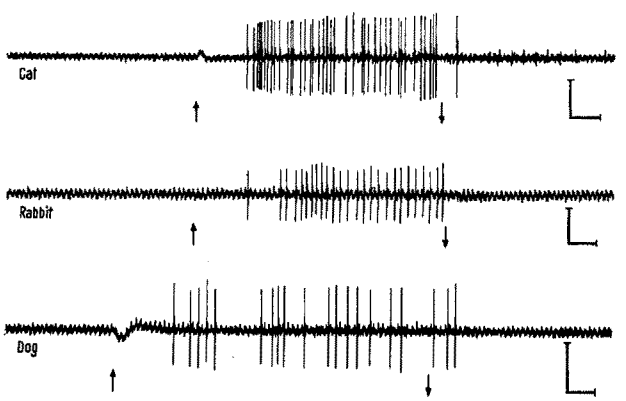
Responses of Single Neurons in the Olfactory Bulbs of Rabbits, Dogs, and Cats to X-Rays[1]

Brief exposure to X-rays has been shown to produce behavioral arousal[2,3] and electroencephalographic (EEG) desynchronization[4,5] in sleeping rats. GARCIA et al.[6] found that X-rays were more effective as a conditioned stimulus when a small-diameter X-ray beam passed through the region of the head containing the olfactory bulb and anterior brain than when the beam passed through other parts of the head. Both HULL et al.[7] and COOPER and KIMELDORF[8] reported that olfactory bulb ablations greatly diminished the number of sleeping rats showing EEG desynchronization in response to X-irradiation. COOPER and KIMELDORF[9] further showed that X-rays evoked responses in single neurons of the olfactory bulb in anesthetized rats. Since such responses could be abolished temporarily by the perfusion of saline through the nasal cavities, or permanently by alcohol perfusion, an effect of X-rays on olfactory receptors was implicated. This conclusion was reinforced by the report[10] that the perfusion of argon or nitrogen through the nasal passages of tracheotomized rats depressed or abolished the response of most olfactory bulb neurons to X-irradiation.

In such investigations as those cited above, the rat has been used almost exclusively. The present experiment was carried out to determine whether responses of single olfactory bulb neurons to X-rays could be obtained in rabbits, dogs, and cats. In addition to the inherent interest in obtaining an answer to this question, there was also a practical reason for undertaking the experiment. Since each species has its own advantages and disadvantages for experimental purposes, it was deemed desirable to have available a variety of species whose olfactory receptors had been proven capable of responding to X-rays, in order to facilitate further research into the mechanism underlying such responses.

Methods. 10 New Zealand white rabbits, 5 mongrel dogs, and 5 cats were used. All animals were adults, although their exact ages were unknown. Rabbits were anesthetized with 1.0 g/kg of urethan, given i.p.; dogs with 35 mg/kg of sodium pentobarbital i.v.; and cats with 30 mg/kg of sodium pentobarbital i.p. The dorsal surface of the olfactory bulb was surgically exposed in rabbits. In dogs and cats a dorsal approach to the olfactory bulb was made, but the bulb was not exposed to direct vision. During radiation exposure trials animals were placed, individually, in a stereotaxic instrument which was located within an electrically shielded cage. A Westinghouse X-ray machine (250 kVP; 15 mA; filtration, 1 mm Al and 0.5 mm Cu; half-value layer equivalent to 1.35 mm Cu) was oriented to pass a horizontal beam through the shielded cage. Whole-animal exposures were made in all cases. The dose rate, measured in air at the skin surface of the head, was 1.0 R/sec. The duration of exposure routinely used was about 2 sec, but varied from about 0.5 to 5.0 sec. Stainless steel microelectrodes, constructed by the method of GREEN[11], and having tip diameters of 0.5–2.0 μ, were used for making extracellular recordings from olfactory bulb neurons. A Grass *P*-6 preamplifier, with cathode follower, was used for amplifying the spike potentials. Graphic recordings of spike potentials were made with a Honeywell 906-C Visicorder oscillograph. An alteration in the discharge frequency of a neuron during X-ray exposure of at least 25%, repeated on at least 2 separate trials, was the arbitrary criterion for a response to irradiation. Sham irradiation trials were carried out by placing a $^1/_4$-inch-thick lead plate between the X-ray machine and the animal, and operating the X-ray unit in the usual manner.

