Role of the Olfactory Bulbs in the Detection of Ionizing Radiation by the Rat

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DINC, H. I. AND J. C. SMITH. Role of the olfactory bulbs in the detection of ionizing radiation by the rat. PHYSIOLOGICAL BEHAVIOR, 1 (2) 139-144, 1966.—The sensitivity to X-rays in the albino rat was studied following destruction of the olfactory bulbs. The two procedures of conditioning an aversion to saccharin solution with X-rays as the unconditioned stimulus and conditioned bar press suppression with X-rays as the warning stimulus were both used in testing the sensitivity to the ionizing radiation. The profound aversion to saccharin flavored water, obtained in rats after a single pairing of X-rays with the saccharin solution, was found to be diminished in animals which had olfactory ablations. The conditioning of sham operated animals and those with frontal lesions resulted in the usual significant saccharin aversion.

The immediate detection of X-rays was profoundly affected by removal of the olfactory bulbs. Animals which had previously been conditioned to suppress a bar-pressing response in the presence of X-rays failed to show this suppression after complete removal of the olfactory bulbs. When ablation was incomplete, with removal of tissue from the frontal lobes, or with sham operations, the animals continued to show the suppression effect at the onset of X-ray exposure.

X-irradiation Conditioned aversion Olfaction Olfactory bulb Saccharin Lesion

Determination of ionizing radiation in rats has been demonstrated both by inference from post-exposure behavior and by the immediate response made during irradiation. Regarding the former it is a well established fact that a profound post-exposure avoidance to saccharin flavored water can be conditioned using X-rays, gamma rays or neutron bombardment as the unconditioned stimulus. An aversion can be conditioned with a single exposure using a total dose as low as 10 r [7], with soft X-rays [20], with fast neutron bombardment [5], in species other than the rat [14], and with different solutions [12]. It has also been shown that the aversion effect can be obtained not only with simultaneous presentations of saccharin and radiation, but also when saccharin precedes or follows the radiation within given time limits [16, 17]. It has been demonstrated that the conditioning is stronger with whole body exposure than with partial body irradiation. With partial body exposure, the abdomen appears to be more sensitive, but some aversion can be conditioned exposing only the head if the dose delivered is quite high [6]. Vision has been ruled out as a necessary site of sensitivity in this conditioning [4]. Although there is some evidence of an effect of X-ray on intestinal motility [3] the use of atropine and physostigmine during the conditioning procedure had little, if any, effect on the conditioned aversion [22].

The second general evidence for the rat's sensitivity to ionizing radiation is an immediate detection made during exposure. This has been demonstrated by several methods. Behavioral arousal and increased heart rate in both normal and aphthalmectomized animals have been demonstrated at the onset of exposure when dose rates of 1.9 r/sec or higher were used [13]. Differential responding in rats in an operant conditioning situation to X-ray stimulation has been demonstrated with dose rates as low as 0.01 r/sec [18].

Desynchronizations in the EEG recording have been reported with the onset of radiation at dose rates of 0.2 r/sec [1, 9]. Efforts to localize the receptor or sensitive area responsible for this immediate detection have indicated that the head of the animal was more sensitive than other parts of the body. This evidence is both behavioral [8, 10, 13] and electrophysiological [1, 9]. More specifically the area of the olfactory bulbs has been shown to be the most sensitive part of the head in the immediate detection of radiation in both the behavioral problem [11] and the electrophysiological technique [2].

Several authors [10, 15] have pointed out the possibility of a dual mechanism for radiation detection. There is some evidence for postulating two mechanisms, since the factors affecting the saccharin aversion and the immediate detection are quite different. It is known for example, that the saccharin aversion effect is independent of the rate of radiation [21] but proportional to the total amount of radiation [7, 19]. The arousal effect on the other hand, is a function of dose rate [13].

Partial body exposures also give different results under the two experimental conditions [6, 11, 12]. There is evidence that the abdomen is the most sensitive area for saccharin aversion while the area of the olfactory bulbs seems to be the most sensitive region in the immediate detection studies.

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2Part of the work included in this report was submitted to Florida State University in partial fulfillment of the Ph.D. Degree. Current address: Faculty of Medicine, Ankara University, Ankara, Turkey.
The visual receptors have been ruled out as the responsible mechanism in both types of detection [6, 13].

