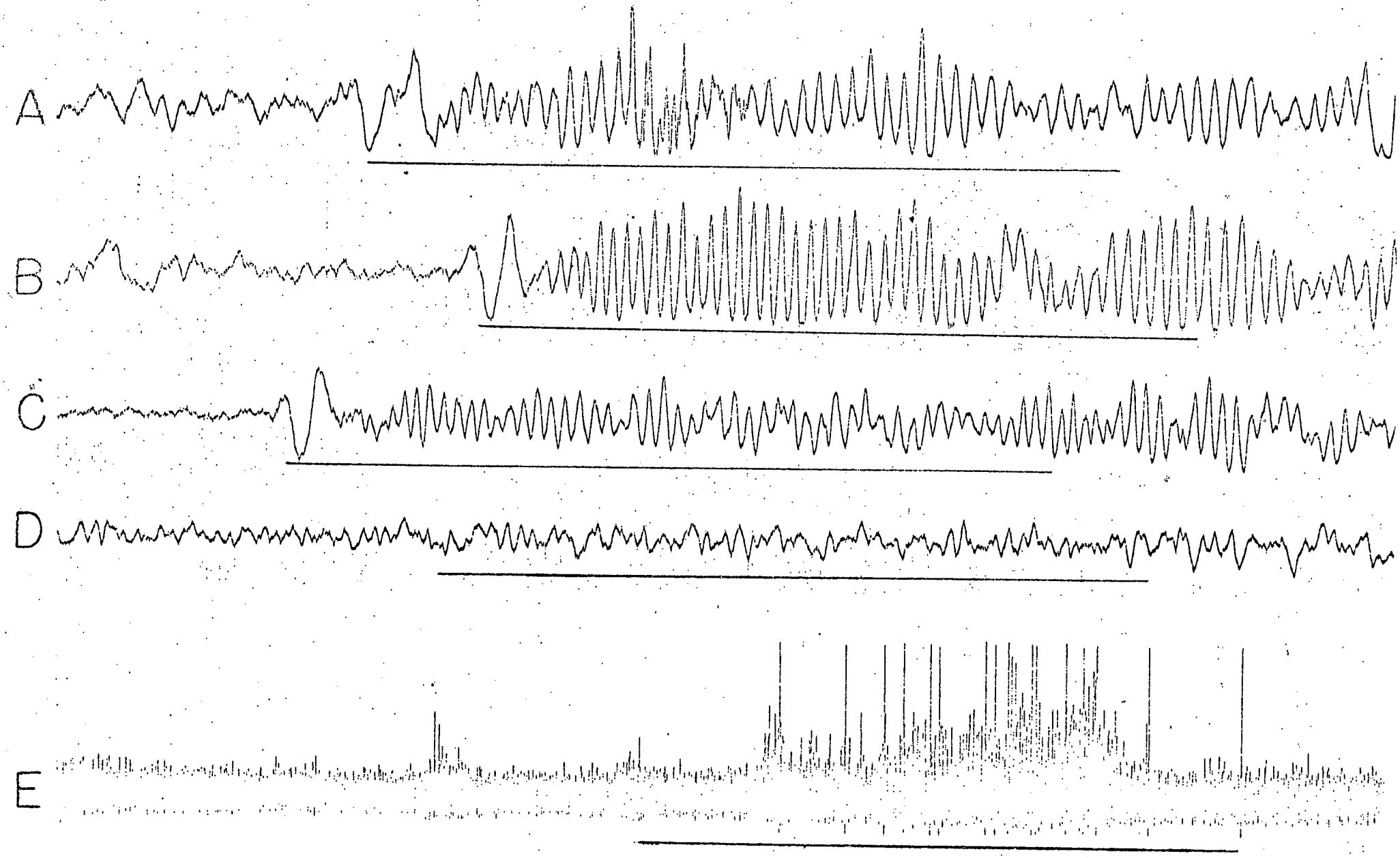

Figure 3. Olfactory bulb unit response to odor following abolition of induced waves. Induced waves: (A) control, (B) after 30 KR, (C) after 55 KR, (D) after 65 KR. (E) is the unit response to cigarette smoke; the activity of two or more units can be seen. Rabbit 6.

