mental pollutants may vary considerably among species of marine planktonic algae. The greater resistance of the one estuarine species, *Dunaliella*, in comparison with the susceptibility of coastal and open-ocean forms like *Skeletonema* and *Coccolithus* and the extreme sensitivity of *Cyclotella*, may reflect the need for adaptability in the face of more unstable conditions close to hand. Although chlorinated hydrocarbons may not be universally toxic to all species, they may exert a dramatic influence on the succession and dominance of individual forms (*10*).

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## Response of Olfactory Bulb Neurons to X-rays as a Function of Nasal Oxygen Concentration

Abstract. *Responses of single olfactory bulb neurons in rats to x-irradiation of the head were examined during the perfusion through the nasal cavities of gas containing various concentrations of oxygen. Response to x-ray was unchanged with oxygen concentrations of from 100 percent down to about 5 percent. Progressively larger response decrements were observed with oxygen concentrations of 2 percent and less.*

The olfactory systems of a variety of animal species, including rats, cats, dogs, rabbits, pigeons, and monkeys, are responsive to ionizing radiation (*1*). Several lines of evidence indicate that the effects of radiation are exerted on olfactory receptors rather than on the olfactory nerves or bulb. For example, in tracheotomized rats breathing room air, responses of olfactory bulb neurons are eliminated or depressed by the nasal perfusion of argon or nitrogen (*2*). Secondly, transection of the olfactory nerves eliminates behavioral responses of pigeons to x-rays (*3*). Finally, localized beta-irradiation of the olfactory mucosa in rabbits produces responses of olfactory bulb neurons (*4*). We have proposed that the effect of ionizing radiation on the olfactory system is an indirect one involving the production of some chemical intermediate such as ozone or hydrogen peroxide (*2*, *4*). Some support for this hypothesis was provided by behavioral experiments with rats. In one (*5*) it was shown that ambient ozone selectively masks the ability of rats to detect x-rays. In another (*6*) conditional responses established with ozone as the conditional stimulus readily transferred to an x-ray conditional stimulus, whereas conditional responses established initially to an odor other than ozone (ethyl butyrate) did not transfer to the x-ray stimulus. The data of this experiment show that the concentration of oxygen within the nasal cavities is an important determinant of the olfactory response to ionizing radiation.

The activity of single olfactory bulb neurons was recorded in adult Wistar rats anesthetized with ethyl carbamate (urethan). The trachea of each rat was severed and both ends were cannulated. The animal breathed room air through the caudal cannula. The rostral cannula was connected to a gas perfusion system which permitted the nasal perfusion of either odorized or unodorized gas containing any concentration of oxygen. Pressurized tanks of nitrogen and oxygen were used, with the desired percentages of the two gases obtained through a system of flowmeters and needle valves. An oxygen meter was included in the perfusion line. The flow rate of gas was 250 to 300 ml/min. The x-ray dose rate was 90 roentgen/min measured in air at the skin surface. All exposures were 3 seconds long and were made with a Westinghouse x-ray machine operated at 250 kv peak and 15 ma, with filtration through 1 mm of aluminum and ½ mm of copper. In most experiments maxi-

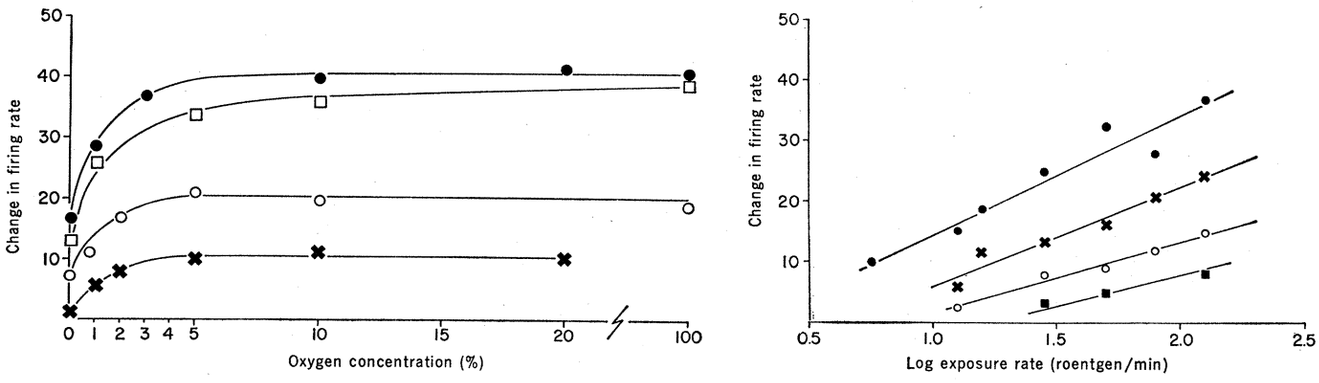


Fig. 1 (left). Change in firing rate of four different olfactory bulb neurons in response to x-ray as a function of nasal oxygen concentration. Change in firing rate is the number of action potentials per second during 3 seconds of irradiation minus the number of action potentials per second for the 3-second period preceding the irradiation. Each point is the mean of two observations. Fig. 2 (right). Change in firing rate of one olfactory bulb neuron as a function of x-ray exposure rate and nasal oxygen concentration. Each point is the mean of two observations. Oxygen concentrations were: 10 percent (filled circles); 2 percent (X's); 1 percent (open circles); 0.5 percent (squares).

mum oxygen concentrations of 10 to 20 percent were used since preliminary experiments had shown that neuronal responses were not affected by increases in oxygen concentration above 10 percent. An attempt was made to test all olfactory bulb units at oxygen concentrations of 10, 5, 3, 2, 1, and 0 percent, but in many cases the cell could not be held long enough to examine satisfactorily its response at all of these concentrations.

