Sensitivity of the Head to X-Ray

Abstract. *Rats have been trained to respond to signals consisting of very low doses of x-ray directed to the head. This stimulus is probably received in the vicinity of the olfactory bulbs and adjacent neural structures.*

Both blind and normal animals appear able to respond to x-rays at the time of exposure. Radiation produces both behavioral arousal and the characteristic electroencephalographic activation pattern (*1*). In instrumental-conditioning studies, where brief exposures serve as conditioned stimuli, responses of short latency (<0.2 second) to the onset of x-rays delivered at 0.050 roentgen per second were observed. The total dose received before activation was, therefore, less than 10 mr. In the threshold range (from 0.01 to 0.40 r/sec) the probability of response is functionally related to the log of the intensity (dose rate). This resembles reactions to stimuli operating by way of peripheral receptors (*2*).

We have attempted to localize the radiation receptor in the rat. Young adult males of a Sprague-Dawley strain served as subjects. The techniques for administering this radiation and recording the behavior have been described (*2*). Briefly, 5-second x-ray exposure was used as a warning stimulus to signal a subsequent shock to the animal's paws. Precautions were taken to deliver the x-rays so that no other stimulus could inadvertently serve as a signal to elicit a false response. The animals were conditioned in a sound-damped chamber with a masking "white noise" background. The x-ray machine (250 kv, 0.5-mm Cu filter, 1.7-mm Cu hvl) remained in continuous operation during an experimental session and the animal was exposed by manual displacement of a lead plate suspended above his compartment, thus eliminating on-off changes in power and noise. The critical control was the interpolation of sham exposures in the training and testing schedules. The sham exposure was identical in all respects to an actual exposure except that the copper filter in the x-ray machine was replaced with a lead plate which blocked the x-rays. Responses were observed during less than 5 percent of the sham exposures; this corresponds closely with the operant or chance level of occurrence computed from random samples of the records during training and testing (Table 1).

Table 1. Mean detection scores and standard errors from a series of exploratory studies with partial body exposures. Detection score = (errorless trials/total trials) × 100.

Area exposed	*N*	r/sec	Mean (%)	S.E. (%)
Head only	10	0.2	84	4
Body only	4	0.2	04	3
Body only	6	0.4	04	3
Body only	3	1.2	00	
Anterior part of head	8	0.4	75	6
Posterior part of head	8	0.4	09	2
Entire rat before test	9	0.2	78	6
Sham test level				
	9		03	2
Operant response level				
	9		04	2

The rats were given a preliminary training consisting of 5 seconds of x-ray exposure (0.2 r/sec) paired with intermittent shocks to the paws during the final 4 seconds of exposure. The shock intensity was adjusted for each animal at the minimum (0.08 to .20 ma) required to produce an observable "startle" response. Typically, the animal received a session of 5 to 10 trials at an unsystematically varied interval between trials averaging from 3 to 5 minutes. Approximately 50 trials were administered in 2 weeks, the accumulated total dose being 50 r. In the beginning of training the animal received 100 percent reinforcement, that is, he was shocked during each exposure. Gradually the schedule was changed so that by the end of the preliminary training he was on a 50 percent variable reinforcement schedule. This procedure permitted observation of the animal's conditioned defensive reactions to the x-ray signal in the absence of electric shock.

Further training was conducted while the thirsty animal was drinking in a narrow (6.3 × 6.3 × 6.3 cm) lucite alley with an electrifiable grid floor. To obtain water the animal had to thrust his head through a hole and stretch out to lap at a water spout (Fig. 2). This alley was designed to confine the animal's head and body in a fixed position while lapping but also to allow him to withdraw easily to avoid the electric shock. The lapping was recorded electronically on a cumulative response recorder. The response rate is constant and rapid (five per second) with little variability in this apparatus (*2*).

The animals were given instrumental avoidance training while drinking in this apparatus. Each was exposed to 5 seconds of x-ray limited to the anatomical area to be explored. If he continued to lap water during exposure, this was considered an error and he was inhibited by shock to the paws administered on a 50 percent variable reinforcement schedule. If the animal stopped lapping within a second of the onset and did not resume until the exposure was terminated, he avoided the shock. The duration of the suppression of lapping was variable, presumably owing to motivational states of the animals (Fig. 1). When an animal reached the criterion of seven errorless out of the last ten trials, he was considered ready for testing with partial body exposure. Most animals reached this level before 15 trials.

