Reports

Electroencephalographic Responses to Ionizing Radiation

Abstract. *Electroencephalographic recordings made from chronically implanted cortical electrodes indicate that ionizing radiation has an immediate effect upon brain wave patterns. X-rays delivered at the rate of 0.2 roentgen per second produce an arousal effect resembling that which occurs as a result of stimulation through peripheral receptor systems.*

An increasing number of reports of diverse origin indicate that small amounts of ionizing radiation produce behavioral and electrophysiological changes in mammals. The implications of these findings with regard to radiosensitivity of neural tissue were major issues at the Symposium on the Effects of Ionizing Radiation upon the Nervous System held recently under the auspices of the International Atomic Energy Agency (*1*). In our laboratory we have demonstrated with instrumental conditioning techniques that rats can detect x-rays. These animals respond to doses as low as 50 mr/sec. The rapidity of the response (<1 second) indicates that total doses of this magnitude are sufficient to produce functional effects (*2*). This is in the range to which patients are routinely exposed during diagnostic x-ray procedures (*3*).

We have observed immediate effects of small doses of x-rays upon the electroencephalogram (EEG). Albino rats were implanted in the cortex with silver ball electrodes. After they had recovered from surgery, EEG recordings were made during sleep and waking states. The usual "desynchronization"—that is, the change from a low-frequency, high-amplitude wave pattern to a high-frequency, low-amplitude pattern was evident when the animals were aroused from sleep with auditory stimuli. Electrode placement from a variety of points on the dorsal convexity of the cortex yielded similar results.

For radiation exposure, the animals were confined to a small plastic chamber enclosed in a larger acoustically and electrically shielded wooden box. Electroencephalographic recordings were made on a two-channel Offner model R dynograph located outside the radiation room. Behavior could be observed through a double-pane window. The animal's compartment was placed directly under an x-ray machine with factors of 250 kv, 0.5 mm Cu filter, 1.7 mm Cu half-value layer. Output was measured by a Victoreen chamber in the animal's compartment and thus includes backscatter.

Since it is well known that novel sound stimuli desynchronize the EEG, several precautions were instituted. First, a speaker emitted a continuous wide-band masking noise within the animal compartment. Second, the x-ray machine remained in continuous operation during an experimental session. A shutter consisting of a plate of lead (3/16 inch thick) suspended between the x-ray tube and the compartment could be moved inaudibly to expose the animal to radiation. Movement of the shutter was monitored by a photoelectric cell. Finally, radiation exposures were alternated with sham exposures. These were identical except that during shams the copper filter in the x-ray machine was replaced with a lead plate which shielded the animal. Sham exposures had no effects upon either behavior or EEG pattern.

In a typical experimental run, the animal was placed in the radiation chamber and the x-ray machine was turned on with the shutter protecting the animal from the beam. We observed behavior until sleep ensued and the EEG displayed the characteristic high-voltage, rhythmic slow waves associated with sleep. Then we exposed the animal to a 10-second radiation period. At a dose rate of 0.2 r/sec the EEG was desynchronized within 1 second and the rats awoke (Fig. 1).

Radiation produces visual effects (*4*). Hence we tested animals blinded by bilateral ophthalmectomy. The results were similar to those observed in intact animals, indicating that the observed effects do not depend on retinal stimulation.

In order to establish the threshold for the EEG response we tested animals with 10-second exposures at 0.5, 0.2, and 0.1 r/sec. Each animal was given no more than one trial per day to prevent habituation. No trial was counted positive unless EEG desynchronization occurred within 2 seconds of the onset of the exposure. The results for six intact and six blind animals, summarized in Fig. 2, indicate that the threshold for arousal by x-ray is between 0.1 r and 0.2 r/sec. Characteristically, EEG desynchronization preceded behavioral arousal and at lower doses sometimes appeared in the absence of behavioral arousal. The fact that the response is dependent on dose is further evidence that it cannot be

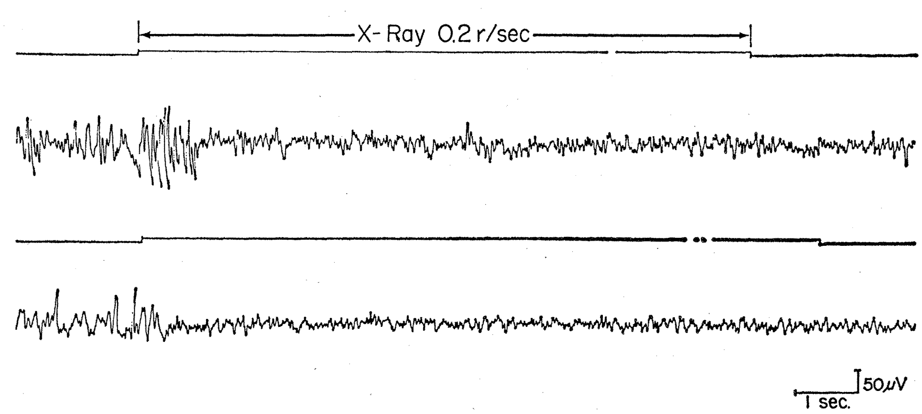

Fig. 1. Electroencephalographic desynchronization (activation) to x-ray recorded from rats with chronically implanted cortical electrodes.