Figure 1 shows the responses of four olfactory bulb units to x-ray as a function of nasal oxygen concentration. The results are representative of all data collected on 17 different units. In all cases, the response to a standard x-ray exposure was the same for all oxygen concentrations above 10 percent. In a few instances there appeared to be a slight depression of response with 5 percent oxygen, but clear response depression occurred only at oxygen concentrations of 2 percent or less. With 2 percent oxygen the mean response of 12 neurons studied was 70 percent of the control response with 10 percent oxygen. At an oxygen concentration of 1 percent the mean response for 13 units was 55 percent of the mean control response for these same units. A mean response amounting to only 30 percent of control values was obtained at 0 percent oxygen (15 units). The curves shown in Fig. 1 closely resemble the "oxygen effect" curves which have been obtained in a variety of other radiobiological experiments (*7*).

With three units the x-ray dose rate, as well as the oxygen concentration, was systematically varied. Representative results obtained from one of these cells are shown in Fig. 2. At any given oxygen concentration the response is approximately a linear function of the logarithm of the dose rate. As the oxygen concentration is reduced, the response function shifts to the right with no appreciable change in slope.

Depressions of normal resting activity of olfactory nerve fibers and olfactory bulb neurons, as well as very small depressions of olfactory nerve response to odor, have been observed with nasal oxygen concentrations of 1 percent or less (*2*, *8*). Therefore, to control for the effects of lowered oxygen concentrations on normal receptor function I examined the response of ten of the units described above to amyl acetate (1/100 of vapor saturation at 30°C). Responses to odor stimulation remained essentially constant at all oxygen concentrations.

We have proposed that ionizing radiation affects the olfactory system through the production of a hypothetical stimulus substance in or near the olfactory mucosa. If this is so, then the data of Figs. 1 and 2 suggest that, within limits, the concentration of this substance is linearly related to the dose rate. As shown in Fig. 2, the neuronal response is approximately a linear function of the logarithm of the dose rate. Since this semilogarithmic relationship between stimulus intensity and response is a rather common property of peripheral sensory systems, we may therefore tentatively conclude that there might be a direct relationship between the dose rate and any hypothetical agent mediating stimulation produced by the ionizing radiation. The data of Figs. 1 and 2 also suggest that the concentration of this hypothetical stimulus substance is positively related to nasal oxygen concentration at very low concentrations. The presence of oxygen within the nasal cavities apparently is not essential. However, the oxygen derived from vascular sources might be quite important or even essential if, as seems probable, ionizing radiation produces an effective olfactory stimulus within the olfactory mucosa itself. I infer from these data that ozone or some other short-lived oxidizing substance probably is the agent mediating olfactory stimulation by ionizing radiation.

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## Spontaneous Rhythmic Contraction of Separated Heart Muscle Cells

Abstract. *Muscle cells that contract spontaneously and rhythmically can be obtained from adult mouse myocardium. Contractions are observed immediately after homogenization in a solution that is ionically similar to intracellular fluid. Contraction frequency varies directly with temperature and decreases as a function of time after homogenization. At 16°C rhythmic relaxation and contraction occur for about 20 minutes. Contractions are dependent on the presence of adenosine triphosphate in the homogenization medium.*

Although partially differentiated rat heart muscle cells in culture show spontaneous contractile activity (*1*) and separated adult muscle fibers can be induced to contract (*2*), spontaneous rhythmic contraction and relaxation by separated mature mammalian cardiac muscle fibers has not been reported. I now describe a method for producing such preparations and comment on the relation of spontaneous contractile behavior to the components of the medium.

When mouse hearts are homogenized in simulated extracellular fluid (*3*) or in a buffered isotonic sucrose solution, microscopic examination reveals the presence of subcellular particles and dense clumps of cell fragments, intact cells, and groups of undissociated cells.

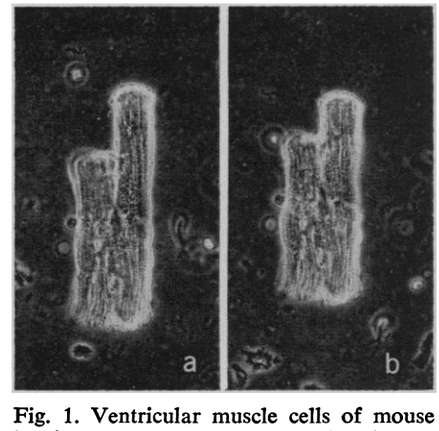


Fig. 1. Ventricular muscle cells of mouse beating spontaneously. Relaxed (a) and contracted (b) state of same cells. These photographs were taken with a high-intensity stroboscopic light source. The condenser was adjusted to be slightly eccentric, and a red filter was used in order to enhance the cross striations. The large size and irregular shapes of this muscle fragment indicate that it is composed of several cells. The contracted cells are about 15 percent shorter than the relaxed cells (both × 400).