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Magnetic Field Conditioned Taste Aversion In Rats

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NOLTE, C. M., D. W. PITTMAN, B. KALEVITCH, R. HENDERSON AND J. C. SMITH. *Magnetic field conditioned taste aversion in rats.* PHYSIOL BEHAV **63**(4) 683–688, 1998.—Conditioned taste aversion is a common classic conditioning procedure used to identify noxious stimuli. When a rat is given a taste solution, the conditioned stimulus (CS), followed by an unpleasant experience, the unconditioned stimulus (US), the rat will avoid consumption of the CS in future presentations. These experiments use the taste aversion procedure to examine the effect of exposure to a high magnetic field. A solution consisting of 3.0 g glucose and 1.25 g saccharin per 1 L of solution (G+S) was used as the CS and a 9.4-T magnet served as the US. In Experiment 1, all rats received a 10 min presentation of the G+S solution followed by either a 30 min exposure to the magnetic field (Magnet, $n = 8$), a 30-min exposure in a container with similar conditions but lacking the magnetic field (Sham, $n = 8$), or no exposure (Control, $n = 8$). The Magnet Group showed a taste aversion on the first day of preference testing ($p < 0.05$). Experiment 2 employed the same US-CS protocol for 3 consecutive days of conditioning. The Magnet Group demonstrated a taste aversion for the postexposure Days 1—8 ($p < 0.01$). There was no difference between the Sham and Control Groups in either experiment. The results of this study clearly demonstrate that the rats associated the G+S solution with the experience of being exposed to the high magnetic field and avoided the solution in subsequent presentations. © 1998 Elsevier Science Inc.

Conditioned taste aversion Magnetic field

INVESTIGATION of biologic responses elicited by magnetic fields has increased in response to questions regarding the safety of conducting clinical imaging and spectroscopy at magnetic field strengths > 2 T (2,7). Excessive tissue heating and abnormalities in electrically excitable nerve, muscle, or myocardial tissue associated with radio frequency and switched gradient magnetic fields, respectively, are well characterized and are matters of concern when addressing patient safety (5,10). Potential pathological effects of static magnetic fields remain obscure although observed perturbations in the EEG pattern of squirrel monkeys (3), rabbits (11), and humans (18) exposed to fields ranging from 0.1 to 9.1 T, as well as, alterations in the cardiac rhythm of rats attributed to induced potentials generated by aortic blood flow in the presence of static magnetic fields (8) demonstrate that possible safety risks need to be addressed (1,2).

Induction effects such as these might explain reports by Weiss et al. (19) that rats placed in a T-maze, one arm of which extended into the bore of a horizontal magnet, would enter the magnet freely at field strengths of 0 and 1.5 T, but would avoid entering in 97% of the trials conducted at 4 T, a field reported by others (15) to induce mild sensory effects in humans including vertigo, nausea, transient visual sensations of flashing lights and a perceived metallic taste following whole-body exposure.

Conditioned taste aversion has proven experimentally useful as a sensitive measure of changes imposed on an organism's internal state. A conditioned taste aversion is a unique learning process by which an animal associates a novel taste (conditioned stimulus) with an aversive stimulus (unconditioned stimulus) so that, on subsequent exposure, the animal shows a decreased consumption of the flavor, interpreted historically as evidence of the deleterious effects of the unconditioned stimulus.

Messmer et al. (13) used the conditioned taste aversion paradigm to test for biologic influences of magnetic resonance imaging (MRI). Rats were given a 10-min exposure to a saccharin solution followed by a 30-min exposure to MRI in a horizontal superconducting magnet with a 1.89-T field. The magnet-exposed rats showed no significant aversion to the saccharin solution. These investigators concluded, therefore, that 30-min exposure to MRI at this field strength failed to reveal any "toxic" effects of magnet exposure.