In a typical test the animal received a maximum of 12 trials under a given exposure condition or until he performed a sequence of 5 consecutive errorless trials. The proportion of errorless trials served as the detection score for the anatomical area exposed under each condition.

In the first exploratory studies, the animals were tested under a number of partial body exposures. Total exposure was prevented by placing lead plates on the roof of the drinking alley. The mean percentages of perfect trials and their standard errors are given in Table 1. The operant behavior before the test and the sham responses based on a random sample of records (*N* = 9) are included for comparison. The results indicate that exposure limited to the anterior region of the head is an adequate stimulus while the posterior portion of the head is relatively insensitive. When the body was exposed with the head shielded, the animals did not detect the x-rays at these intensities (dose rate). Approximately one-half the animals used had been previously ophthalmectomized. The response of the blind animals was similar to that of the visually intact animals; thus the retina as the site of action could be eliminated.

In a second series of experiments, a narrow (0.45 cm) beam was aimed at points in the animal's head. To accomplish this the x-ray was directed through a collimator consisting essentially of a 0.45-cm hole bored in a shield composed of two (0.45 cm) lead plates separated by (0.6 cm) of wood. This collimator was placed approximately 0.6 cm from the rat's head and about 75 cm from the x-ray tube target

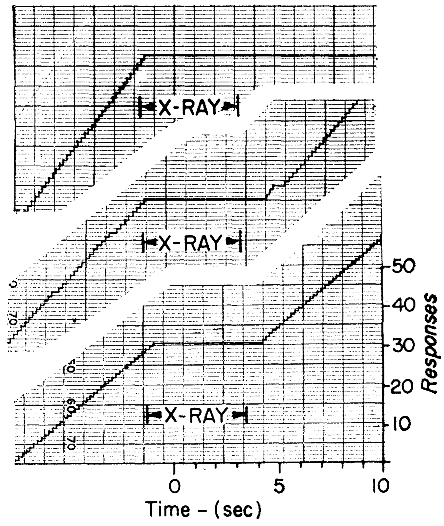


Fig. 1. Examples of conditioned avoidance responses to x-ray in the cumulative lapping record of rats. The animals have detected the x-ray signal and have stopped drinking during the 5-second exposure, thus avoiding a shock to the paws.

at the 0.4 r/sec setting. Under these conditions it produced sharply defined radiographs when its beam was passed through a lucite panel and a wax model designed to simulate the rat's head in the drinking compartment. Dosimetric monitoring indicated that radiation scatter was below detectible amounts at a point approximately 0.3 cm from the collimated beam. In the experimental procedure, the collimator was positioned while the animal was lapping at the water spout. External features of the head served as reference points (eyes, ears, mouth) to obtain equivalent fields from animal to animal and from session to session. Nine animals which had served as subjects in pilot studies were tested with collimated beams. These tests were repeated with ten more previously untested animals. The results were so similar that the data from both groups were pooled. Again, one-half the animals were ophthalmectomized.

