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Sex and estrous cycle differences in the behavioral effects of high-strength static magnetic fields: role of ovarian steroids

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Cason, Angie M., Megan DenBleyker, Kimberly Ferrence, James C. Smith, and Thomas A. Houpt. Sex and estrous cycle differences in the behavioral effects of high-strength static magnetic fields: role of ovarian steroids. *Am J Physiol Regul Integr Comp Physiol* 290: R659–R667, 2006. First published October 13, 2005; doi:10.1152/ajpregu.00305.2005.—Advances in magnetic resonance imaging are driving the development of higher-resolution machines equipped with high-strength static magnetic fields (MFs). The behavioral effects of high-strength MFs are largely uncharacterized, although in male rats, exposure to 7 T or above induces locomotor circling and leads to a conditioned taste avoidance (CTA) if paired with a novel taste. Here, the effects of MFs on male and female rats were compared to determine whether there are sex differences in behavioral responses and whether these can be explained by ovarian steroid status. Rats were given 10-min access to a novel saccharin solution and then restrained within a 14-T magnet for 30 min. Locomotor activity after exposure was scored for circling and rearing. CTA extinction was measured with two-bottle preference tests. In *experiment 1*, males were compared with females across the estrous cycle after a single MF exposure. Females circled more and acquired a more persistent CTA than males; circling was highest on the day of estrus. In *experiment 2*, the effects of three MF exposures were compared among intact rats, ovariectomized females, and ovariectomized females with steroid replacement. Compared with intact rats, ovariectomy increased circling; estrogen replacement blocked the increase. Males acquired a stronger initial CTA but extinguished faster than intact or ovariectomized females. Thus the locomotor circling induced by MF exposure was increased in females and modulated by ovarian steroids across the estrous cycle and by hormone replacement. Furthermore, female rats acquired a more persistent CTA than male rats, which was not dependent on estrous phase or endogenous ovarian steroids.

conditioned taste aversion; locomotor circling; estrogen; progesterone

ADVANCES IN MAGNETIC RESONANCE imaging (MRI) are leading to the development of more powerful MRI machines capable of producing higher-resolution images, but the sensory, neural, or somatic effects of high-strength static magnetic fields (MFs) on mammals and humans are virtually unexplored. MF exposure under 2 T does not appear to be detectable by humans and has been reported to have no effect on a variety of behavioral tasks in rats (28, 42, 45, 56, 65). However, there have been reports of vertigo and nausea in humans between 4 T and 8.4 T using MRI machines, suggesting stimulation of visceral or sensory systems (29, 56).

Our laboratory has discovered that a 30-min exposure to a 7, 9.4, or 14 T MF has behavioral and neural effects in male rats and mice (25, 37, 44, 59). At the behavioral level, MF exposure suppressed normal rearing and induced tight locomotor circling in a counterclockwise direction for the first few minutes after magnet exposure. The direction of circling was dependent on the orientation of the MF, such that when the rat was exposed head-up toward the positive pole, he circled in a counterclockwise direction in the horizontal plane when viewed from the dorsal perspective. Exposure head-down toward the negative pole induced horizontal clockwise circling as viewed dorsally (25). Furthermore, we observed that when the MF exposure was paired with a novel taste solution (glucose + saccharin), a conditioned taste avoidance (CTA) or aversion was induced similar to that seen after pairings of taste and rotation or motion sickness (3, 7, 16, 21, 25, 27, 37, 44). At the neural level, MF exposure induced significant c-Fos immunoreactivity, a marker of neuronal activation, in specific vestibular and visceral nuclei within the rat brainstem (59).

The circling seen in rats immediately after MF exposure is strikingly similar to the locomotor circling seen after unilateral hemilabyrinthectomy (33). The suppression of rearing behavior after magnet exposure is similar to the suppression of rearing seen after horizontal whole-body rotation (47). MFs and vestibular stimulation by rotation induce similar c-Fos patterns in the rat brain stem (30–33, 40). Because these results demonstrate parallel responses to vestibular activation and magnet exposure, they suggest that rats may be experiencing a vestibular disturbance similar to the self-reports of humans after MF exposure (56).

Although sex differences in the effects of high-strength, static MFs have not been previously reported, sex differences have been reported in all three dependent measures (circling, rearing, and CTA) used to determine the behavioral effects of MF exposure. Becker and colleagues (5, 6, 54) have shown that females display more rotational behavior than males following amphetamine treatment and that estrous stage and ovarian status modulate this behavior. In general, female rats show increased locomotor and rearing behavior compared with males. Gonadectomy in male rats has no effect, but in female rats, it decreases locomotion and rearing (61, 62). Sex differences have also been reported in lithium chloride-induced CTA, particularly in that males require a longer time to extinguish CTA (8, 10, 50, 64, 66, 67). Sex differences might exist in MF responses that might be of clinical significance, particularly if high-strength MFs interact with the vestibular system. There is some evidence that the women are more sensitive than men to vestibular stimulation. Women are more

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prone to orthostatic intolerance (19, 51, 53) and motion sickness than men (14, 48), and women report a high level of vertigo and dizziness during the premenstrual period (1). Thus, as MFs in clinical MRI machines increase in strength, it is possible that women might experience more vestibular side effects than men.

Our laboratory has collected preliminary data suggesting that females might be more sensitive to the effects of MFs than male rats (26). There has been no formal test, however, of the role of endogenous steroids in modulating the responses to MFs. Therefore, the present study compared the behavioral effects of MF exposure in male and female rats to determine whether there are sex differences in the behavioral responses to high-strength MF exposure and whether ovarian steroids (estradiol and progesterone) in female rats affect the behavioral response.

We examined the role of sex and ovarian hormones in two ways. First, we compared the response of female rats at three stages of the estrous cycle to the response of male rats after all had received MF exposure. This allowed us to observe sex differences and variations in female behavior that correlated with changing levels of ovarian hormones across the estrous cycle.