Conditioned taste aversion learning has proven to be a very sensitive procedure for studying the effects of ionizing radiation exposure and a variety of drug treatments. It should be noted, however, that equating an observed conditioned taste aversion with a specific qualitative interpretation of a stimulus (such as toxicity) is tenuous because there have been demonstrations of failure to produce conditioned taste aversions to known poisons such as strychnine (14), and success in obtaining conditioned taste aversions to compounds that are self-administered (9). A more acceptable interpretation would be that the animal can perceive, either

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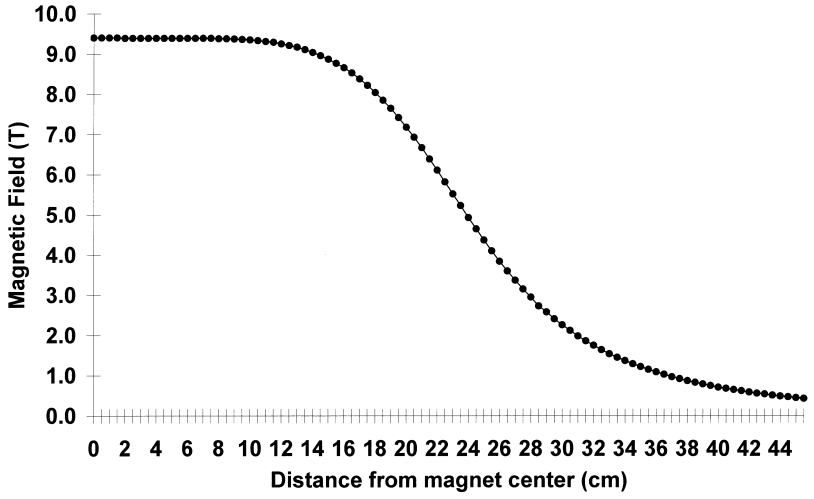

FIG. 1. Strength of the magnetic field measured in Teslas (T) from the center of the superconducting Oxford Instruments 400/89 magnet. 1 T = 10,000 Gauss. The 10-Gauss line is located 2.4 m from the center of the magnet.

directly or indirectly, the presence of the unconditioned stimulus and associate its presence with the novel taste.

Since Weiss et al. (19) have shown a strong aversive response to a 4-T magnet, it seems likely that the lack of a conditioned taste aversion learning seen by Messmer et al. (13) was due to the lower strength of the magnetic field. In the present study we give evidence for the formation of a conditioned taste aversion to a palatable glucose + saccharin solution upon exposure to a static 9.4-T magnetic field using a vertical bore superconducting magnet.

EXPERIMENT 1

Subjects

Twenty-four male Sprague–Dawley rats were housed individually in transparent plastic cages in a temperature-controlled colony room at the National High Magnetic Field Laboratory (NHMFL) in Tallahassee, FL. All animals were on a 12:12 light cycle with lights on at 0700 h, had access to Purina Rat Chow 5001 and deionized water ad lib. Four days prior to the Conditioning Day, all rats were placed on a water deprivation schedule with restricted access to water for 10 min per day starting at 0900 hours.

Apparatus

Magnetic field exposures were conducted using a superconducting Oxford Instruments 400/89 magnet (vertical bore of 6.7 cm) with a fixed-field strength of 9.4 T. As seen in Fig. 1, when measured along the vertical axis of the bore, the magnetic field degrades from 9.4 at the center to 9.3 T over ± 11 cm. The magnetic field was orientated vertically with the north pole at the bottom of the magnet.