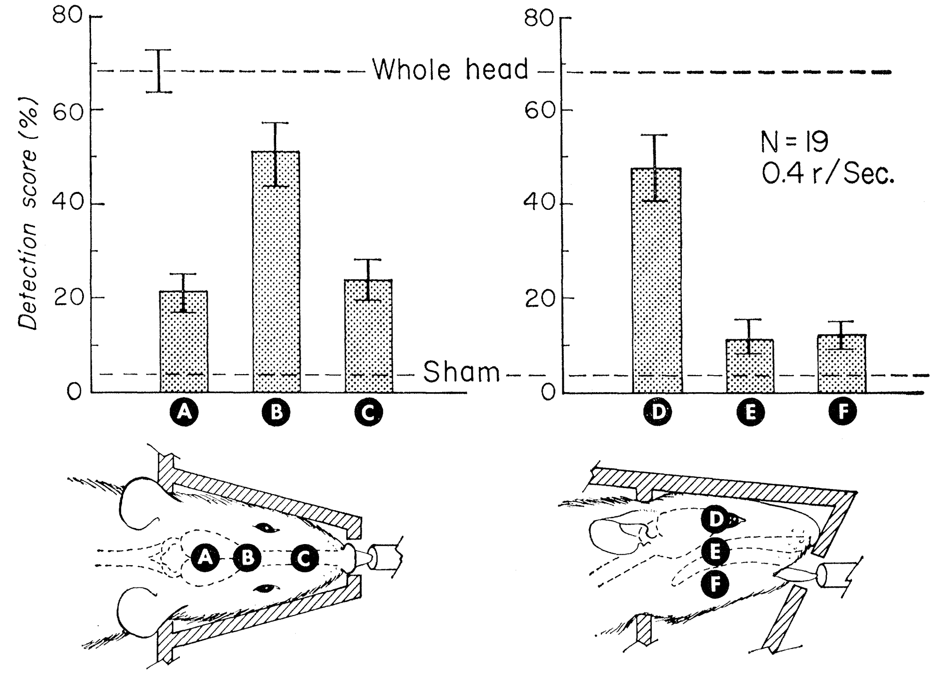
Three tests with a vertical beam were made first. Figure 2*A*. illustrates three entry sites of the collimated 0.45-cm beam. Sub-groups of animals were first assigned to each site and then rotated in a balanced order so that each animal was eventually tested at every site. The 5-second exposure at site *B* produced results of greater statistical significance than exposure at site *A* or site *C*. This beam (*B*) passed down through the olfactory bulbs and the frontal brain area, the nasal passages, and the oral cavity. When the beam was directed at a more rostral position (*C*), where it passed through the nasal passages and oral cavity but not through the brain tissue, there was a drop in sensitivity. A similar loss in sensitivity was observed when the beam was passed through a more posterior region of the head (*A*). This beam passed through the posterior cerebrum, the diencephalon, and the pharynx.

The animals were then tested with a horizontal beam aimed to intersect the path traversed by the vertical beam (*B*), which had yielded the highest probability of detection. Figure 2*B*. illustrates the three sites tested along this path. Significantly greater detection was obtained at the highest site (*D*) in the olfactory bulb area of the brain. When the horizontal beam passed through the nasal (*E*) and oral (*F*) regions, there was a marked loss of sensitivity. There was no apparent difference between the blind and intact animals' responses to either the vertical or horizontal beam.

Unfortunately, histological verification of the path of the beam was not possible. The paths described are estimates subject to the errors inherent in the method of positioning the animal's head and directing the beam with external reference points. Nevertheless, the data clearly indicate an extremely radiosensitive area in the anterior region of the brain. The olfactory bulbs are the most prominent anatomical features here, and this may mean that radiation detection is mediated by way of olfactory system. The amplitude of response of the olfactory system is within certain limits proportional to the logarithm of the intensity of the stimulus. Radiation detection is also a logarithmic function. It is interesting to note that theories of electromagnetic radiation or molecular vibration have been proposed for olfaction (*3*) and that x-rays produce fluorescence in animal tissue.

The radiation response does not appear to be due to a secondary effect, such as smelling of ozone, because there is a loss in sensitivity when the beam is passed through the nasal and oral passages anterior or ventral to the bulb area. Moreover, the rapidity of the onset and offset of the response is an argument against mediation by way of a relatively stable product lingering within the animal compartment. It appears more likely that radiation produces a direct effect upon the olfactory system or upon related neural structures known to take part in arousal.

Radiation limited to this sensitive olfactory brain region is not as effective as radiation of the entire head. Moreover, a significant, though lower,



A.-Vertical beam B.- Horizontal beam

Fig. 2. Relative effectiveness of a 0.45-cm x-ray beam as a signal when directed at different sites in the heads of rats. The bars indicate the mean detection scores plus and minus 1 standard error. Each animal was tested at each site first with the horizontal beam and then with the vertical one. Detection score = (errorless trials/total trials) × 100.

degree of sensitivity is apparent when the collimator is aimed at other areas of the head (Fig. 2). This may mean that there are other areas in the head which are also sensitive to x-ray. An equally tenable hypothesis is that there is only a single sensitive area in the olfactory brain area and that whole-head exposure is a more reliable method of stimulating the critical area. The apparent sensitivity of other head areas may be explained by inadvertent radiation of the sensitive area by scattered x-rays or by minor errors in positioning of the collimator. This question might be resolved by surgical lesion studies. Observations of behavioral arousal and electroencephalogram desynchronization in rats with the entire head shielded and the body exposed have been reported (*4*); this effect was abolished by spinal transections (*4*). The apparent contradiction may mean that radiation arousal operates by way of diverse mechanisms. At least two other mechanisms are known to be responsive to extremely low doses of radiation. One is the well-known retinal effect (*5*). In addition, radiation of the abdomen operates as an aversive stimulus to produce avoidance reactions (*6*).