Second, we compared ovariectomized rats with and without chronic estrogen and progesterone replacement to intact females and males after MF exposure. This allowed us to distinguish the organizational effects of sex (which persist after gonadectomy) from the activational effects of ovarian steroids (induced by hormone replacement) on the response to the magnetic field.

Rats were maintained on a water restriction schedule and given 10-min access to a novel saccharin solution before one or three exposures to a 14-T MF for 30 min. Three responses were measured: *1*) the acute induction of locomotor circling, *2*) the acute suppression of rearing, and *3*) the expression of a CTA to saccharin.

METHODS

All procedures and experiments were reviewed and approved by the animal care and use committee of the Florida State University.

Subjects

Male and female Sprague-Dawley rats (200–225 g; Charles River Laboratories, Wilmington, MA) were housed individually in polycarbonate cages in a temperature-controlled colony room at the National High Magnetic Field Laboratory at The Florida State University. The rats were maintained on a 12:12-h light-dark cycle with lights on at 7:00 AM. All procedures were conducted during the light cycle. The rats had ad libitum access to Purina Rat Chow and deionized, distilled water except where specified otherwise.

Magnet

The magnet exposure was conducted in a superconducting magnet with a vertical bore designed for biochemical nuclear magnetic resonance studies. The 14-T magnet was a 600-Mhz Bruker Cryo magnet with an 89-mm bore and fixed field strength of 14.1 T. It contained a shim magnet extending along the magnet bore for approximately ±15 cm from the magnet core, which was used to stabilize the magnetic field and give a central core field of uniform strength. The magnetic field was oriented vertically, so that the positive pole was at the top of the magnet. The magnet was operated without radiofrequency pulses, so rats were exposed only to static magnet fields.

Conditioning Procedure

Rats were conditioned against saccharin by pairing it with MF exposure using a procedure similar to earlier experiments (25, 44). Nine days before conditioning day, the rats were placed on a water-restriction schedule. In the first day of water restriction, rats had access to water for 3 h/day. The period of access was diminished over 8 days to 30 min/day as described below. In *experiment 1*, the initial period of water access was 3 h/day, and the period of water access was diminished over 8 days to 1 h/day with a 30-min supplement in the late afternoon to minimize disruption of estrous cycling. In *experiment 2*, the initial period of water access was 3 h/day, and the period of water access was diminished over 8 days to 30 min/day without supplement. The conditioned stimulus was a solution of 1.25 g of sodium saccharin mixed in 1 liter of deionized, distilled water. The rats were allowed 10-min access to saccharin. If the rat failed to drink 2 ml during this 10-min presentation of saccharin, the bottle was returned to the cage for an additional 5 min. Because the amount of saccharin consumed might affect CTA magnitude and because female rats tended to drink less saccharin than males, intakes were matched post hoc to ensure that all groups consumed the same amount of saccharin (i.e., males and ovariectomized females with high intakes, and females with low intakes were excluded; see Table 1 for intakes).

Immediately after saccharin access, rats were placed in a Plexiglas restraint tube that had an inside diameter of 56 mm and an outside diameter of 64 mm. A cone shaped plug with a 1-cm hole at the apex was inserted in the rostral end of the restraint tube to accommodate the head of the rat and to allow fresh air for breathing. A second plug was inserted in the caudal end of the restraint tube and could be adjusted to restrain the movement of the rat. It had a 1-cm hole in the center to accommodate the rat's tail. When in the tube, the rat was almost completely immobile. Restrained rats were carried individually to the 14 T magnet, where the rat was inserted head-up into the bottom of the vertical bore of the magnet. The rat was quickly raised through the magnet until the head of the rat was in the core of the magnetic field. Rats remained in the 14-T MF for 30 min.

To control for restraint and handling, additional rats were sham-exposed. Sham-exposed rats were allowed 10-min access to the

Table 1. *Mean saccharin intake before sham- or magnet-exposure after matching intake across groups by excluding rats with high or low intakes*

Groups	Saccharin Intake During Conditioning (g)	
	Sham-Exposed Rats	Magnet-Exposed Rats
	Experiment 1	
Male	5.8±0.5 (n=8)	5.7±0.6 (n=8)
Proestrus	6.1±0.5 (n=10)	5.1±0.7 (n=7)
Estrus	6.5±0.6 (n=10)	5.4±0.6 (n=8)
Metestrus	6.1±0.6 (n=8)	4.9±0.7 (n=12)
Excluded rats		
Male	11.8±0.7 (n=8)	11.7±0.9 (n=8)
Proestrus	4.1±0.7 (n=6)	
	Experiment 2	
Male	10.0±1.8 (n=6)	7.3±1.4 (n=6)
Intact	10.1±1.4 (n=6)	7.2±0.5 (n=8)
OVX	10.4±1.8 (n=6)	7.8±0.4 (n=10)
OVX + E	9.4±1.2 (n=6)	8.1±0.8 (n=6)
OVX + EP	9.5±1.3 (n=6)	6.1±0.2 (n=6)
Excluded rats		
Male	14.2±1.4 (n=6)	12.0±0.5 (n=6)
Intact	7.4±1.4 (n=6)	4.2±0.7 (n=4)
OVX	13.3±1.6 (n=6)	13.3±1.7 (n=2)

Values for saccharin intake are presented as means ± SE; n is number of rats.

saccharin solution and were inserted into identical restraint tubes. Then, the sham-exposed rats were inserted vertically into an opaque polyvinylchloride pipe with dimensions and conditions similar to those of the bore of the 14-T magnet. The sham apparatus was located in the same room as the 14-T magnet, but placed outside the 5 Gauss field. One magnet-exposed and one sham-exposed rat were conditioned simultaneously. Rats were not habituated to the restraint tube before their first magnet or sham exposure.