Several of the above studies have indicated a possible role for the olfactory system in the detection of X-rays. Although the region of the olfactory bulbs have been found to be the most sensitive area in only the immediate detection problems, an aversion to saccharin solution can also be conditioned by head-exposure only.

The purpose of the present research was to study the role of the olfactory system in both types of detection by studying the changes in sensitivity to radiation after elimination of the olfactory bulbs.

**EXPERIMENT I**

This experiment was designed to study the effects of the removal of the olfactory bulbs on conditioning an aversion to saccharin fluid using X-rays as the unconditioned stimulus.

**Method**

**Subjects.** The subjects for this experiment were 56 adult male albino rats of the Sprague-Dawley strain.

**Apparatus.** The radiation source was a G. E. Maxitron 300 X-ray Therapy Unit, operated at 250 kVp, and 15 mA with 3 mm aluminium filtration. To ensure equal dose for all animals, a turntable rotating at a speed of 1.25 rev/min was placed under the head of the X-ray machine. Subjects were put on this turntable in plexiglass boxes measuring $3 \times 3 \times 7$ in. The source to target distance was adjusted so as to yield a dose rate of 30 r/min. The dosimetry was done with a Victoreen Radocon Model 575, using a Thimble Chamber Model 602.

**Procedure.** The subjects were randomly divided into three groups of 38 (Group I), 7 (Group II), and 11 (Group III). All subjects were housed in individual cages with food and water available at all times until the day prior to radiation. Prior to the radiation exposure the animals in Group I underwent surgery for removal of the olfactory bulbs. The operations were performed under ether anesthesia, and the bulbs removed by suction with a glass aspirator.

The animals in Group II were sham operated. The procedure was the same as for the actual operation up to the point of suction of tissue. At this point, however, the glass aspirator was not connected to the faucets vacuum pump while being held in contact with the bulbs.

The animals in Group III underwent the same operation as those in Group I, except that the tissue was removed from the prefrontal lobes.

All subjects were given 12 days for recovery before the continuation of the experiment. At the completion of this period of recovery, the subjects were gentled and their water bottles removed. Twenty-four hr later, they were given a sham irradiation for the purpose of habituation to the experimental conditions. The sham exposure procedure started by giving the subjects their water bottles for 20 min. Then they were placed in Plexiglass boxes and put on the turntable under the X-ray machine. All sounds associated with the operation of the machine were present, but the voltage to the X-ray tube was not turned on. The subjects remained on the revolving turntable for the same length of time as would be necessary for their actual exposure. Following the sham irradiation, each subject was returned to its home cage and left there under water deprivation for 24 hr.

At the end of the 24 hr period all animals were given saccharin flavored water (0.1%) by wt. for 20 min. Immediately following this step, the subjects in Group I were randomly divided into three subgroups of ten, twenty-four and four, and exposed respectively to 300 r, 100 r, and 0 r. The sham operated animals in Group II were divided into two subgroups of five and two, and received 100 r and 0 r respectively. Animals in Group III were divided into two subgroups of ten and one, and exposed to 100 r and 0 r. The purpose of sham exposing some of the animals in each group (0 r subgroups) was to test for any possible effect of the surgical procedures alone on the saccharin preference of rats, without the effect of radiation.

A 48 hr preference test was started for all animals 24 hr after exposure. During the preference testing each subject had access to two bottles attached to its home cage, one of them containing tap water, the other saccharin flavored water. At the end of the first 24 hr the positions of the bottles were exchanged in order to account for the effect of position habits in case they did develop. The preference of subjects was assessed in terms of S-scores [7] where: $S = (\text{Saccharin consumption}/\text{Total liquid consumption}) \times 100$.

At the termination of the experiment all subjects were sacrificed for verification of the locus of surgery and amount of tissue removed. The brains were fixed by perfusing the animals with 150 ml of Ringer's solution, followed by 150 ml of 33% formalin solution.

**Results**

The results of the different surgical procedures are illustrated in Fig. 1. Brain A is that of a sham operated animal with its olfactory bulbs intact. The brain of a subject with frontal lesions is represented by B. Brain C is an example of the cases where removal of the olfactory bulbs was attempted but was not complete. Finally, D shows the brain of an experimental subject after complete removal of the bulbs.