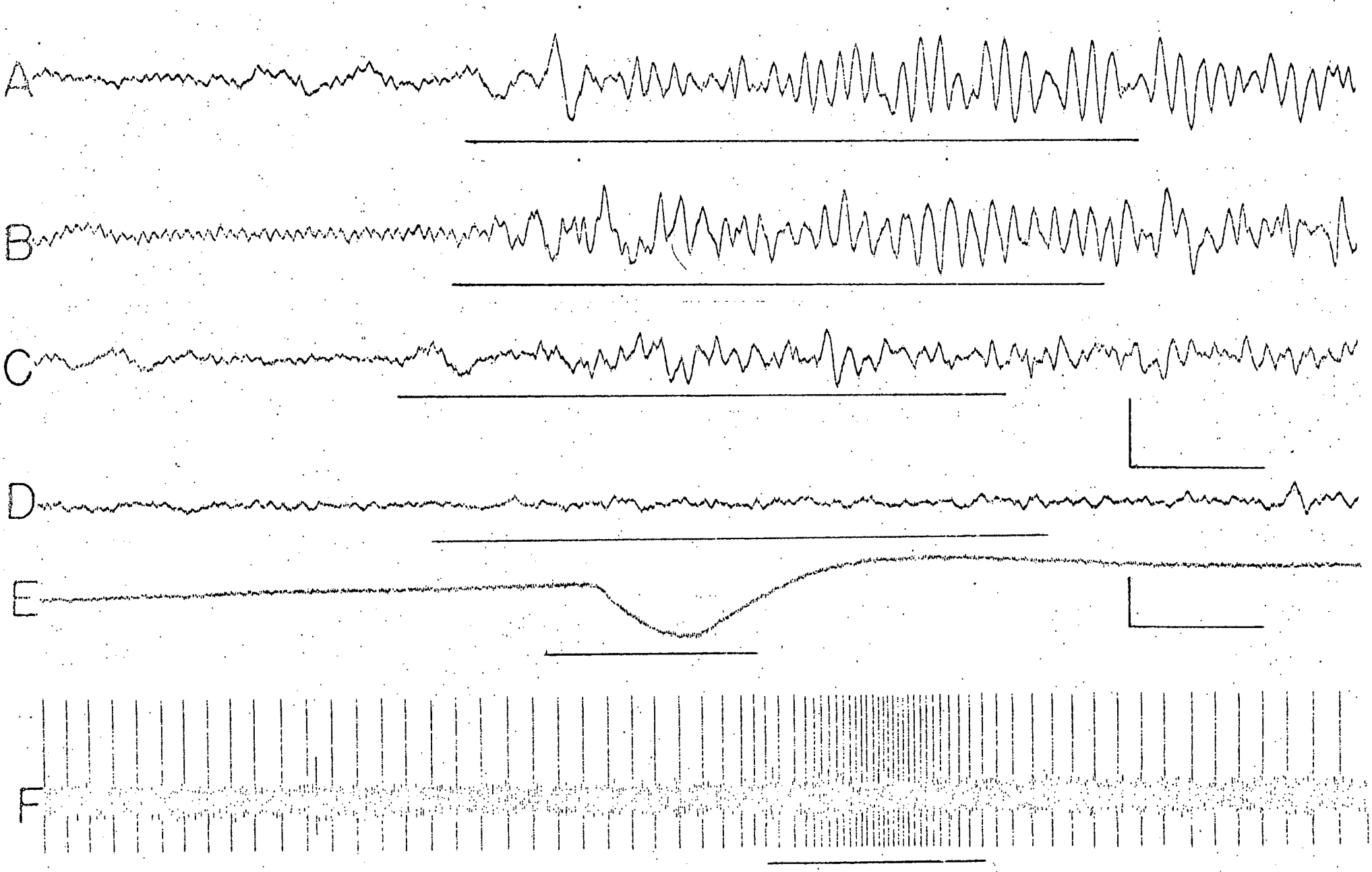

Figure 4. Electro-olfactogram (E) and mucosal unit response (F) to amyl acetate following complete abolition of induced waves and olfactory bulb unit response. Induced waves: (A) control, (B) after 25 KR, (C) after 45 KR, and (D) after 60 KR. Rabbit 13. Calibrations: Induced wave - 0.5 mv and 200 msec; EOG - 0.5 mv and 1 sec; unit potential - 0.1 mv and 1 sec.