Locomotor Activity

Following magnet or sham exposure, the rostral plug of the restraint tube was removed; and the rat was allowed to emerge into an open polycarbonate cage (37 cm wide × 47 cm long × 20 cm high). The floor of the cage was covered with chip bedding. The locomotor behavior of each rat was recorded on videotape for 2 min after release into the cage. Then, the rat was returned to its home cage and returned to the animal facility. Rats were not acclimated to the novel testing chamber. An observer blind to the rats' treatment and hormonal condition scored the videotapes later. Instances of tight circling behavior, latency to rear (latency to the first rear), and rearing behavior (both forepaws on the side of the cage) were quantified. Circles were counted if the rat moved continuously around a full circle with a diameter less than the length of the rats' body. Partial circles or circles interrupted by stationary pauses were not counted.

Preference Tests

Two-bottle, 24-h preference tests were begun on the day after the last conditioning trial to test for magnetic field-induced CTA. Each day, two bottles were placed in the cages, one containing the saccharin solution and the other containing distilled water. The position of the two bottles was reversed each day. Fluid consumption was measured every 24-h and a preference score was calculated as a ratio of saccharin intake to total fluid intake. The preference tests were continued for 12 postconditioning days. Because saccharin access during the preference tests was not paired with magnet exposure, the preference tests constituted extinction trials. A CTA was considered significant if the preference score of the magnet-exposed group was significantly less than the preference score of the sham group. A CTA was considered extinguished when the average saccharin preference of magnet-exposed rats was not significantly different from the sham-exposed rats. Thus two measures were taken: *1*) first-day preferences, which show the initial magnitude of the CTA; and *2*) preferences across the extinction trials, which show the persistence and rate of extinction of the CTA.

Statistical Analysis

All data were analyzed using Statistica software (Statsoft, Tulsa, OK), as described below. When the ANOVA revealed a significant difference, Tukey's honestly significant difference was performed to determine significant differences between specific groups.

Experiment 1. Single Pairing of Saccharin and Magnet Exposure Across the Estrous Cycle

The purpose of *experiment 1* was to evaluate the effects of a single pairing of saccharin and magnet exposure across the female rat's estrous cycle and to compare these effects to males. Estrous cycles were monitored daily throughout the entire experiment by vaginal lavage taken 2 h after lights-on. Vaginal smears were microscopically examined to determine the stage of the estrous cycle. Phases of the cycle were identified using standard criteria (18, 38). The day of proestrus (P) was characterized by large clumps of nucleated epithelial cells. The day of estrus (E) was characterized by large clumps of cornified cells, and the day of metestrus (also known as the day of diestrus I) was characterized by the predominance of leukocytes interspersed with nucleated and cornified cells. These days were chosen to coincide with different levels of circulating ovarian steroids, particularly estrogen. The circulating levels of estradiol and progesterone reach a peak during the day of proestrus, are the lowest on the day of estrus, and are intermediate on the day of metestrus. Diestrus was not tested because circulating estrogen levels are intermediate while progesterone levels are low (18). Only rats with at least two normal 4-day cycles before being placed on water deprivation and that maintained normal 4-day cycles were used in this study.

Rats were individually housed and placed on a water restriction schedule. The initial period of water access was 3 h/day, and the period of water access was diminished over 8 days to 1 h/day with a 30-min supplement in the late afternoon to minimize the disruption of estrous cycling. Female rats were divided into three groups based upon estrous stage on the day of conditioning: proestrus ($n = 23$), estrus ($n = 18$), and metestrus ($n = 16$). A total of 33 males were conditioned (see Table 1). One half of the rats from each group received MF exposure, while the other half received sham exposure. Locomotor behavior was recorded for 2 min as described above. The rats were returned to their home cages and given ad libitum access to water. On the following day, the first 24-h, two-bottle preference test between saccharin and water began.

Statistical analysis. To determine whether there were differences in locomotor variables (number of circles, latency to rear, or number of rears) among rats treated on different phases of the estrous cycle, we compared male rats to female rats in three estrous stages: proestrus, estrus, and metestrus. Because none of the sham-exposed rats circled, a one-way ANOVA was calculated for the number of circles in MF-exposed rats only. For rearing, factorial ANOVAs (2 × 4) were calculated comparing latency to rear and number of rears. The two main factors were exposure (magnet or sham) and group (male, proestrus, estrus, and metestrus). To determine the effects of estrous stage on CTA acquisition, a repeated-measures ANOVA was calculated comparing the preference scores of MF- and sham-exposed rats across 12 days of two-bottle preference tests. The two main factors were group and test day (the repeated measure).

Experiment 2. Three Pairings of Saccharin and Magnet Exposure in Male and Female (Intact and Hormone-Treated) Rats

Experiment 1 showed that there were sex and estrous cycle differences in circling behavior and CTA after MF exposure. *Experiment 2* was designed to determine the role of ovarian hormones in these differences in response to MF exposure. To increase the magnitude of MF-induced effects, rats received three pairings of saccharin and MF exposure across 3 days.

A total of 72 female rats and 24 male rats were individually housed as above. Bilateral ovariectomy was performed through an intra-abdominal approach under halothane anesthesia in 48 female rats. A midline 2-cm incision was made and the uterine horns externalized and clamped with a hemostat below the fallopian tubes. The ovaries were excised by scalpel, and the incision sutured closed. During the same surgery, Silastic capsules were inserted subcutaneously in the neck of 24 rats. Twelve rats were implanted with Silastic capsules containing crystalline estradiol (OVX+E), and 12 rats were implanted with Silastic capsules containing estradiol and progesterone (OVX+EP). The estradiol (E) capsules were 5 mm in length with 1.57 mm (ID), and the progesterone (P) capsules were 35 mm in length with 3.35 mm (ID). Identical implants produced serum E concentrations of 157 ± 80 pg/ml and P concentrations of 6 ± 1 ng/ml in previous studies using rats of the same strain and size (20). (Note that the expected E levels obtained would be somewhat higher than the maximum level of E during proestrus; the expected P levels would be equivalent to endogenous P levels at the time points tested in *experiment 1*.) The remaining 24 ovariectomized (OVX) rats did not receive Silastic capsules.

