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Humoral Mediation of Radiation-Induced Motivation in Parabiont Rats

Abstract. *Parabiont rat pairs with a skin–vascular anastomosis were used to test for a radiation-induced transferable humoral factor which would motivate aversive conditioning in a nonirradiated (shielded) recipient. The shielded partner, by tasting saccharin-flavored fluid shortly after its partner was x-irradiated (360 roentgens), was aversively conditioned to saccharin. This was interpreted as evidence for a humoral motivating factor.*

Exposure to ionizing radiation has been used as a motivating stimulus in the conditioning of aversive reactions; this noxious effect has been demonstrated in several species and in a variety of learning situations (*1*, *2*). An aversion to drinking saccharin-flavored water has been obtained in rats by pairing their tasting of saccharin (the conditioned stimulus) with a single irradiation. There are indications that radiation-induced motivation may be initiated by non-neural (humoral) consequences of exposure rather than by prompt neural stimulation. Aversive conditioning has been obtained when the saccharin was presented during irradiation at the rate of 0.7 mr/sec (*1*), which is much lower than the rates that have been used thus far in eliciting prompt reactions (*2*). A conditioned saccharin aversion has also been obtained by presenting the saccharin within the first few hours after exposure at higher rates (*3–5*). Such conditioning after exposure does not depend on trace effects of prompt neural stimulation since conditioning has been obtained with this arrangement in rats that were deeply anesthetized (ethyl ether) during their irradiation (*6*).

Our investigation was designed to test for humoral mediation of radiation-induced motivation. Unrestrained parabiont rat pairs, united by vascular anastomosis, were used to determine whether irradiation of one partner would produce a transferable systemic humoral factor which would motivate the conditioning of a saccharin aversion in the nonirradiated (shielded) partner.

Male littermates of the specific pathogen-free strain of Sprague-Dawley rats bred at the U.S. Naval Radiological Defense Laboratory were paired by weight on removal from their litter at 24 ± 3 days of age and the parabiosis operation was usually performed 1 to 2 days later. A skin-to-skin union, forming a common lateral skin flap, was made with the Bunster-Meyer technique (*7*), modified by deleting the abdominal muscle union. The skin suture clips were removed 7 to 9 days after the operation. Losses among these animals resulted primarily from parabiosis intoxication (*8*, *9*) and, much less frequently, from infection, physical separation, or chronic fighting. These losses amounted to 43 percent and always occurred within 3 weeks after the operation. The remaining pairs were subjected to the irradiation-conditioning procedure at 37 to 44 days of age. Within 24 hours after completion of the conditioning test, patency of the vascular anastomosis was tested in each pair by counting the activity in blood samples from both partners 1 hour after injection of Fe^{59}-labeled red cells into the irradiated partner. To serve as physical and behavioral controls approximating the parabiont condition without anastomosis, additional animals were physically tied in pairs by joining lateral skin folds with wound clips. Losses among tied pairs arose from tie separation and, sometimes, from chronic fighting. These losses amounted to 31 percent.

Separate measurement of drinking behavior for each partner was provided by a twin-tunnel device leading from the living cage to the drinking spouts (Fig. 1). For a period of 4 to 5 days prior to the start of experimental treatment the animal pairs were habituated cooperatively to use the tunnels in drinking.

A double-chambered radiation exposure unit was used to provide nearly complete shielding of the test partner of each pair. One member of each pair, randomly selected as the right- or left-hand partner, was exposed to 360 r with the x-ray machine operated at 250 kv (peak) and 15 ma (half-value layer, 1.5 mm Cu). The exposure rate, measured in air within the

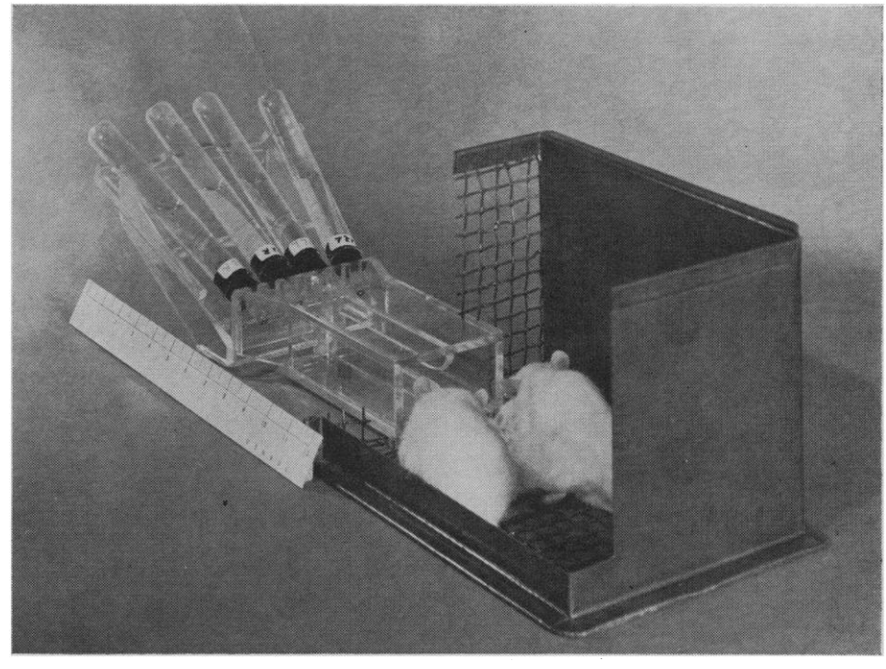

Fig. 1. Twin tunnels used to provide a separate drinking station for each partner of conjoined rat pairs. Shown in position on living cage (cut-away view). The septum between tunnels is slotted to accommodate the skin flap that unites the partners. The length of the tunnels suffices to preclude drinking at the incorrect station. Two drinking tubes, as used in the saccharin preference test, are shown at each station, and center positions are available for single-tube presentations. One-foot (30.5 cm) scale shown.

exposure chamber, was 24 r/min. During exposure, the shielded animal was exposed to less than 0.13 r/min, primarily through scatter to the abdominal region.