A Plexiglas tube (5 cm in diameter) was used to restrain rats during magnet exposures. The top of the tube was fabricated in a cone shape to accommodate the head of the rat with a 1-cm hole at the cone's apex to allow for fresh breathing air. An open-centered plug which accommodated the tail was inserted into the bottom of the tube in order to restrain movement. When the rat was properly restrained in the tube, this chamber could easily be inserted into the magnet and elevated to the magnet's core. For "sham" exposure, rats were placed in the same restraining tubes described above and inserted into a large opaque tube with approximately the same dimensions as the bore of the magnet. The sham tube was placed in the same room as the magnet, beyond the point where the magnetic field strength was 5 gauss. A thermocouple was placed between the rat's body and the restraining tube wall to record body temperature during both magnet and sham exposures. The mean temperatures for the Magnet and Sham Groups were 32.5 ± 1°C and 34.1 ± 1°C, respectively.

Procedure

On the Conditioning Day (starting at 0800 hours), the animals were run in eight squads of three, one rat from each of three experimental groups: Magnet (n = 8), Sham (n = 8), and Control (n = 8). Each of the three rats was given access to a bottle containing a glucose + saccharin (G+S) solution (30 g glucose and 1.25 g saccharin per 1 L of solution) in their home cages for 10 min. Following the drinking period, the Magnet Group rat was immediately placed in a restraining tube and vertically inserted upward into the bore of the magnet and exposed at the core of the 9.4-T magnetic field for 30 min. At the same time, the Sham Group rat was placed into a restraining tube and vertically inserted into the sham tube for the same 30-min period. The Control Group rat from each squad remained in the home cage. The remaining seven squads of rats were treated consecutively in an identical fashion. After the magnet and sham exposures, animals were returned to their home cages and given ad lib access to food and water. Approximately 24 h after their respective exposures, the rats were given 2-bottle preference tests between water and G+S. These tests were conducted 24 h/day for the next 9 days.

Data Analysis

Preference scores were calculated in the following manner

$$(\text{G+S consumption})/(\text{G+S consumption} + \text{water consumption}) \times 100$$

Because the distributions were not normal, significance of group preference score differences was determined using a nonparametric analysis of variance Kruskal–Wallis test (12). In cases where significant group differences were found, Dunn's multiple comparison test (6) served as a post-hoc test to identify which group differences resulted in the significance.

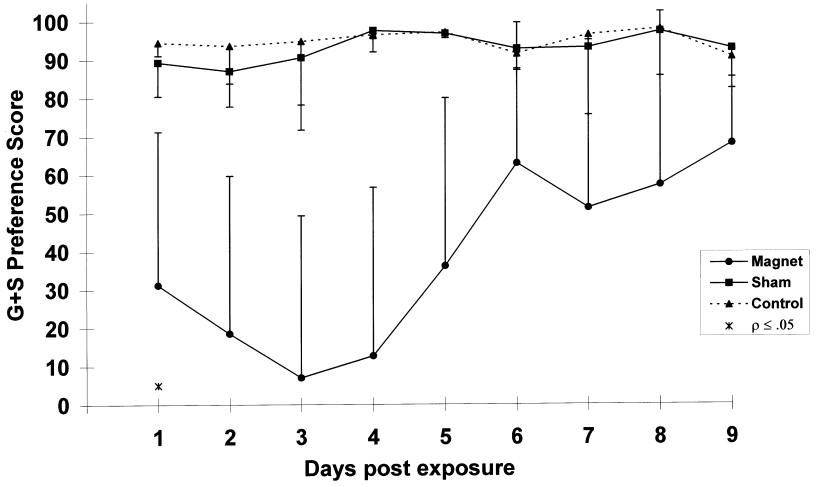

FIG. 2. Median percentage preference scores of G+S versus water for the Magnet (closed circle), Sham (closed square), and Control (closed triangle) Groups following one pairing of a G+S solution with a 30-min exposure to a 9.4-T magnetic field. The error bars represent one semi-interquartile range (SIQR).