Two weeks after surgery, all rats were placed on a water restriction schedule. The initial period of water access was 3 h/day, and the

period of water access was diminished over 8 days to 30 min/day without supplement. Female rats were divided into four groups based upon surgical and steroid treatment on the day of conditioning: intact, OVX, OVX+E, and OVX+EP.

Rats received three pairings of saccharin with MF or sham exposure. After the first pairing and locomotor videotaping, the rats were returned to their home cages and remained on water restriction until the next day, when the second conditioning trial was started. This procedure was repeated for a third day of conditioning. After the third pairing of saccharin + MF (or sham) and locomotor recording, the rats were returned to their home cages and given ad libitum access to water. On the following day, the first 24-h, two-bottle preference test between saccharin and water began. These 24-h tests were continued for 12 days.

Statistical analysis. To detect significant differences in locomotor variables, a two-way, repeated-measures ANOVA was calculated for each variable (number of circles, number of rears, and latency to rear) after 14-T MF exposure on three consecutive conditioning days. The two main factors were group (intact male, intact female, OVX, OVX+EP, OVX+E) and conditioning day (the repeated measure). The effects of ovarian steroids on CTA expression were determined by two-way repeated-measures ANOVA comparing the preference scores of sham-exposed rats and magnet-exposed rats across 12 test days. The two main factors were group and test day (the repeated measure)

RESULTS

Experiment 1

Estrous cycling. Water deprivation, MF exposure, or restraint did not disrupt the estrous cycle of female rats. All female rats maintained normal 4-day estrous cycles throughout preference testing.

Conditioning day intakes. Males as a group consistently had higher intakes (8.7 ± 0.6 g) compared with females (5.6 ± 0.2 g). Therefore, conditioning day intakes were matched across groups to control for saccharin intake on conditioning day. Seventeen male rats (8 magnet, 9 sham) with the highest intakes and 6 female rats in proestrus on the day of sham exposure with the lowest conditioning day intakes were dropped from the study. After these exclusions, there were no significant differences in conditioning day intake among the remaining rats regardless of estrous stage, sex, or exposure group (average across all groups = 5.7 ± 0.2 g; see Table 1).

Locomotor effects. There was a difference among estrous stages in the number of circles induced by MF exposure [$F(3,27) = 3.80$, $P < 0.05$]; see Fig. 1*A*). MF-exposed estrus females circled significantly more than MF-exposed males ($P < 0.01$). Females in proestrus and metestrus displayed intermediate levels of circling compared with intact males.

There was a significant effect of group [$F(3,58) = 3.30$, $P < 0.05$] and exposure [$F(1,58) = 200.23$, $P < 0.001$] on latency to rear, but no significant interaction (see Fig. 1*B*). Latency to rear was longer in MF-exposed rats than sham-exposed rats. Female sham-exposed rats, regardless of estrous stage, had shorter latencies to rear than male sham-exposed rats.

There was a significant effect of exposure on number of rears [$F(1,58) = 218.64$, $P < 0.001$], such that MF exposure effectively suppressed rearing in all groups compared with sham-exposed rats (see Fig. 1*C*). There was no significant effect of group on the number of rears and no significant interaction with exposure.

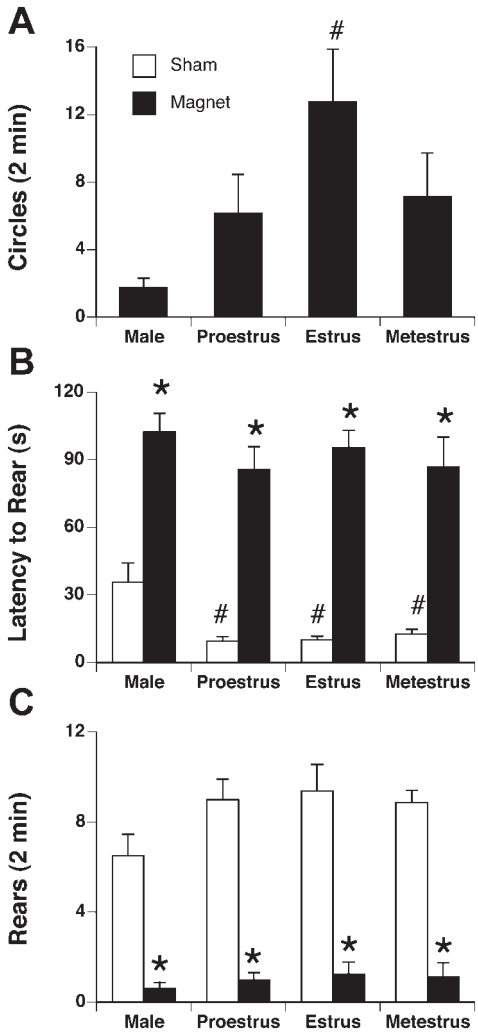

Fig. 1. Estrous cycle influences on locomotor behavior after a single pairing of saccharin and 14-T magnetic field (MF) (solid bars) or sham exposure (open bars) for 30 min. *A*: circling (means ± SE) was significantly greater in female MF-exposed rats in estrus than in male MF-exposed rats. No sham-exposed rat circled. *B*: latency to rear (means ± SE) was significantly longer in MF-exposed rats compared with sham-exposed rats; female sham-exposed rats, regardless of estrous stage, had shorter latencies to rear than male sham-exposed rats. *C*: MF exposure, regardless of sex and estrous stage, suppressed rearing compared with sham exposure, regardless of sex and estrous stage. Female sham-exposed rats reared more than male sham-exposed rats. *$P < 0.05$ vs. sham-exposed rats; #$P < 0.05$ vs. same treatment in males.