The post-mortem verification of the lesions in the subjects of this experiment revealed almost complete removal of the bulbs in all cases where removal was attempted, except one. The damage to the bulbs being negligible in that case, the results of that particular subject were discarded before analysis of the data. Figure 1 D is typical of the remaining cases where little olfactory tissue was left. No systematic relationship was found between the presence of the small amount of remaining olfactory bulb tissue and the size of S-scores. The results presented in Fig. 2 therefore, are averaged S-scores of all subjects belonging to the described groups. It can be seen that all sham exposed animals (0 r), whether sham operated, with olfactory bulbs removed, or with frontal lesions, displayed the typical high preference of rats for saccharin solution. Of the subjects receiving 100 r, those having undergone sham operation, and those with frontal lesions showed the usual profound aversion to saccharin solution following the conditioning procedure, yielding mean S-scores of 4 and 12 respectively. Subjects with their olfactory bulbs removed, on the other hand, demonstrated a weaker aversion, giving an average S-score of 37. In the group of animals without olfactory bulbs which were exposed to 300 r, the aversion effect appeared greater with a mean S-score of 24.

A Kruskal–Wallis test performed on the S-score of the three groups of bullectomized animals receiving 0, 100 r, and 300 r, showed the difference to be significant beyond the 0.01 level ($H = 10.38; df = 2$). The difference between the 100 r and the 300 r groups was not significant.

A comparison was made with the Mann–Whitney Test between the different groups receiving 100 r. The sham
FIG. 1. Four representative brains following the surgery. Brain A represents a sham operated animal; In Brain B, tissue has been removed from the frontal lobes; C is a typical example where the olfactory ablation was incomplete; and D represents a complete olfactory ablation.
operated group and the group with frontal lesions were not different \( Z = 1.50 \). They were therefore pooled for comparison with the bulbectomized group receiving 100 r, and the difference was found to be significant beyond the 0.05 level \( Z = 1.99 \).

**EXPERIMENT II**

The second experiment was designed to study the effects of the removal of the olfactory bulbs on the animal's immediate response to X-ray exposures. In one group of animals the response to X-rays was demonstrated, the bulbs were removed and the response to the radiation was noted after the surgery. In a second group, the bulbs were removed prior to X-ray exposures and an attempt was made to demonstrate the acquisition of the response in the bulbectomized animals.

**Method**

**Subjects.** The subjects were 19 adult male albino rats of the Sprague-Dawley strain.

**Apparatus.** The apparatus consisted of a standard Foringer operant conditioning rat chamber, delivering liquid reinforcement. The grid floor permitted the administration of an electric shock to the paws of the subjects. The shock was generated by an AC power supply. A grid scrambler was used to insure that the subjects did not avoid the shocks by standing on rods of same polarity.

The wiring of the control panel permitted the counting of the responses during two consecutive 15 sec periods when a switch was operated by the experimenter. The events were recorded by a high speed polygraph recorder and on counters.

The apparatus and conditions of radiation were the same as for Experiment I. A silently operating hydraulic shutter was added to the X-ray machine for making possible the delivery of radiation for short periods of time without having to turn the machine on and off, thus eliminating the noises associated with those events.

**Procedure.** All subjects were brought to 80 per cent of their ad libitum body weight and kept at that weight throughout the experiment, except for the recovery period following surgery. The reinforcer used during the experimental sessions was a sucrose solution prepared with commercial granulated sugar at a concentration of 16% by wt.

Following the shaping procedure, the animals were run on a continuous reinforcement (CRF) schedule for a 20 min session, then shifted to a variable interval schedule (VI 1 min). Each subject was run every day for a 30 min session until the baseline achieved stability. At this point the animals were divided into two groups of 14 and 5. For the one group of 14, training for conditioned suppression started, with radiation used as the warning stimulus. The training consisted of giving the animal, while it was working in the experimental chamber, a radiation exposure of 15 sec duration followed by a shock of 300 msec to the paws. The intensity of the shock was manipulated for each animal until an optimal value was obtained. By optimal value is meant one which permitted the observation of suppression without causing complete breakdown of the baseline. These values ranged between 30 and 40 V.