Results

On the Conditioning Day all of the rats drank the G+S solution before their respective exposures. The mean consumption of the G+S solution for the Magnet, Sham, and Control Groups were 13.5, 13.3, and 11.5 g, respectively. Differences in mean consumption values were not significant ($H = 1.005$) at the $p < 0.05$ level. The data in Fig. 2 represent the median preference scores and semi-quartile intervals for all groups during the nine days of the 24-h two-bottle preference testing. Values for the 24-h median and semi-interval quartile ranges for water and G+S intake on the first postexposure testing day are: Magnet Group: water = 25.2 ± 14.4 g, G+S = 11.9 ± 16.2 g; Sham Group: water = 6.2 ± 2.2 g, G+S = 45.8 ± 19.9 g; Control Group: water = 4.3 ± 1.2 g, G+S = 64.8 ± 10.9 g. The Magnet-exposed group showed a taste aversion ($H = 8.705$, $p < 0.05$) only on the first day of preference testing. As shown in Fig. 3, within the Magnet-exposure group the individual responses of the rats varied from showing no taste aversion (Rats 1, 5, and 6) to profound taste aversion (Rats 2, 4, and 7) which remained strong through Day 8 of postexposure testing. Dunn's multiple comparison post-hoc tests revealed no differences between the preference scores of the Sham and Control Groups.

EXPERIMENT 2

Data from previously published ionizing radiation studies demonstrate that conditioned taste aversion formation, while possible after a single radiation exposure, is more pronounced following multiple exposures given on consecutive days (16). To test whether this observation was true of high magnetic field induced taste aversions, an experiment in which multiple exposures to 9.4 T on 3 consecutive days was conducted.

Subjects

A naive set of 24 male Sprague-Dawley rats was used in Experiment 2. These animals were housed at NHMFL under

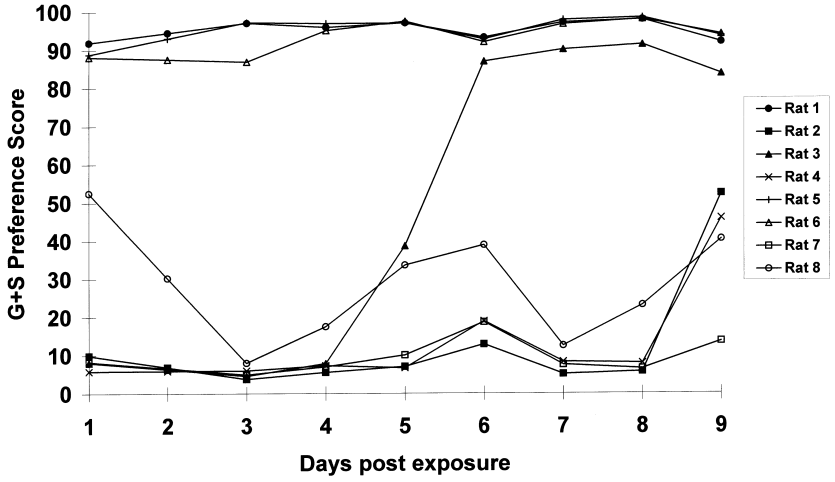

FIG. 3. Individual percentage preference scores of G+S versus water for the rats in the Magnet Group following one pairing of a G+S solution with a 30-min exposure to a 9.4-T magnetic field.