The sequence of irradiation, conditioning, and testing was essentially that employed with single animals for conditioning of a saccharin aversion after exposure (*4, 6*), except that the shielded test animal was presented with saccharin fluid following irradiation of its partner. The main experimental group, AS, consisted of anastomosed rats that were shielded during irradiation and were then presented with the saccharin fluid (0.1-percent solution of saccharin sodium, by weight). Two control groups were used: group A, composed of shielded anastomosed rats that were presented with nonflavored water (and, therefore, lacked the saccharin taste experience), and group S, composed of shielded tied rats that experienced the saccharin (but lacked the possibility of humoral transfer). The saccharin-flavored and nonflavored fluids were presented for a 1-hour period starting 40 minutes after irradiation. The grey back and side walls of the cage were covered with black metal sheeting immediately after irradiation for the purpose of enhancing the distinctiveness of the conditioning situation. Also, water deprivation was instituted 12 hours before presentation of saccharin to promote drinking, and deprivation was continued for a 3-hour period after presentation of saccharin.

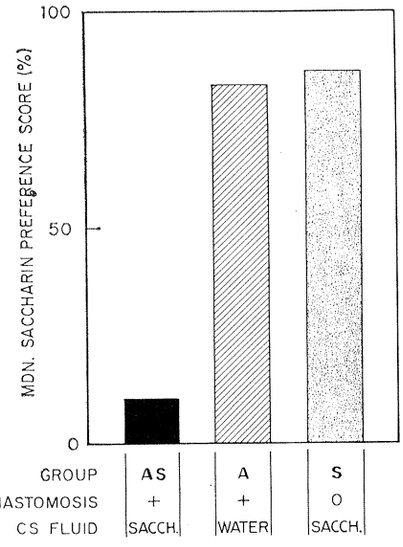

Fig. 2. Aversive conditioning to saccharin fluid obtained in shielded parabiont rats that experienced the taste of saccharin after the partner was exposed to 360 r of x-rays (24 r/min). Preference score

$$\left(\frac{\text{saccharin fluid}}{\text{total fluid}} \times 100\right)$$

was calculated from 24-hour consumptions in two-bottle test started 1 day after conditioning. MDN., median; AS, experimental group; A, control group (anastomosed); S, control group (tied); and CS, conditioned stimulus.

The relative preference for saccharin-flavored or nonflavored water was used as the test of conditioning. The preference test was started 1 day after the conditioning treatment and was continued for 24 hours. The amount of saccharin fluid consumed, expressed as a percentage of the total fluid consumption, served as the preference score. The location of the saccharin bottle, to the animal's right or left, was assigned so as to balance any side-preference among groups.

Although the behavior of irradiated partners of test animals was not of direct interest in this test, they also were tested to determine the adequacy of the situation for producing conditioning after exposure. Irradiated partners of A-group animals were presented with saccharin after irradiation, while those of the AS- and S-group animals received nonflavored water. As expected, the irradiated partners of A-group animals exhibited a strong conditioned aversion to saccharin in the subsequent preference test.

The study was completed with six replications of the experimental design, with 38 anastomosed and 17 tied pairs. Behavioral data were discarded from two pairs that showed failure of anastomosis patency, from one tied pair that developed fighting behavior, and from eight pairs in which one or both partners failed to consume a measurable amount of fluid during the period of conditioned-stimulus presentation. Data from the remaining pairs, 16, 14, and 14 in groups AS, A, and S, respectively, were used in the analysis.

Results of the preference test of conditioning in the shielded partners are summarized in Fig. 2. The experimental group, AS, in which the postulated humoral factor and the saccharin-taste experience were jointly available, rejected the saccharin fluid. The control group A, which was subjected only to the same humorally mediated effects of irradiation as group AS, exhibited a high preference for saccharin. A similarly high saccharin preference was exhibited by control group S, which lacked the humoral influence and was subjected only to the saccharin-taste experience. The chance probability for the reduced saccharin preference of the AS group, compared with that of the combined control groups, was < 0.002, according to the U-test analysis (*10*). Furthermore, these saccharin preferences were not related to total fluid consumptions in the preference test. The median fluid consumptions were 24, 32, and 26 grams for the AS, A, and S groups, respectively. It can be concluded, therefore, that the aversion to saccharin in the AS group resulted from conditioning which was motivated by humoral effects of irradiation.

Our results show that a noxious effect of irradiation can be mediated by a systemic humoral factor. To produce conditioning in the shielded animal, the factor must persist long enough to be transferred and to initiate motivation in the recipient. In order to motivate behavior, the factor also must convey information which in some manner, perhaps directly, affects the central nervous system.

This humoral motivating factor has, as yet, been identified only by behavioral analysis. The behavioral effect conceivably might reflect toxicity resulting from products of cellular breakdown or even a loss from the vascular system of a substance normally present. However, a more complete specification of the humoral factor, its source, fate, and dose dependence, depends on further investigation.

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