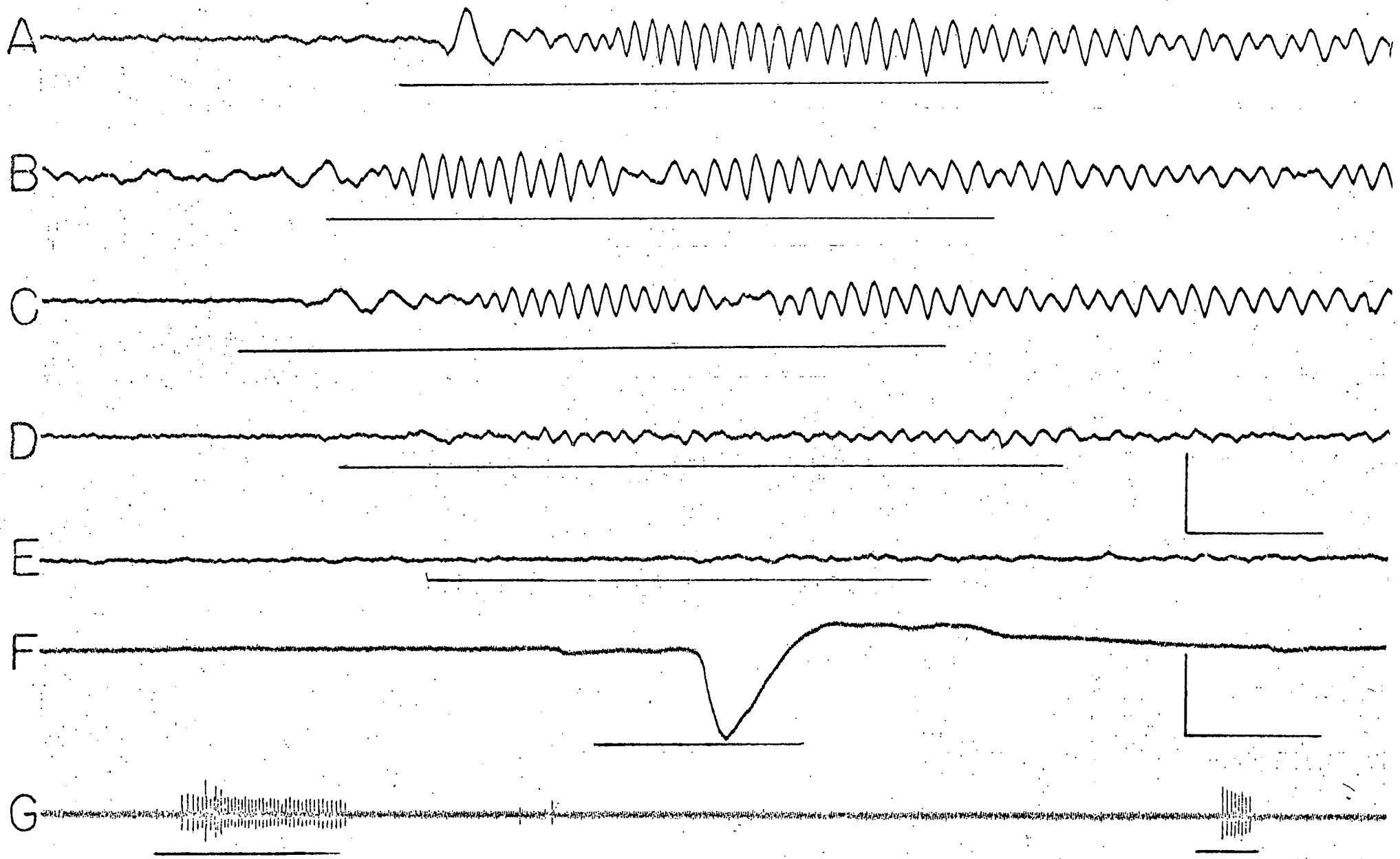

Figure 5. Electro-olfactogram (F) and mucosal unit response (G) to cigarette smoke after abolition of induced waves and olfactory bulb unit response. Induced waves: (A) control, (B) after 30 KR, (C) after 55 KR, (D) after 65 KR, (E) after 70 KR. The unit potentials in (G) are believed to be from a receptor. Calibrations: Induced waves - 1 mv and 200 msec; EOG - 1 mv and 1 sec; unit potentials - 0.1 mv and 1 sec. Rabbit 17.

APPENDIX I

RECEPTOR ORIGIN OF THE OLFACTORY BULB RESPONSE TO IONIZING RADIATION[1]

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Running Head: ORIGIN OF OLFACTORY BULB RESPONSE TO RADIATION

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ABSTRACT

Responses of olfactory bulb neurons to 250 KVP x-rays were examined in anesthetized rats in relation to the intensity and duration of irradiation. In anesthetized rabbits the olfactory epithelium was surgically exposed and the responses of olfactory bulb units to both 250 KVP x-rays and beta rays (strontium-yttrium) were studied. In rats it was found that (a) the firing rate of an olfactory bulb unit is a linear function of the logarithm of the dose rate, (b) for a threshold response the product of dose rate and exposure duration is a constant, (c) for the most responsive units the threshold dose rate is 10 mR/sec and the threshold dose 5 mR, and (d) the minimum response latency is approximately 125 msec. In rabbits beta-irradiation produced responses of olfactory bulb neurons if, and only if, the beam was focused directly upon the olfactory epithelium. The population of units responsive to beta-irradiation was identical to that responsive to x-irradiation. These data indicate that responses of the olfactory system to ionizing radiation are of receptor origin.