Radiation was delivered by manual operation of the hydraulic shutter, and was always made to correspond to the second of the 15 sec periods during which the additional counters were activated. In this manner it was possible to keep a record of the number of responses made during the 15 sec preceding, as well as those made during radiation exposure. This information permitted the calculation of the suppression ratio which was used as the index of suppression. The suppression ratio was obtained by the following formula:

\[
\text{Suppression ratio} = \frac{(\text{pre-radiation responses}) - (\text{radiation responses})}{(\text{pre-radiation responses}) + (\text{radiation responses})}
\]

With this formula a value of 0.00 corresponds to an instance of no suppression, and a value of 1.00 to perfect suppression with no response during exposure to radiation.

The suppression training was continued until five consecutive trials were obtained with suppression ratios of at least 0.70. Training was also considered complete if four consecutive trials yielded suppression ratios of 1.00, i.e., perfect suppression. Each subject was given a minimum of five shutter control trials for testing the possibility of dis-
crimination by sounds associated with the movement of the shutter. These control trials consisted of replicating the steps of an actual trial, including the opening of the shutter, but with the voltage to the X-ray tube turned off. No shock was administered at the end of these trials. There were also sham trials to check the stability of the baseline. They consisted of taking the number of responses during two consecutive 15 sec periods, at random intervals within the session.

Training was terminated and the subjects eliminated from the experiment if a suppression ratio of at least 0.70 was not obtained after 15 trials.

At the termination of training, eight of the 14 subjects underwent surgery for removal of the olfactory bulbs, as described in Experiment I. Of the remaining six, three were sham operated, and three had tissue removed from their frontal lobes. The number of surviving animals from the three groups were six, two, and two in that order. The surgery was followed by a minimum of 12 days of recovery with ad libitum food and water. At the end of this period, the subjects were brought once more to 80 per cent of their ad libitum body weight and tested for suppression. The testing was terminated, either when one of the criteria used in training was reached, or when the subject failed to give a suppression ratio of at least 0.70 after eight trials. The shutter control and the baseline control trials were given during the test sessions as well.

Three of the remaining five animals had the olfactory bulbs removed and the other two were sham operated prior to any training with the X-ray warning stimulus. Twelve days after surgery these animals were once more brought to 80 per cent of their ad libitum body weight and trained for suppression with X-rays as the warning stimulus.

After the post-operative testing, the fifteen remaining subjects were sacrificed, and the brains fixed with the technique described in Experiment I.

Results

In the first group of 14 rats, the conditioned suppression effect was generally very easily obtained. Only one subject with a comparatively unstable baseline failed to reach the criterion at the end of the fifteenth trial which was accepted as the limit for discarding a subject. All other animals demonstrated the effect much earlier in training. During

![Graph showing suppression ratios for four typical animals prior to and following the surgical procedure indicated. Suppression values obtained during baseline control and shutter control trials are also presented for comparison with the radiation exposure trials.](image-url)
DETECTION OF X-RAYS BY THE RAT

post-operative test sessions for this group, suppression generally appeared at the first or second exposure to radiation in the cases where it appeared at all.

The pre- and post-operation suppression ratios for one of the sham operated animals (10 A) under the three conditions of baseline control, shutter control, and radiation exposure are presented in the upper left of Fig. 3. The values in the figure were obtained by averaging for each subject the ratios of the criterion trials for radiation exposure, and all ratios for the control trials. As can be seen from the figure the suppression ratios are above 0.86 for the radiation exposure trials, but within the limits of —0.10 and 0.04 for the control trials. That shutter control trials do not differ systematically from the baseline control trials is evidence for the fact that the suppression effect is due solely to the detection of radiation, and not to any other cues associated with the movement of the shutter.

In the upper right of Fig. 3 are similar data for a subject with frontal lesions (3 A). No difference can be observed between the results obtained under this condition and those obtained with sham operation. The suppression effect is again very clearly present before and after the operation. The suppression ratios for radiation exposure trials are both 1.00, while those for the control trials fall within the limits of —0.17 and 0.08.

The post-mortem verification of the brain lesions revealed that in three out of the six cases where removal of the olfactory bulbs was attempted, the operation had been only partially successful, and part of the olfactory bulb had remained attached to the brain. The amount of remaining tissue was comparable in all three cases to that seen on brain C in Fig. 1. Since, unlike Experiment I, the present results showed a drastic difference between the data of subjects with complete removal of the bulbs and those with some tissue remaining, their results are presented separately.