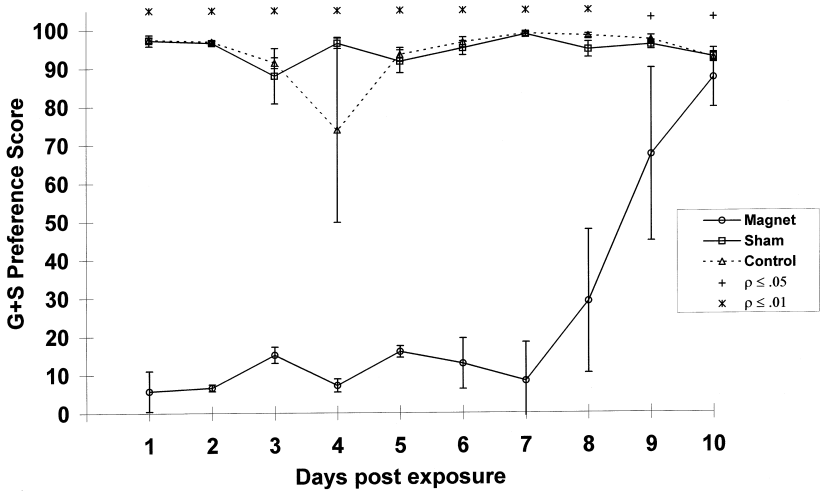

FIG. 4. Median percentage preference scores of G+S versus water for the Magnet (open circle), Sham (open square), and Control (open triangle) Groups following three consecutive days of pairing a G+S solution with a 30-min exposure to a 9.4-T magnetic field. The error bars represent one semi-interquartile range (SIQR).

similar conditions as the animals in Experiment 1. The rats were tested at 60 days of age. Four days prior to the testing day, all of the rats were placed on a water deprivation schedule and given restricted access to water for 10 min per day.

Procedure

The same procedure that was used in Experiment 1 prior to the Conditioning Day was employed in Experiment 2; however, in Experiment 2 the rats were given three consecutive Conditioning Days. As in Experiment 1, the 24 rats were separated into 3 experimental groups called: Magnet ($n = 8$), Sham ($n = 8$), and Control ($n = 8$). The testing began at 0800 hours each Conditioning Day. On the first Conditioning Day the animals were run in 8 squads of three, one from each of the three groups. Each of the three rats was given access to a bottle containing the G+S solution in their home cages for 10 min. At the end of the drinking period, the Magnet Group rat was immediately placed in a restraining tube and vertically inserted upward into the bore of the magnet. The rat was exposed at the core of the 9.4-T magnetic field for a period of 30 min. At the same time, the Sham Group rat was placed into a restraining tube and vertically inserted into the sham tube for the same 30-min period. The Control Group rat from each squad remained in the home cage. The remaining seven squads of rats were treated consecutively in an identical fashion. After the magnet and sham exposure period the animals were returned to their home cages where they remained on the water deprivation schedule described previously. On the second and third Conditioning Days, rats were treated in the same manner as on the first day except that the 8 squads of rats were run in different orders. After the third conditioning period, the rats were given ad lib access to food and water. Approximately 24 h after the third conditioning trial the rats were given two bottle preference tests between water and G+S. These tests were conducted 24 h per day for the next 10 days.

Results

On each Conditioning Day all of the rats drank the G+S solution before their respective exposures. The mean consumption of the G+S solution across all three days for the Magnet, Sham, and Control Groups was 12.4, 13.1, and 15.0 g, respectively. Differences in mean consumption were not significant ($H = 2.645$) at the $p < 0.05$ level. The median preference scores for Experiment 2 are shown in Fig. 4. Values for the 24-h median and semi-interval quartile ranges for water and G+S intake on the first postexposure testing day are: Magnet Group: water = 33.2 ± 4.9 g, G+S = 2.3 ± 1.5 g; Sham Group: water = 1.4 ± 0.4 g, G+S = 48.4 ± 5.3 g; Control Group: water = 1.8 ± 0.4 g, G+S = 70.2 ± 19.4 g. The animals in the Magnet-exposure group demonstrated a taste aversion ($H > 9.2$, $p < 0.01$) for the postexposure Days 1 through 8. On Days 8, 9, and 10, taste aversion extinction became evident for some animals in the Magnet-exposure group. However, the Magnet Group still demonstrated a taste aversion that was significant at the $p < 0.05$ level for Days 9 and 10. As demonstrated in Fig. 5, the variability of the preference scores for the Magnet-exposed group was dramatically reduced in Experiment 2 as compared with the variability in Experiment 1. Although Rat 6 showed no taste aversion, the remaining 7 rats showed a robust taste aversion through Day 5 postexposure. After Day 5, more variability was seen as the rats began to show extinction. Dunn's multiple comparison post-hoc tests found there were no significant differences between the preference scores of the Sham and Control Groups.