Index words: x-ray Beta-ray olfaction rat rabbit

INTRODUCTION

It has long been known that ionizing radiation is an effective visual stimulus under appropriate conditions (12), but only within the last few years has ionizing radiation been shown to be a potent stimulus to the olfactory system. In 1962 it was found that rats could be aroused from sleep by briefly exposing them to x-rays (10) and, in subsequent work, it was demonstrated that an effect of x-rays on the olfactory system was responsible for this arousal reaction (1, 7, 9). In behavioral experiments with rats in which x-ray was used as a conditional stimulus, it has also been shown that the olfactory system mediates x-ray detection (5).

Since this work was done other experiments have provided strong, though by no means conclusive, evidence that the effects of x-rays on the olfactory system are exerted in the periphery, probably at the receptor level. Microelectrode recordings have shown that some olfactory bulb neurons respond, usually with an increase in firing rate, when rats, cats, dogs, and rabbits are exposed to x-rays (2, 3). However, such responses evidently were not the result of an effect of the x-rays on the olfactory bulb itself since they were abolished in tracheotomized rats by the nasal perfusion of physiological saline or alcohol (2) and, in most cases, were depressed or abolished by the nasal perfusion of nitrogen or argon (4). A peripheral locus of action of x-rays was also suggested by behavioral experiments in which ozone in ambient air was shown to mask selectively the response

to x-rays (8).

In some of the experiments reported in this paper in which the olfactory system in rabbits received localized beta-irradiation it is shown that the responses of olfactory bulb neurons result from some influence of the ionizing radiation exerted on the olfactory epithelium. Experiments with rats show that the response varies quantitatively with certain radiation parameters in ways characteristic of a sensory response.

METHODS

Rat Experiments. Twenty-six adult Wistar rats anesthetized with ethyl carbamate (Urethan) were used. The dorsal aspect of the olfactory bulb was surgically exposed and each animal was tracheotomized to eliminate cyclic variations in olfactory bulb unit activity associated with respiration. Unit activity was recorded with stainless steel microelectrodes and monitored with an oscilloscope and audio system. Permanent records were made with a Honeywell Visicorder oscillograph. A Westinghouse x-ray machine operated at 250 KVP (1 mm Al and $\frac{1}{2}$ mm Cu filtration) was used to make all exposures. A 5 mm-thick lead plate having a 1.5 cm opening was secured to the x-ray portal. The shutter was comprised of a piece of lead 5 mm thick and 2 cm in diameter, which was cemented to the plunger of a solenoid. The shutter was spring-loaded so that it remained in the closed position unless the solenoid was activated. One side of the power line to the solenoid was broken and connected to normally-open contacts

of a relay which could be closed for any desired period of time by one of the sweep circuit outputs ("+ gate") of an oscilloscope. A light mounted between the x-ray tube and the shutter activated a phototube when the shutter was open, thereby providing an accurate indication of exposure duration. On the basis of the phototube output and associated dosimetry, the values for exposure duration were estimated to be accurate to within about $\pm$ 10 percent. The dose rate was varied by altering the distance between the x-ray tube and the rat and by manipulating the tube milliamperage. The x-ray machine was oriented so that the olfactory apparatus would be included in all exposures.

When searching for an olfactory bulb unit responsive to x-rays, exposures of long duration and high intensity were employed (1-2 sec; 1-1.5 R/sec). With responsive units a series of exposures of different duration were made with the dose rate held constant. Then another dose rate was selected and another series of exposures made. The dose rates employed ranged from 0.005 R/sec to 2.0 R/sec and the exposure duration from 50 msec to 2 sec, although no attempt was made to vary either the dose rate or the exposure duration continuously over the ranges used. The dose rates usually employed were 1.5, 1.0, 0.7, 0.5, 0.3, 0.2, 0.1, 0.05, 0.03, 0.01, and 0.005 R/sec; the exact values varied slightly from day to day. The exposure durations usually used were 2.0, 1.0, 0.7, 0.5, 0.2, 0.1, and 0.05 sec. Both the dose rate and the exposure duration were varied according to ascending, descending, and

random sequences although it was rarely possible to use all of these sequences for any one unit. In determining thresholds, no more precision was attempted than that which could be achieved using the standard exposure values given above. For instance, if a unit responded with a consistent latency and frequency to 3 out of 5 exposures at a dose rate of 0.2 R/sec but failed to respond at 0.1 R/sec, then the threshold dose rate was taken as 0.2 R/sec.