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Conditioned Discrimination in the Planarian

Abstract. *To demonstrate classical conditioning in the planarian in a situation uncontaminated by the possible artifacts of pseudoconditioning or sensitization 30* Phagocata gracilis *were successfully trained, by the use of directional shock as the unconditioned stimulus, to turn in one direction to a light, and in the opposite direction to vibration. Ten similarly trained planaria tested by an independent observer who did not know the previous training conditions experienced by any animal showed similar results.*

Thompson and McConnell's report of classical conditioning in the planarian (*1*) has revived interest in the learning abilities of this primitive organism. The literature has been reviewed recently by Jacobson (*2*). However, there has been concern with the possibility that much, or all, of the evidence for classical conditioning in the planarian might really be an artifact of the experimental situation. A basic source of possible artifact lies in the tendency of a response which has been repeatedly elicited to become "dominant," or more probable of occurrence (*3*). If the response is one elicited by the unconditioned stimulus (UCS), the tendency to make this same response to any other stimulus, in the absence of associative pairing, has been termed pseudoconditioning. Moreover, if the response is one directly (innately) elicited by the conditioned stimulus (CS), the increased probability of response has been called sensitization (*3*), and is of concern here because the light used as a CS by Thompson and McConnell can, innately, elicit the criterion response of contraction or turning. In addition to their classical conditioning group, Thompson and McConnell ran control groups which were exposed, respectively, only to the CS, only to the UCS (electric shock), and to no stimulation whatever. Neither the CS-only nor the UCS-only group showed ultimate response levels significantly higher than that of the no-stimulation group. On the other hand, Halas, James, and Knutson (*4*) found that a CS-only group, in a situation similar to Thompson and McConnell's, did give significantly more responses than a no-stimulation control.

We were interested in showing classical conditioning in a situation where there could be no question of artifactual "conditioning" due to sensitization or pseudoconditioning. Exploiting the planarian's marked galvanotropism, we were able to concurrently condition homologous, mutually exclusive responses to two different CS's and thus not only demonstrate conditioning but also test the planarian's ability to form a conditioned discrimination.

The subjects were 30 large *Phagocata gracilis,* obtained as needed during the study from a local stream. All were run within 24 hours of capture.

A plastic petri dish, 8.8 cm in diameter by 2.5 cm deep, filled to a depth of 2 cm with aged tap water, constituted the experimental chamber. A white base, with a reference grid to aid in evaluating the response, was glued to the underside of the chamber. This assembly was then firmly secured to the cover of a Johnson Speed-X constant-frequency buzzer, the vibration from which served as one CS. A commutator, 12.5 cm above the chamber, supported both a clear 12-watt light, which served as the second CS, and two nonpolarizing, platinum electrodes which extended down into the water on opposite sides of the chamber. By rotating the commutator, the electrodes could be oriented across any diameter of the chamber. The UCS, electric shock, was supplied by a filtered 8.5-volt d-c power supply in series with a variable resistance and a milliameter. Since the planaria showed differential sensitivity to the UCS, the current was individually adjusted during the first

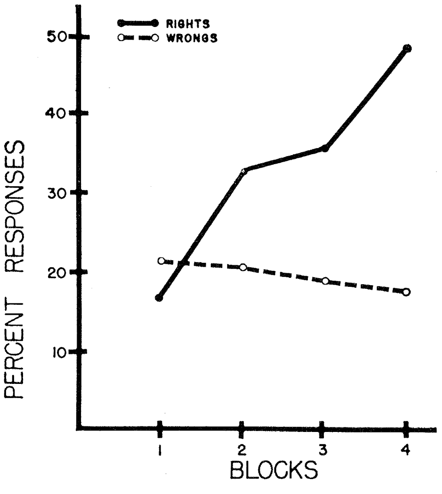

Fig. 1. Percentage of correct and incorrect test-trial responses (of amplitude greater than 22.5 degrees) in successive blocks of 50 training trials.