CTA effects. There was no significant difference among the preference scores of male and female rats that were sham-exposed on the day of proestrus, estrus, or metestrus. Therefore, their data were combined into one sham-exposed group for analysis. The remaining four groups were the male MF-exposed rats, and female MF-exposed rats, conditioned on one of three stages of the estrous cycle: proestrus, estrus, or metestrus.

After MF exposure, rats acquired significant CTAs that were not different among groups on the first day (see Fig. 2*A*) but that extinguished at different rates across test days (see Fig. 2*B*). A Student's *t*-test comparing the initial magnitudes of CTA between included males (low intake) and excluded males (high intake) showed that there was no difference ($P = 0.80$) in saccharin preference on the first day of two-bottle testing

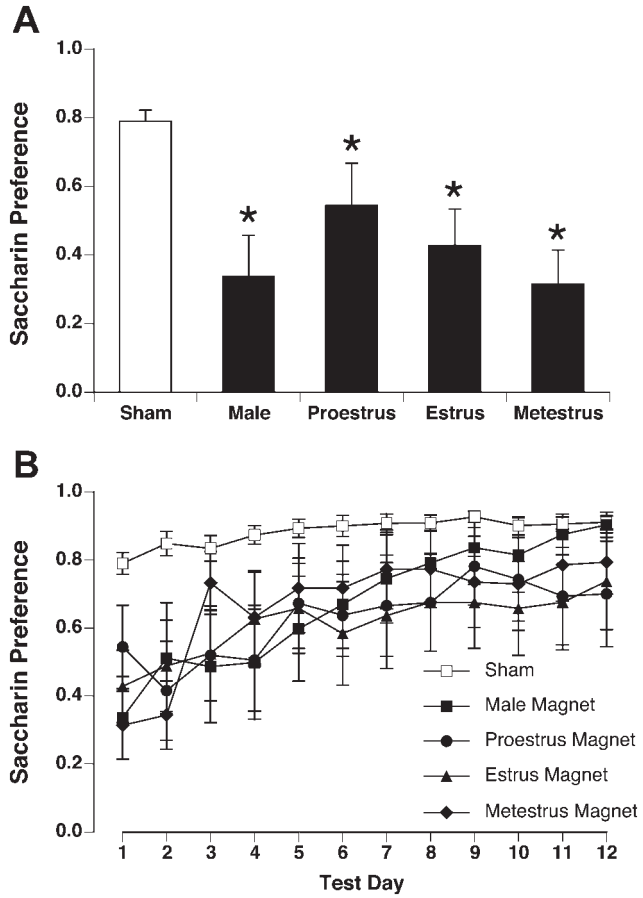

Fig. 2. Estrous cycle influences on conditioned taste avoidance (CTA) after a single pairing of saccharin and a 14-T MF or sham exposure for 30 min. *A*: on the first day of two-bottle testing, MF-exposed rats (solid bars) showed significantly lower preference scores (means ± SE) than sham-exposed rats (open bars), but there was no effect of estrous stage. **P* < 0.05 vs. sham-exposed rats. *B*: CTA of male MF-exposed rats (■) extinguished faster (after 8 days) than female MF-exposed rats, regardless of estrous stage. Proestrus (●), estrus (▲), and metestrus (◆) CTA of female MF-exposed rats failed to extinguish across the 12 test days measured.

(see Table 1). Repeated-measures ANOVA revealed a significant interaction between treatment group and test day [$F(44,682) = 3.68$, $P < 0.001$]. In particular, the CTA of the male group extinguished after 8 days of two-bottle testing, while the proestrus, estrus, and metestrus MF-exposed groups failed to completely extinguish across the 12 test days measured (i.e., their preference scores remained significantly below the preference scores of sham-exposed rats).

Experiment 2

Conditioning day intakes. To ensure that groups did not differ in saccharin intake on conditioning day, intakes were matched post hoc by discarding the lowest or highest intakes within groups until intakes were equal among groups. Because male and OVX rats typically had higher conditioning intakes (10.6 ± 0.1 g) than intact and hormone-treated rats (7.9 ± 0.1 g), 12 male rats (6 magnet, 6 sham) and 8 OVX rats (2 magnet, 6 sham) were dropped from the study. In addition, 10 intact females (4 magnet, 6 shams) were excluded from the study because they had lower conditioning day intakes than the other rats. After post hoc matching, overall mean saccharin intake was 7.4 ± 0.30 g (see Table 1).

Locomotor effects. Because none of the sham-exposed rats circled, the number of circles was compared among MF-exposed groups only (see Fig. 3*A*). There was a significant effect of group [$F(4,31) = 3.21$, $P < 0.05$] and conditioning day [$F(2,62) = 15.88$, $P < 0.001$] but no interaction. After MF exposure, OVX rats circled significantly more than intact females on conditioning *day 1* ($P < 0.05$). The amount of circling by OVX rats decreased over conditioning days, however, so that OVX rats circled less on conditioning *day 3* than conditioning *day 1* ($P < 0.01$), and thus OVX rats did not circle more than intact rats on conditioning *days 2* and *3*.