Rabbit Experiments. Twenty-two adult New Zealand white rabbits anesthetized with Urethan were used. The trachea was cannulated, the dorsal surface of the olfactory bulb surgically exposed, and the olfactory epithelium exposed on one side by the removal of the nasal bone and underlying membrane. Usually the olfactory epithelium on the contralateral side was destroyed. Microelectrode recordings of olfactory bulb unit activity were made as described above, 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 animal.

X-irradiation was carried out with a Westinghouse x-ray machine operated at 250 KVP ($\frac{1}{2}$ mm Cu and 1 mm Al filtration). The dose rate was 1.5 R/sec and only the head of the animal was irradiated. The beta source was a strontium-yttrium medical applicator 5 mm in diameter which delivered about 28 REP/sec at the surface of the applicator. During irradiation the

applicator was held about 1 cm from the surface to be irradiated.

RESULTS AND DISCUSSION

Unit Responses to X-Rays in Rats. Figure 1 shows the relationship between the firing rate of 5 olfactory bulb neurons (from 5 different rats) and the x-ray dose rate. All data for this figure were derived from exposures having a duration of about 2 sec, which is sufficiently long for a unit to reach its maximum firing rate at any dose rate used. Each point represents the number of action potentials recorded during a 1 sec interval beginning 0.5 sec after the shutter was opened. As can be seen, within limits the firing rate of these units is linearly related to the logarithm of the dose rate, a relation commonly encountered in sensory physiology (Weber-Fechner function). Similar data were obtained with six other units. Hunt and Kimeldorf (11) showed that the percentage of rats which could be aroused from sleep by x-rays is a function of the dose rate, and Garcia, et al (6) obtained similar results using electroencephalographic desynchronization as an index of arousal. These observations are pertinent in the present context because subsequent research showed that the capacity of x-rays to produce an electroencephalographic arousal reaction depends upon an intact olfactory system (1, 9).

(Figure 1)

The relationship between stimulus duration and stimulus intensity for a threshold response is shown for two different olfactory bulb units in Fig. 2. Each plotted point represents the threshold dose rate for that particular exposure duration.

Thus, the shorter the x-ray stimulus duration the greater must be the dose rate for a threshold response. Data from three other units yielded similar results. For these data the product of stimulus intensity and stimulus duration is approximately equal to a constant which again is a classical sensory function (Bunsen-Roscoe relation).

(Figure 2)

Three units were studied during the presentation of stimuli at different frequencies using a dose rate of 1.5 R/sec and a stimulus duration of 50 msec. One of these units showed distinct and separate responses to stimuli up to a frequency of 7/sec, at which point "flicker fusion" occurred. Stimulus frequency was not studied systematically but these observations are mentioned to illustrate the good control over olfactory response which can be achieved using ionizing radiation as a stimulus.

Threshold dose rate determinations were made with 30 units for the purpose of establishing an approximate minimum rate for the most responsive units. Twenty-six of the units tested had thresholds ranging from 0.05 to 0.3 R/sec, but 3 responded at 0.03 R/sec and 1 at 0.01 R/sec. Under the conditions of this experiment, then, a dose rate of 0.01 R/sec is taken as the threshold of the most responsive units, a value which approaches the threshold rate of 0.004 R/sec found by Morris in behavioral experiments (13). The threshold dose for the most responsive units was found to be approximately 0.005 R, which was determined

during the study of the one unit which responded at a dose rate of 0.01 R/sec. The minimum response latency observed at the highest dose rates used was about 125 msec, which is in agreement with the figure previously reported (2).

Although the locations of the electrode tips were not verified histologically in these experiments, the amplitude and shape of the spike potentials and the micrometer depth recordings indicated that most of the responsive units studied were in the external plexiform layer. In previous work on rats (2) associated histological observations showed no rostrocaudal or dorsoventral localization of responsive units although most were located within the external plexiform layer.

Unit Responses to X- and Beta-Rays in Rabbits. Figure 3a shows the response of an olfactory bulb unit to x-irradiation of the head and 3b to localized beta-irradiation of the olfactory epithelium. The activity of two or more units is shown in these records, the only one of interest here being that which produced the largest spikes. It is apparent from these records that beta rays, which have not previously been shown to affect the olfactory system, are effective olfactory stimuli. In Fig. 3c beta-irradiation of the olfactory epithelium is shown to be ineffective when a piece of cardboard 1 cm in diameter and about 3 mm thick was placed between the beta source and the olfactory epithelium. Beta-irradiation of the surface of the olfactory bulb also produced no responses as can be seen in Fig. 3d. In ten units so studied,

no response to beta irradiation was ever obtained when the olfactory epithelium was shielded or as a consequence of olfactory bulb irradiation. These data make it evident that for beta rays to produce a response the active surface of the beta applicator must be pointed directly at the olfactory epithelium.