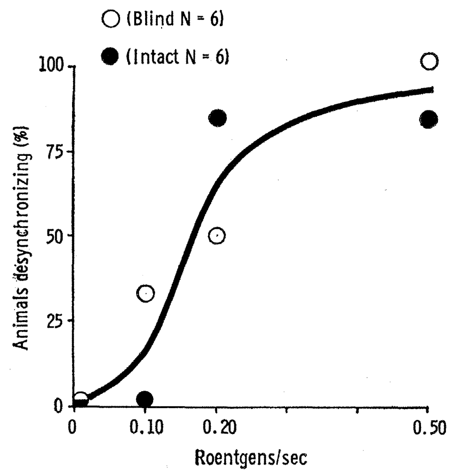

Fig. 2. Threshold determinations based on frequency of animals exhibiting EEG desynchronization (activation) as a function of x-ray dose rate during a 10-second exposure.

attributed to extraneous stimuli associated with the x-ray machine and the shutter.

We studied habituation of the response by subjecting other animals to repeated 10-second exposures at 0.2 r/sec during a single experimental run. After each exposure the animal was allowed to return to sleep for several minutes before it was exposed again. This procedure was repeated until the animal no longer responded to the x-rays. Five days later the animals were tested again. The results (Fig. 3) indicate the animals become habituated rather rapidly to the stimulus of radiation at this dosage. After several days, spontaneous recovery of the response occurs, followed by a more rapid habituation. This pattern is characteristic of responses to repeated mild stimulation through a peripheral afferent system. Electroencephalographic desynchronization, again, appeared to be a more sensitive measure than behavioral arousal, since it lingered in the records after all behavioral manifestations of arousal were absent.

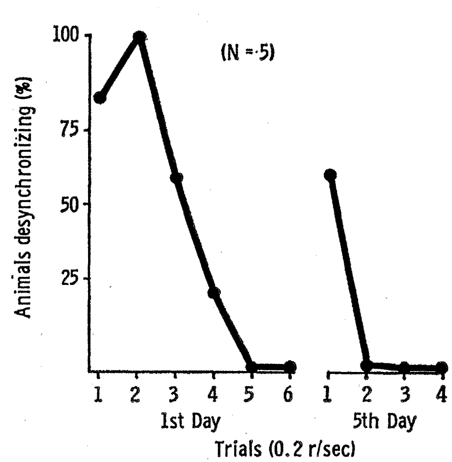

Fig. 3. Habituation of the EEG desynchronization (activation) to repeated 10-second exposure to x-rays (0.2 r/sec). Spontaneous recovery of the response occurred after a 4-day "rest" period.

We also explored the possibility that exposures of longer than 10 seconds' duration might have a summation effect. Another group of four animals was exposed to 10 minutes of 0.1 r/sec radiation, a dose which appeared to have little arousal effect with a 10-second exposure. Continued exposure at this "subthreshold intensity" increased the mean amount of desynchronization in the EEG record from a pre-exposure level of approximately 16 percent to 34 percent during the 10-minute exposure period. In the 5 minutes immediately following radiation exposure, the mean amount of desynchronization increased even further (48 percent). The amount of behavioral arousal observed during these periods substantiated the EEG evidence indicating that prolonged exposure produces a summation effect. Furthermore, these data are in accord with previous work which indicates that chronic exposure to extremely low intensities (0.0007 r/sec for 4 hours) will produce a conditioned aversion in the rat (*5*).

Russian investigators have reported EEG changes resulting from low levels of radiation. These have been interpreted as "depressions" of cortical electrical activity apparently due to a direct effect upon the central nervous system (*6*). In a recent article entitled "Evidence for direct stimulation of the mammalian nervous system with ionizing radiation" (*7*), Hunt and Kimeldorf reported behavioral arousal from sleep within 12 seconds in response to x-rays (0.25 r/sec). In general our data agree with the empirical findings reported in these papers. Our lower thresholds and faster reaction times are probably due to: a greater sensitivity of the EEG when compared with behavioral indices and depth of sleep, which is an important factor, for we have demonstrated even lower thresholds (0.05 r/sec) in active alert animals with the conditioned suppression technique (*2*).

However, we do not believe that these electrographic changes (and the associated behavioral events) imply a direct effect upon neural tissue. On the contrary, such changes are usually the result of arousing action of stimuli operating via a peripheral receptor. Our data demonstrate a prompt response to radiation which is functionally related to intensity (dose-rate) and which habituates rapidly. This suggests to us the intriguing hypothesis that sensitive radiation receptors may exist within the mammalian system (*8*).

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References and Notes

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In vitro Culture of the Flagellate Protozoan Hexamita salmonis

Abstract. *Trophozoites of* Hexamita salmonis, *asserted pathogen of juvenile salmonid fishes, were isolated from two species of Pacific salmon hosts and cultured repeatedly in an organic medium saturated with nitrogen. Primary isolates and serial subcultures usually exhibited five- to tenfold population increases per passage.*

The culture technique reported here was developed as an essential prelude to experimental evaluation of the polymastigine flagellate *Hexamita salmonis* (Moore, 1922), a common resident of the intestinal tract of trouts and salmons less than a year old. The basic objectives of this study were to produce routinely vigorous, populous, and axenic primary and secondary cultures which could be harvested for inoculation into disease-free test fishes. These objectives were met, and *H. salmonis,* under the