Discussion

The present experiments demonstrate that of the rats given one pairing of the G+S solution with a 30-min exposure to a 9.4-T magnetic field, only four rats of the eight in the Magnet Group showed a statistically significant aversion to the sweetened solution, when compared with the Sham or the Control Groups; however, this aversion was evident through the fourth day of preference testing. In contrast, when three daily pairings of the G+S solution and the 30-min magnet exposure were made, seven out of eight of the rats showed a profound taste aversion that lasted through Day 7 of preference testing. The magnitude of the taste aversion demonstrated in the 3-day exposure test was very similar to the taste aversion seen with one pairing a solution and a 100-rad whole body exposure to γ-rays (16).

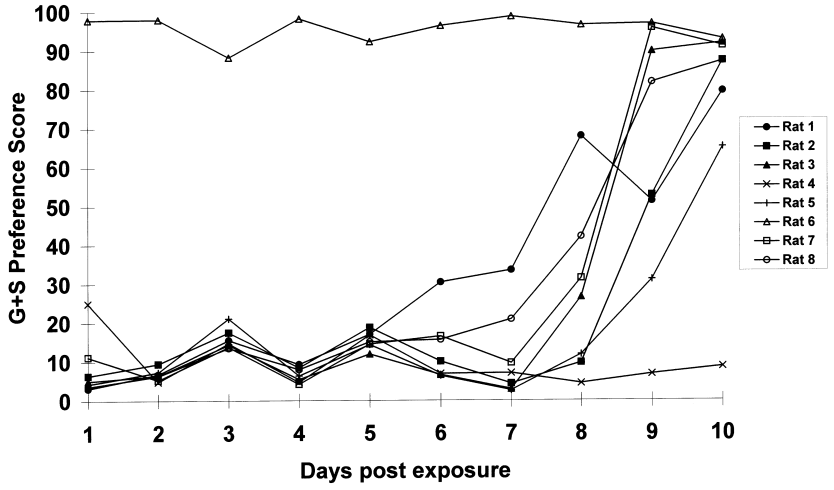

FIG. 5. Individual percentage preference scores of G+S versus water for the rats in the Magnet Group following three consecutive days of pairing a G+S solution with a 30-min exposure to a 9.4-T magnetic field.

Messmer et al. (13) were unable to demonstrate a taste aversion when they presented one pairing of saccharin-flavored water with a 30-min exposure to a 1.89-T magnet. These investigators pointed out that a longer exposure, or repeated exposures may result in a conditioned taste aversion. Considering the results of the present experiment, it seems likely that there is a threshold involving the strength of the magnetic field and the repetition of magnetic field exposure for producing a taste aversion. Weiss et al. (19) demonstrated such a threshold effect using a T-maze, one arm of which extended into a horizontal magnet. The rats would readily enter the magnet when the strength was 1.5 T, but avoided the "magnet" arm of the T-maze when the strength of the magnet was 4 T.

It would be premature to assume that the "detection" of a magnetic field as shown by Weiss et al. (19) and the conditioned taste aversion of a 30-min magnet exposure are mediated by the same biologic effect. By analogy, it has been shown that the reception mechanisms for "immediate detection" of ionizing radiation and the use of these rays as an unconditioned stimulus in a taste aversion experiment are quite different. The immediate detection is dependent on head exposure and has been shown to be mediated by olfactory and/or visual mechanisms (4). In addition, the immediate reaction of a rat to the onset of a radiation exposure is dependent upon the rate of irradiation. On the other hand, when used as an unconditioned stimulus in a taste aversion experiment, head irradiation is not a necessary condition and the total exposure, not rate of exposure is critical (17). Whether longer exposures to lower strength magnets will result in the same magnitude of taste aversion as short exposures to high strength magnets awaits further research.