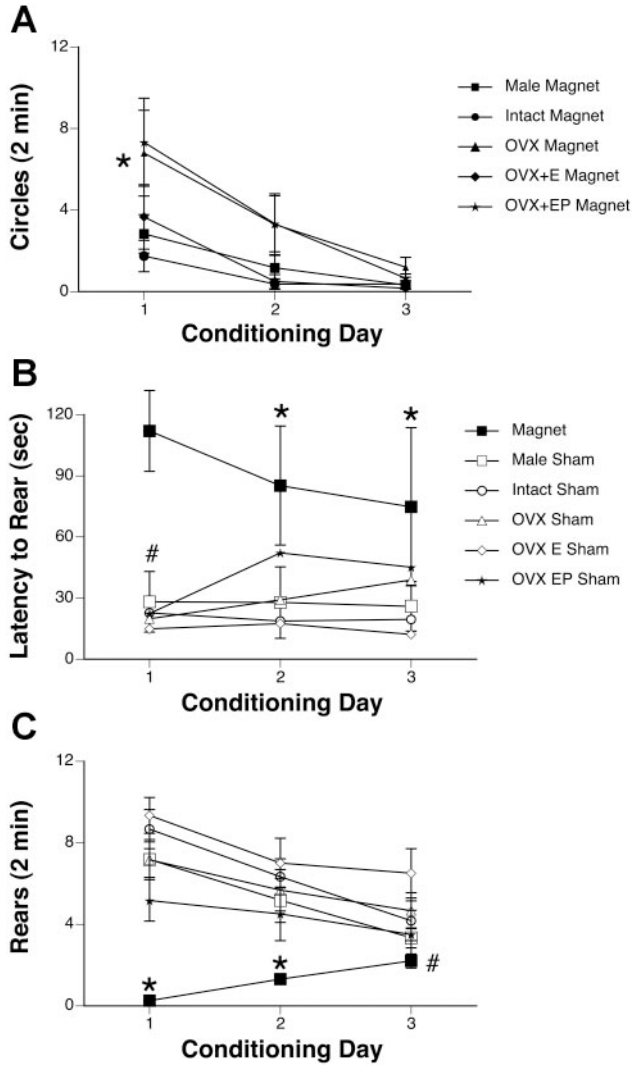

Fig. 3. Influence of ovarian steroids on locomotor behavior after three pairings of saccharin and 14-T MF or sham exposure for 30 min. *A*: among MF-exposed groups, circling (means ± SE) was significantly greater in OVX rats (▲) compared with intact female (41) MF-exposed rats. No sham-exposed rats circled. **P* < 0.05 vs. intact female. *B*: all sham-exposed rats had a shorter latency to rear (means ± SE) compared with MF-exposed rats on conditioning *day 1*. Latency to rear was longest on *day 1* in MF-exposed rats and decreased across conditioning days. **P* < 0.05 vs. same group, conditioning *day 1*; #*P* < 0.05 vs. MF-exposed rats on conditioning *day 1*. *C*: all sham-exposed rats reared significantly more than the MF-exposed group on *days 1* and *2*. The amount of rearing was increased in MF-exposed rats on conditioning *day 3*. **P* < 0.05 vs. sham-exposed rats; #*P* < 0.05 vs. conditioning *day 1*.

Latency to rear was long in MF-exposed rats (>1.5 min) and short in sham-exposed rats (<30 s; see Fig. 3*B*). Because latency to rear was not significantly different among MF-exposed rats, the data from MF-exposed rats were combined into one group and compared with sham-exposed groups. There was a significant interaction of group and conditioning day [$F(10,120) = 3.69$, $P < 0.001$]. There was no difference across conditioning days in the latency to rear of sham-exposed rats. In MF-exposed rats, however, latency to rear was longest on *day 1* and decreased across conditioning days ($P < 0.001$).

Because the number of rears was not significantly different among MF-exposed rats, the data from MF-exposed rats were combined into one group and compared with sham-exposed groups. MF exposure almost completely suppressed rearing, regardless of ovarian and steroid status (see Fig. 3*C*). There was a significant interaction of group and conditioning day [$F(10,120) = 8.39$, $P < 0.001$]. Sham-exposed rats, regardless of group, reared significantly more compared with all MF-exposed groups on conditioning *days 1* and *2*. The amount of rearing increased across conditioning days in MF-exposed rats such that by conditioning *day 3*, only OVX E sham-exposed rats reared significantly more than MF-exposed rats.

CTA effects. Because there was no significant difference in preference scores among the sham-exposed rats, their preference scores were combined and only one sham group was used for analysis.

All groups acquired a strong CTA after three pairings of saccharin with 30-min 14-T MF exposure, as shown by decreased saccharin preference during two-bottle testing compared with sham-exposed rats (see Fig. 4*A*). The CTA of the male and OVX EP MF-exposed group was greater than the CTA of intact female MF-exposed rats on the first day. A Student's *t*-test comparing the initial magnitudes of CTA between included males (low intake) and excluded males (high intake) showed that there was no difference ($P = 0.82$) in saccharin preference on the first day of two-bottle testing.

During extinction, there was a significant interaction between group and test day [$F(55,660) = 4.57$, $P < 0.001$]. The initial magnitude of the MF-induced CTA was greatest in males. Across the 12 days of preference testing, post hoc analysis revealed a significantly lower preference for saccharin in males than intact females on the first 8 days, OVX rats on test *days 3–5*, OVX E rats on test *days 2*, and *4–7*, and OVX+EP rats on test *days 4–6*. MF-exposed intact females had higher preference on *days 1* and *2* compared with MF-exposed OVX+EP females. None of the MF-exposed groups completely extinguished their CTA during the 12 preference test days, but by *day 9*, the male group had increased its initial low preference to a preference intermediate to (and not different from) the intact female group. Thus male rats acquired a stronger CTA initially, but extinguished faster to the same preference level as intact females by *day 9*.