(Figure 3)

A total of 180 units were tested for response to both x- and beta-irradiation. Twenty units responded and all those which responded to one type of radiation also responded to the other type. Therefore, it appears that the same population of neurons is involved in the response to both x- and beta-irradiation.

The results of both the rat and rabbit experiments reported in this paper support the hypothesis that ionizing radiation acts as a stimulus to olfactory receptors. As pointed out previously, only a relatively small proportion of olfactory bulb neurons respond to ionizing radiation (2, 3) and it therefore seems probable that a specific population of receptors is involved. If this interpretation is valid then it is most likely that the effective stimulus is some radiation-produced chemical substance such as hydrogen peroxide or ozone which is generated in the vicinity of the receptors and acts in a normal physiological fashion.

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FOOTNOTE

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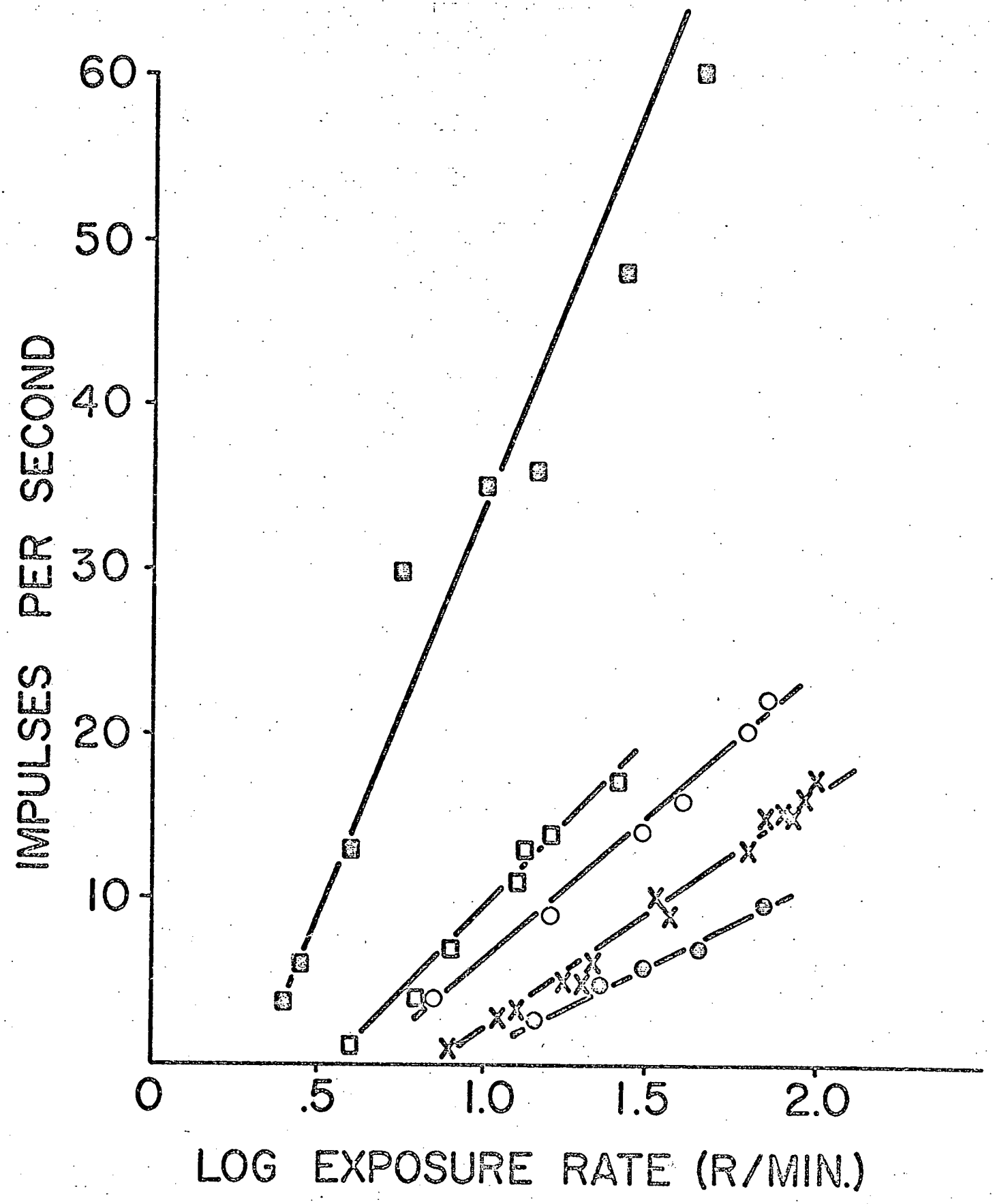
FIGURE CAPTIONS

Fig. 1. Firing rate of olfactory bulb neurons in the rat as a function of the x-ray dose rate. Each of the lines was visually fitted to data obtained from the study of one neuron and each of the points is a raw datum.