Weiss et al. (19) concluded that the detection of the magnetic field by the rats in their T-maze probably resulted from movement of the rat through the magnetic field gradient. From a preliminary experiment performed in our laboratory, we have produced evidence that the magnetic field-conditioned taste aversion is not the result of passing through the gradient, but is dependent on total exposure in the uniform field. In this pilot study, 22 male rats were assigned to four groups. A Magnet Group ($n = 6$) received a 10-min access period to saccharin-flavored water followed by a 30–min exposure to the 9.4-T magnet as described previously. A Sham Group ($n = 5$) received a 30-min period in the sham tube after 10 min drinking the saccharin solution. A Gradient Group ($n = 5$) received 10-min access to the saccharin followed by a 30-min exposure in the gradient field of the 9.4-T magnet. They were inserted approximately 15 cm into the bore of the magnet where the field strength varied from 8.9 T at the head to 3.3 T at the tail of the rat. The Eddy Current Group ($n = 6$) received 10 min of saccharin and then was raised and lowered through the gradient to the core of the magnet five times. A string from a motorized crank was tied to the Plexiglas exposure tube, allowing the rat to be raised to the core of the magnet and lowered in an approximately sinusoidal 10-s cycle. The time to pass through a majority of the gradient was approximately 2.5 s in either direction. The time in the core was approximately 5 s. The Sham, Gradient and Eddy Current Groups showed no difference in their average preference scores for the saccharin-flavored water over 13 days of testing (86, 85, and 89%, respectively), as compared with the Magnet Group receiving 30-min exposure, whose average preference score was significantly lower (45%). Movement of the rat through the gradient may be quite important for the detection of the magnet as demonstrated by Weiss et al. (19), but it is unlikely that movement through the gradient into the magnetic field is the unconditioned stimulus that produces the conditioned taste aversion.

From the current research we know little about the duration of the effects of the magnet exposure. Experiments in which the taste solution was presented following, rather than preceding, the magnet exposure could yield important information about any lingering effects. To ensure that the results of the current research represent a conditioned taste aversion and not some lingering effect of the magnet exposure, future experiments will include a group of rats that receive the magnet exposure without experiencing the taste of the G+S solution.

The availability of high power magnetic fields (≥ 1.5 T) for use in clinical diagnostic settings has increased the importance of studies seeking to identify and understand biologic responses to these levels of magnetic field exposure. Because electromagnetic radiation effects upon an organism are largely influenced by that organism's size, the sensitivity of the exposed tissues and the duration of exposure, extrapolation of findings from experiments using laboratory animals to the major human parallel of magnetic resonance imaging should be cautiously pursued. In our experiments, conditioned taste aversion was observed in response to both a single 30-min exposure to a 9.4-T static magnetic field, as well as to three consecutive 30-min exposures at the same static field

strength. These exposures are quite different than those incurred in the clinical use of MRI, which are generally of shorter duration, of lesser field strength and involve static magnetic fields in addition to gradient and radiofrequency magnetic fields.

As was pointed out earlier, although several exposures in the core of the 9.4-T magnet are sufficient to result in a conditioned taste aversion, it cannot be concluded that some sort of "toxicity" is caused by the magnetic field exposure. Taste aversions have been conditioned with a variety of drugs that are not considered toxic but simply put the rat in some altered state (9). Therefore, it is important to understand the mechanism producing an unconditioned effect to the exposure in a magnetic field.

This study produced data to suggest that a 9.4-T magnetic field is sufficient to condition a taste aversion in a rat. Furthermore, this study demonstrated that the magnetic field conditioned taste aversion could be strengthened by increasing the number of exposures in the magnetic field. This manipulation of the magnetic field conditioned taste aversion is consistent with the properties of other conditioned taste aversion paradigms. Future research is planned to isolate the mechanisms underlying the unconditioned effect of magnetic field exposure.

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