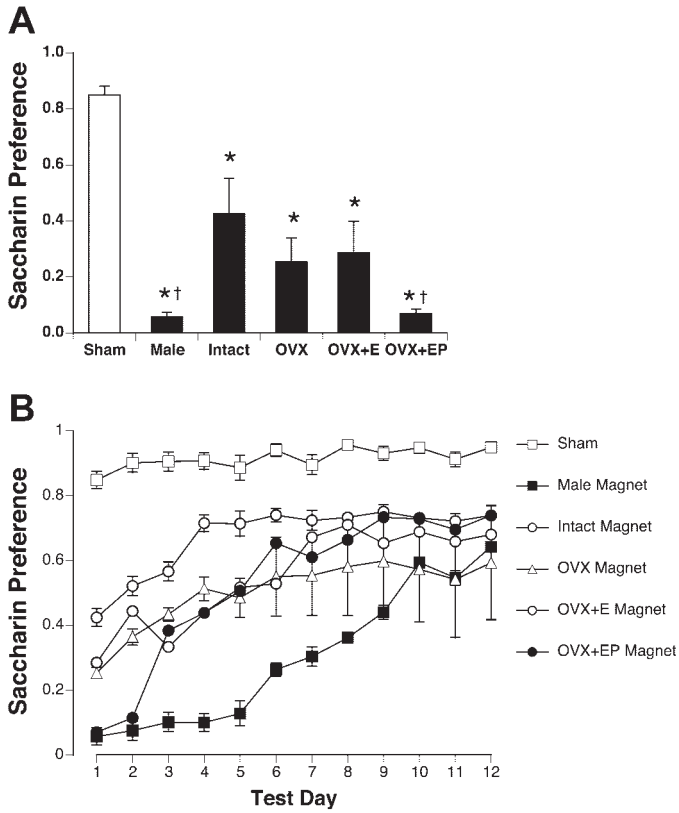

Fig. 4. Influence of ovarian steroids on CTA behavior after three pairings of saccharin and 14-T MF or sham exposure for 30 min. *A*: on the first day of two-bottle testing, MF-exposed rats (solid bars) had significantly lower preference scores (means ± SE) than sham-exposed rats (open bars). Male and OVX EP MF-exposed rats had a greater initial CTA compared with intact MF-exposed females. *$P < 0.05$ vs. sham-exposed rats; †$P < 0.05$ vs. intact female. *B*: preference scores of male MF-exposed rats were significantly lower than that of intact female MF-exposed rats on *days 1–8*, but increased faster to the same preference level as intact females by *day 9*.

DISCUSSION

In this study, as in previous reports (25, 44), exposure to a 14-T static MF had significant effects on locomotor behavior and the acquisition of CTA in rats. Furthermore, differences in the responses of female and male rats were observed.

MF exposure significantly reduced rearing behavior and induced tight counterclockwise circling in all groups tested. Intact females, especially females with the lowest levels of estrogen (estrus females), displayed more circling behavior than males. Compared with intact rats, OVX increased circling; E decreased circling in OVX rats. Thus females were more sensitive to the effects of MF exposure on circling and both endogenous and exogenous E appeared to be inhibitory to circling. (The amount of circling in intact female rats was variable between *experiments 1* and *2*, perhaps because the more strict water restriction schedule in *experiment 2* generally makes intact females acyclic, with intermediate levels of endogenous E.)

When saccharin was paired with MF exposure, all groups developed a CTA, as evidenced by decreased saccharin preference during two-bottle tests. After one pairing, male and female rats acquired CTAs of similar magnitude; however, males extinguished but females failed to extinguish saccharin preference within the time observed (9 days). After three pairings, males had greater CTA than intact and OVX females, but while male rats extinguished rapidly, the saccharin preferences of females plateaued and they did not completely extinguish within 12 days. E and EP increased extinction rate only transiently in OVX females. Therefore, the most robust sex difference in MF-induced CTA was seen in the slower rate of

extinction in females, which was insensitive to levels of E at the time of conditioning.

The difference in initial magnitude of CTA after 1 or 3 MF exposures suggests that males and females have different stimulus-response curves for MF-induced CTA acquisition. The sex difference in rate of extinction was constant across 1 or 3 exposures, however.

In summary, the response to MFs was sensitive to both sex and ovarian steroids. Circling varied across the estrous cycle and was sensitive to OVX and steroid replacement, which suggests that the locomotor response may be acutely modulated by ovarian steroids (an activational effect) (2, 4, 49). Because CTA was unaffected by estrous cycle and only weakly affected by OVX and steroids, the CTA response may be determined more by sex or developmental history (an organizational effect) than the acute effect of ovarian steroids. (These experiments do not rule out an activational effect of testosterone on CTA response, however. It is possible that high levels of testosterone in male rats at the time of conditioning and extinction contributes to their rapid extinction rate).

The increased circling behavior after MF exposure in female rats in this study could be due to sex differences occurring in the vestibular system or dopaminergic system, among other places. The similarities in circling and suppression of rearing in response to both MFs and vestibular pertubation suggest that MFs may be acting upon the vestibular system. On the other hand, the circling behavior following MF exposure may be an indirect result from activation of other motor systems. In particular, the circling behavior also closely resembles the amphetamine-stimulated circling seen in 6-hydroxydopamine-lesioned rats. Unilateral lesions within the striatum, ascending dopaminergic pathways, cerebellum or vestibular nuclei can induce circling or susceptibility to circling after drug or vestibular stimulation (58). It is impossible to localize the site of magnetic field action from the intact rat's behavioral response alone because of the complicated reciprocal connections of the vestibular and motor systems.

There is no literature on sex differences in circling after vestibular lesions. There is evidence for sex differences in striatal function and drug-induced rotational behavior, however, (5, 6, 54). Female rats exhibited more diurnal rotational behavior (i.e., measured by rotometer) than male rats following amphetamine treatment, and estrous cycle differences were found such that females in estrus, diestrus, and proestrus displayed more rotational behavior after amphetamine treatment than males (6, 54). Furthermore, it was found that ovariectomy decreased diurnal rotation and estrogen treatment (estradiol benzoate; 4 days of 5 μg in 0.1 ml peanut oil) restored rotational behavior immediately following the cessation of treatment and 4 days later (but not 24 h after treatment), suggesting that estrogen has both immediate and long-term effects (5).