Results and Discussion. The Figure shows the responses to irradiation of a single olfactory bulb neuron in each of the 3 species examined in this experiment. In each case the record represents a response to irradiation which is near the maximum response frequency observed for that species. As can be seen in the Figure, the spike frequency increases in order from the dog, through the rabbit, to the cat, which had the highest response frequency. It should be pointed out that the amount of data obtained in this study does not permit a rigorous quantitative statement regarding these response frequencies. Furthermore, the thickness of the skull and overlying tissues is different for the various species of animals used, so that the effective radiation exposure rate at the level of the olfactory receptors and olfactory bulb no doubt varied considerably between species; this may very well be responsible for the differences in response. However, within these limitations, it may be stated that the maximum increase in firing rate (spike frequency during irradiation minus the spike frequency before radiation) observed for the dog was 20–25 spikes/sec, for the rabbit 35–50 spikes/sec, and for the cat 50–70 spikes/sec. Increases in firing rate of 80–100 spikes/sec have been observed in the rat at a radiation exposure rate of 1 R/sec. The firing rate of 1 neuron in the dog and 2 neurons in the cat was depressed during irradiation.



The responses of single olfactory bulb neurons to X-irradiation in the cat, rabbit, and dog. In each record the arrow pointing upward indicates the beginning of irradiation and the arrow pointing downward the cessation of irradiation. Calibration: 1 mV and 100 msec.

[1] This study was supported through funds provided by the Bureau of Medicine and Surgery, U.S. Navy, and the Defense Atomic Support Agency. The opinions and assertions contained herein are those of the authors and are not to be construed as the official views of the Navy Department.

[2] E. L. HUNT and D. J. KIMELDORF, Science, N.Y. *137*, 857 (1962).
[3] E. L. HUNT and D. J. KIMELDORF, Radiat. Res. *21*, 91 (1964).
[4] J. GARCIA, N. A. BUCHWALD, G. BACH-Y-RITA, B. H. FEDER, and R. A. KOELLING, Science, N.Y. *140*, 289 (1963).
[5] G. P. COOPER and D. J. KIMELDORF, Science, N.Y. *143*, 1040 (1964).
[6] J. GARCIA, N. A. BUCHWALD, B. H. FEDER, R. A. KOELLING, and L. TEDROW, Science, N.Y. *144*, 1470 (1964).
[7] C. D. HULL, J. GARCIA, N. A. BUCHWALD, B. DUBROWSKY, and B. H. FEDER, Nature, Lond. *205*, 627 (1965).
[8] G. P. COOPER and D. J. KIMELDORF, Int. J. Radiat. Biol. *9*, 101 (1965).
[9] G. P. COOPER and D. J. KIMELDORF, Radiat. Res. *27*, 75 (1966).
[10] G. P. COOPER, D. J. KIMELDORF, and G. C. MCCORLEY, Radiat. Res., in press.
[11] J. D. GREEN, Nature, Lond. *182*, 962 (1958).

The Table shows the number of units tested in each species, and the number and percentage of such units which responded to irradiation. Here again, reservations concerning these values must be made because of the relatively small number of units studied. Also, no attempt was made to record from homologous areas of the olfactory bulb in the various species; sampling of units was random. It would appear, however, that a greater percentage of olfactory bulb neurons in the dog responded to irradiation as compared with the rabbit and cat. Previous work[9] resulted in percentages for the rat which are approximately the same as those observed for the rabbit and cat in this study.