Fig. 2. The minimum duration of x-ray exposure required to produce a threshold response is plotted against the associated x-ray dose rate for two different olfactory bulb units. Each point is a raw datum.

Fig. 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 response when a piece of cardboard was placed between the beta source and the olfactory epithelium. D: failure of response to beta-irradiation of the olfactory bulb. Solid lines beneath tracings indicate periods of irradiation.


60
50
40
30
20
10
IMPULSES PER SECOND
0
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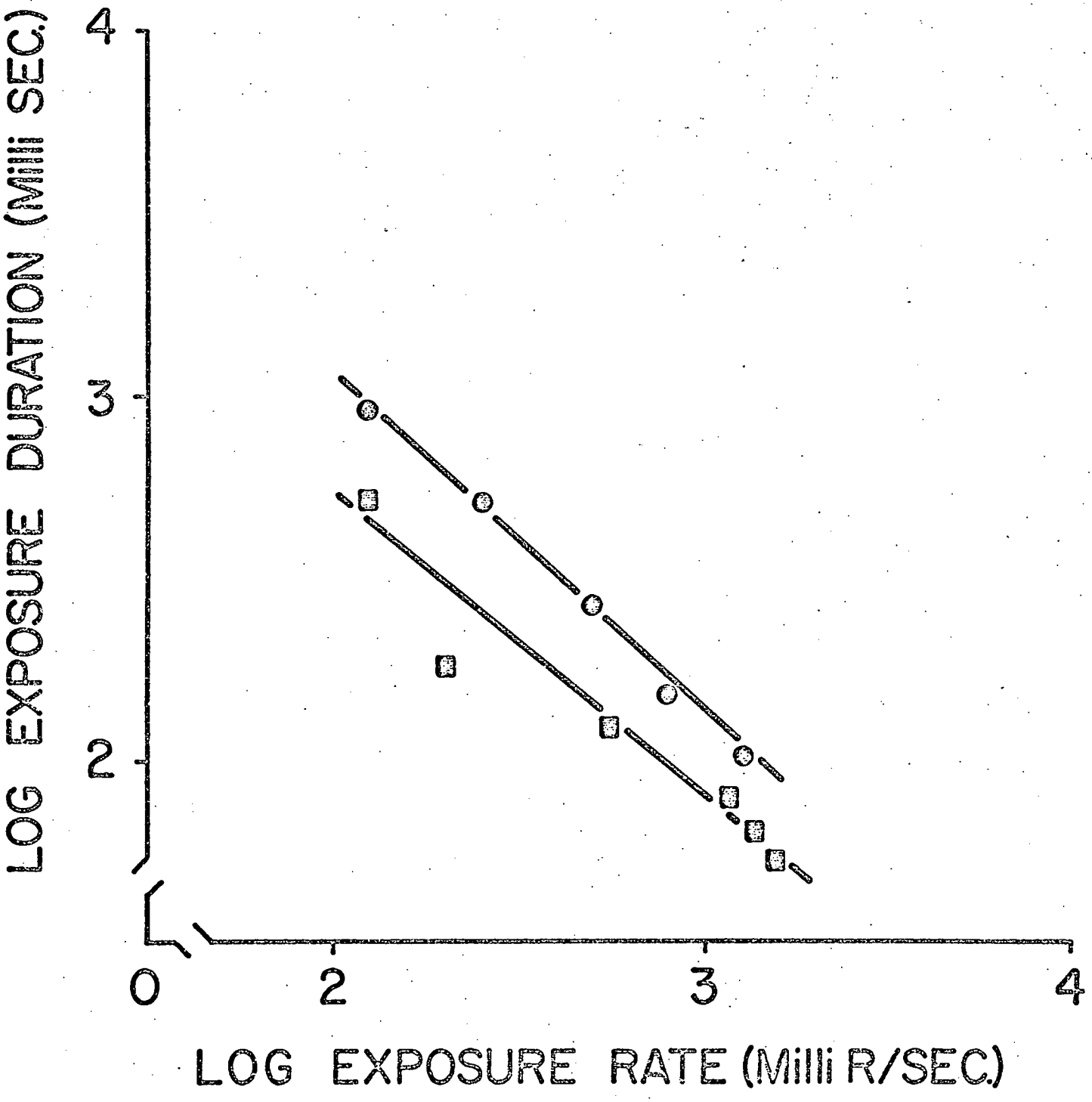
1.0
1.5
2.0
LOG EXPOSURE RATE (R/MIN.)