Thus estrous stage and ovarian steroid status modulate both MF-induced circling and amphetamine-induced circling. Ovariectomy and E appear to act in the opposite direction in these two designs, however: E enhances amphetamine-induced rotation while E attenuates MF-induced circling. One possibility is that E is acting on two separate systems. In the case of amphetamine-induced circling, it has been suggested that E enhances amphetamine-stimulated dopamine release in the striatum (5). In MF-induced circling, E could act on a separate neurotransmitter system, e.g., in the vestibular system.

We observed a higher incidence of circling after magnet exposure in female rats than male rats. Sex differences have been reported in the basal activity levels of rats, which might contribute to the observed differences in locomotor circling. These differences are influenced by steroid hormones and are most apparent in novel environments (15, 41), and the test chamber in our experiment was novel to the rats. However, we did not observe basal differences in the number of rears or latencies to rear of sham-exposed male and female rats in *experiment 1*. Sex differences in activity levels could also reflect differences in anxiety levels. Leret and colleagues (36) showed that female rats were more active and less anxious than males when tested in open-field test followed by an elevated-plus maze. Furthermore, it has been shown that exposure to a weak magnetic field or an opiate antagonist improves spatial performance in female deer mice and may be due to a decrease in anxiety (34).

In this study, we also found a sex difference in MF-induced CTA, in that females failed to extinguish CTA in the same time as males. In agreement with previous studies, MF exposure induced CTA when a novel, saccharin solution was paired with 14-T exposure (25, 37, 44). The fact that MFs can induce CTA learning is also consistent with activation of the vestibular system. Other studies have shown that vestibular stimulation (e.g., whole-body rotation) or labyrinthectomy can induce CTA (3, 7, 16, 21, 27).

There have been no reports of sex differences in vestibular system-induced CTA, but other studies have shown sex differences in lithium chloride-induced CTA. As in our study, sex differences have been seen primarily in extinction rates; but unlike our study, males have been reported to extinguish more slowly than females (8, 10, 17, 50, 64, 66, 67). A specific role for sex steroids in the modulation of extinction rates has been demonstrated in a few studies, but no study has examined cyclic variation across the estrous cycle. Testosterone has been shown to slow extinction, whereas estradiol has been shown to accelerate extinction of CTA in gonadectomized male and female rats (8, 9, 17, 57, 66, 67). Thus, in LiCl-induced CTA, sex steroids have an activational effect on extinction rate, whereas in MF-induced CTA there is an organizational sex difference.

The differences between our study using MFs and previous findings with LiCl could be due to a difference in extinction mechanisms related to the unconditioned stimulus (i.e., sex and steroid status might affect extinction of LiCl-induced CTA differently than extinction of MF-induced CTA). Alternatively, the results from the present study could be specific to MF-induced CTA or due to differences in testing parameters [e.g., one-bottle vs. two-bottle tests (50, 66, 67) or sucrose vs. saccharin (9, 57).] It may be relevant that we matched saccharin intakes between male and female groups by excluding rats with the highest and lowest intakes during conditioning. Although this controlled for sex differences in saccharin intake (see Table 1), it violated the assumption of random assignment of subjects to the various experimental groups, which may have affected the results.

In conclusion, circling behavior in females after MF exposure varied across the estrous cycle and was increased by OVX and decreased by E replacement. These results suggest an activational effect of estrogen to inhibit circling in females.

Conversely, although females acquired a weaker CTA after MF exposure that extinguished more slowly than males, OVX and steroids only weakly affected CTA induced by MFs in female rats. Therefore, the CTA response may be determined more by sex than acute steroids, suggesting an organizational effect of sexual development. Further studies are needed utilizing castrated males and neonatal hormone manipulations to confirm an organizational effect.

If the MF effect is mediated by the vestibular system, then the differences in circling behavior suggest a sex difference in the vestibular system that is modulated by estradiol. This effect would require the presence of estrogen receptors (ERs) within the vestibular system. Although there is some evidence for the presence of ovarian steroid receptors within the vestibular apparatus and central vestibular relays, their localization and subtype have not been conclusively determined.

In the periphery, ER-α and ER-β immunoreactivity has been reported in the nuclei of inner and outer hair cells of mouse and rat, suggesting that estrogens might affect the inner ear (60) (but see Ref. 43). Centrally, the ER and progesterone receptor (PR) have been found within the rat brain stem (11, 12, 23, 35, 39, 52, 63), but little work has been done to localize the ER and PR throughout the central vestibular relays of the brain stem network (12). No electrophysiological or c-Fos studies have been conducted to determine whether there are sex differences in the cellular response to vestibular stimuli.

There is some evidence for, but little systematic investigation of, sex differences in the human vestibular system. A few sex differences have been reported in response to stimuli that perturb the vestibular system, such as orthostatic intolerance and motion sickness (19, 22, 24, 46, 51, 53, 55). Premenopausal women are more prone to orthostatic intolerance and its symptoms (lightheadedness, nausea, vomiting, dizziness, and syncope) than men (19, 51, 53), and young women are the most commonly affected (47). In addition, orthostatic intolerance is common in astronauts after spaceflight, and its symptoms occur and reoccur more often in female astronauts (19, 22, 24, 55). Other studies have shown sex differences in motion sickness history and circularvection latencies (the illusion of self-rotation). Women report greater incidence of motion sickness history than men, and males exhibit significantly longer circularvection latencies after viewing a rotating optokinetic drum (14, 48).

Although suggestive, none of these studies have conclusively demonstrated a sex difference or a role for ovarian steroids in human vestibular function. For example, few of these studies have demonstrated variations in vestibular function across the ovarian cycle, although there is a higher occurrence of nausea, vomiting, vertigo, and dizziness during the premenstrual period (1) and reduced postural stability at times in the menstrual cycle when there are low levels of circulating estrogen and progesterone (13). Nonetheless, our results suggest that if high-strength MF can perturb the human vestibular system, then exposure to high-strength MF might have a greater effect on women than men.

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