All previous work on the response of olfactory bulb neurons to X-irradiation has been done on the rat. Similarly, rats have been used exclusively in behavioral studies which have demonstrated the ability of X-rays to arouse animals from sleep. With the exception of an experiment by TSYPIN and GRIGOR'YEV[12], in which rabbits were used, rats have been used in studies showing immediate EEG changes as a result of brief exposure to low-dose ionizing radiation. The present study demonstrates that olfactory bulb neurons of the dog, rabbit, and cat, as well as those of the rat, are capable of responding to X-irradiation. On the basis of previous work done on the rat[9,10], it is probable that these responses are the result of an effect of X-rays on olfactory receptors.

The total number of olfactory bulb neurons studied in the rabbit, dog, and cat are shown, along with the number and % of such units which responded to X-irradiation

	No. of neurons tested	No. of neurons responsive to X-rays	% of neurons responsive to X-rays
Cat	95	16	17
Rabbit	128	19	15
Dog	71	17	24

Zusammenfassung. Die durch Röntgenstrahlen (1 R/sec) erzeugten elektrischen Impulse einzelner Nervenzellen der Geruchszwiebel wurden in narkotisierten Katzen, Kaninchen und Hunden gemessen. Als typische Reaktion wurde eine kurzfristige Erhöhung der elektrischen Impulse gefunden. Somit sind die von Röntgenstrahlen ausgelösten elektrischen Impulse des Geruchsystems kein artspezifisches, sondern vielmehr ein allgemeines Phänomen.

G. P. COOPER and D. J. KIMELDORF

Physiology-Psychology Branch, U.S. Naval Radiological Defense Laboratory, San Francisco (California 94135, USA), August 29, 1966.

[12] A. B. TSYPIN and YU. G. GRIGOR'YEV, Bull. exp. Biol. Med. USSR, *49*, 21 (1960).

Survival of Stressed Rats Following Experimental Cardiac Necrosis

It has been demonstrated that isoproterenol produces infarct-like myocardial necrosis in rats[1]. The functional capacity of such hearts, as measured by cardiac output and work during overloading produced by polyvinylpyrrolidone infusion, is greatly reduced[2]. The present study was initiated to observe the effect of stress on the survival of rats with myocardial necrosis.

Male albino rats of the Carworth CFN strain were used. Weanlings were maintained on a normal chow diet, except for one group on a high fat diet[3]. All the animals weighed 350–400 g. Isoproterenol was injected at 3 mg/kg s.c. for 2 days and the rats were stressed on the third day. Stress consisted of hypoxia in a covered glass jar, swimming in 10 °C water or restraint by tying in a supine position for 24 h. All animals were autopsied at death, or 24 h after surviving the experimental procedure, and the hearts examined by a previously described method[1].

Isoproterenol produced grossly evident myocardial damage in each rat, while stress alone, or with the high fat diet plus restraint, did not result in myocardial injury. However, there was no significant difference in the length of survival between isoproterenol-treated and control animals following hypoxia, swimming or restraint (Table), indicating that the presence of myocardial damage did not increase the myocardial sensitivity to cellular anoxia sufficiently to affect survival. No animals died

The effect of stress on the survival of isoproterenol-treated rats

Treatment	No. of rats	Survival (range)
Isoproterenol	20	None dead
Isoproterenol + hypoxia	6	109 min[a] (90–136)
Hypoxia	6	112 min (92–118)
Isoproterenol + swimming (10 °C)	6	17 min[a] (13–25)
Swimming (10 °C)	9	20 min (17–25)
Isoproterenol + restraint	18	None dead
Restraint	18	None dead
Isoproterenol + high fat diet + restraint	14	3 dead[b]
High fat diet + restraint	12	None dead

[a] Not significantly different from stress alone at $P = 0.05$ using the Student t test. [b] Animals died 12, 15 and 22 h after the end of restraint.

[1] G. RONA, G. I. CHAPPEL, T. BALAZS, and R. GAUDRY, Archs Path. *67*, 443 (1959).
[2] M. BEZNAK, Can. J. Biochem. Physiol. *40*, 25 (1962).
[3] T. BALAZS, M. R. SAHASRABUDHE, and H. C. GRICE, Toxic. appl. Pharmac. *4*, 613 (1962).