The suppression ratios of one of the subjects with incomplete removal of the bulbs (6 B) are seen in the lower left of Fig. 3. The suppression effect is still clearly observable after the operation, just as in the cases of sham operation or frontal lesions. The suppression ratios for the radiation exposure trials are both 1.00, while control trials give ratios falling within the limits of —0.07 and 0.09.

The data for one of the subjects (8 A) with complete removal of the bulbs are presented in the lower right of Fig. 3. A dramatic change is observed between the pre- and post-operative results. The suppression ratio for radiation exposure trials goes down from 0.95 to 0.06. The values for the control trials fluctuate between 0.35 and —0.09.

The results reported are representative of all the subjects in each of the different groups.

In the five animals which received no suppression training prior to surgery, only the two sham operated animals reached the criterion of learning. One of the sham operated animals reached the criterion of perfect suppression in 13 trials and the second animal in 15 trials. The three bulbectomized animals failed to reach the criterion and were terminated after 15 trials with no suppression. The mean suppression ratio for these three animals on the last four trials was 0.21.

DISCUSSION

The removal of the olfactory bulbs had a disruptive effect on both the conditioned suppression and the saccharin aversion phenomena, supporting the view already presented in the literature that these structures are instrumental in the immediate detection of radiation [2, 11]. The possibility of their being the only mediators, however, is not defensible on the basis of the present data. The bulbs seem to be of utmost importance for the immediate detection displayed in conditioned suppression, since their complete removal results in the failure to suppress X-ray as a warning stimulus. In addition, animals which have had the bulbs removed prior to training fail to learn the use of X-ray as a warning stimulus. The situation is quite different in the case of saccharin aversion. It is undeniable that the olfactory bulbs also play a role in this phenomenon. The evidence for this role is the weaker aversion displayed by the bulbectomized animals when compared to control animals receiving an equal amount of radiation. This weakening of the aversion effect cannot be attributed to the loss of preference for saccharin due to surgery, because operated but sham irradiated animals continue to show the high preference for saccharin, typical of rats.

On the other hand, there is also strong evidence indicating that radiation can still be detected after the removal of the bulbs under the conditions of saccharin aversion. The fact that the average S-score of the bulbectomized animals receiving 100 r is significantly lower than that of the sham irradiated animals, clearly demonstrates that these animals continue to detect radiation. The presence of the olfactory bulbs therefore, is not a necessary condition for the detection of radiation under the conditions of saccharin aversion as it is in conditioned suppression.

It can be concluded therefore that the possibility of a dual mechanism for radiation detection, suggested by Kimeldorf et al. [15], and Garcia et al. [10], is definitely supported by the present data.

The mechanism responsible for the remaining sensitivity to radiation in the bulbectomized animals of the saccharin aversion study is still unknown. The intestinal motility hypothesis is a possibility worth investigating. But in view of the present results, detection via the olfactory system should be considered in future studies attempting to determine the second reception mechanism of the aversion effect.

Another difference between the saccharin aversion phenomenon and that of conditioned suppression seems to be the fact that incomplete removal of the bulbs has no effect on suppression, whereas the weakening of the aversion effect occurs even in animals with incomplete removal of the bulbs, in the case of aversion. It should be brought to attention, however, that the amount of non-removed tissue differed from one situation to the other, being larger in all cases of suppression. In none of the aversion cases was the remaining tissue of the size seen in the three suppression animals. It is possible to speculate, therefore, that if the remaining tissue had been comparable in size to that observed in conditioned suppression cases, no effect would have been observed in saccharin aversion either. By the same reasoning it is also possible to state that if there were some suppression animals where the remaining tissue was smaller, i.e. comparable in size to that seen in aversion animals, the conditioned suppression effect might have disappeared in spite of the incompleteness of bulb removal.

An effort was also made by the present investigators to determine the role of the peripheral olfactory system in the radiation detection mechanism. Towards this aim, the animals were nasally treated by a zinc sulfate solution in an attempt to destroy the sensory cells in the nasal mucosa. This treatment had no effect on either saccharin aversion or conditioned suppression. However, the histological results
showed only partial destruction of the mucosa making it impossible to discard the peripheral olfactory system as a possible mediator in radiation detection. On the other hand, if the peripheral olfactory system is hypothesized as the receptor site for radiation, it is difficult to account for the complete lack of change in sensitivity to radiation in the cases where histology showed the destruction to be comparatively extensive.

REFERENCES