Fig. 1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4
3
2
0
2
3
4
LOG EXPOSURE RATE (Milli R/SEC.)

Fig. 2

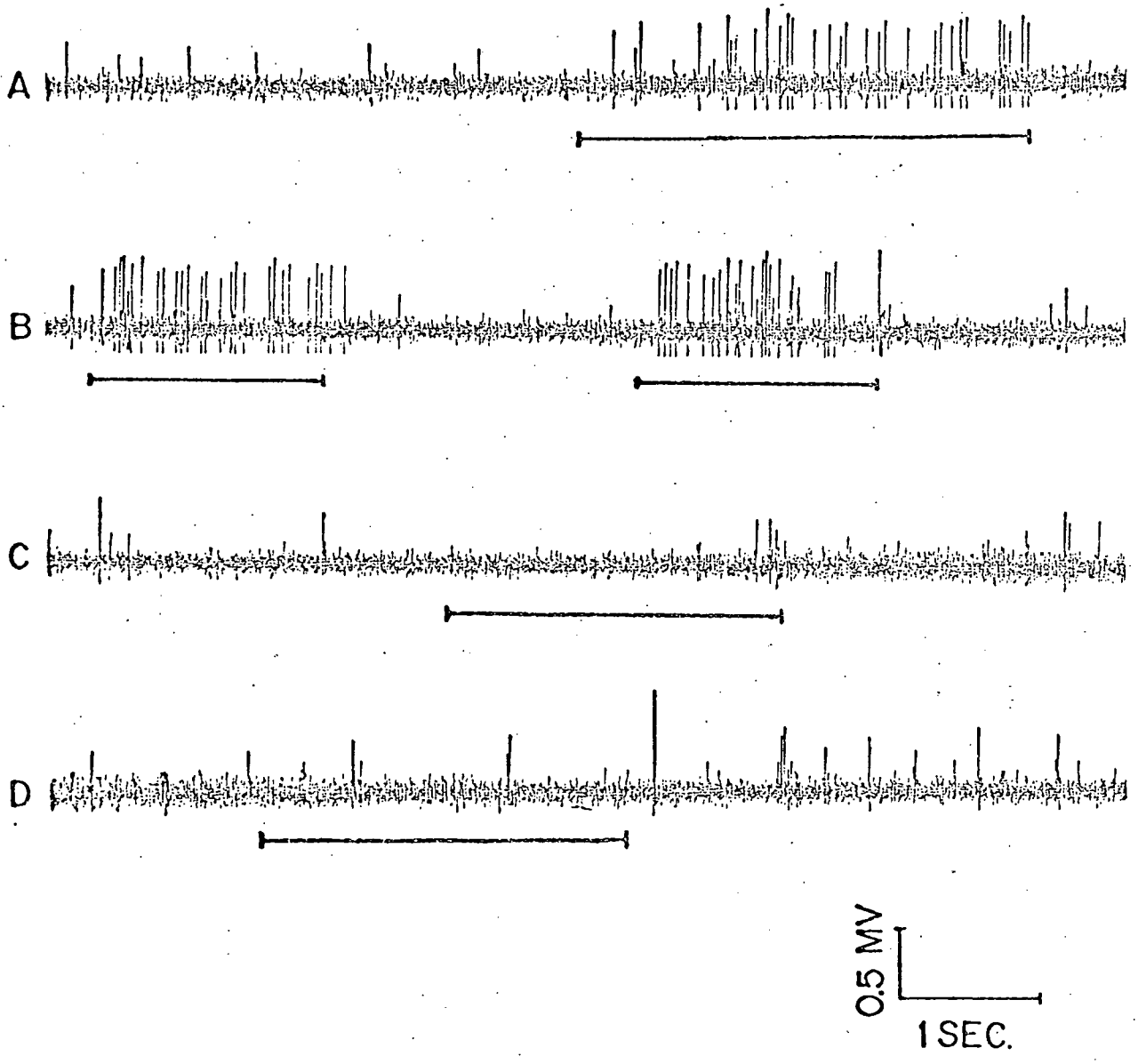

Fig. 3