Original Research

Biological Effects of Long-Duration, High-Field (4 T) MRI on Growth and Development in the Mouse

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The effects of long-duration, high-field magnetic resonance imaging (MRI) on fetal growth and postnatal development in mice were studied. Seven experimental groups of pregnant ICR mice were exposed for 9 hours on day 9 and/or day 12 post coitus (pc) to magnetic fields (4 T static, 5 T/sec switched gradient, and 0.2 W/kg radiofrequency at 170 MHz) associated with MRI conditions. Two experimental groups (sham and exposure groups) were exposed to a combination of ultrasound (day 9 pc, 3.25 MHz, focused) and MRI-associated fields (day 12 pc). No statistically significant changes in fetal growth were observed in the animals exposed to only MRI or ultrasound fields. However, in the combined ultrasound and MRI-exposed group, the fetal weight and crown-rump length were reduced compared with the sham and cage controls. These results suggest that MRI and ultrasound exposure well in excess of current clinical conditions can exert biological effects if applied at sensitive stages of fetal development. J. Magn. Reson. Imaging 2000;12:140–149. © 2000 Wiley-Liss, Inc.

Index terms: magnetic resonance; testis development; ultrasound; daily sperm production; fetal growth, motor skills

HUMAN EXPOSURE to static and time-varying magnetic fields is likely to increase with the continued development of high-field MRI systems (1). Currently, very high-field MRI systems (6–9 T) are under development at research sites around the world, and approximately 50 3–4 T systems are now in operation (2). These high-field systems have great potential to enhance the clinical use of MRI, MR spectroscopy, and functional (f)MRI; however, they also increase the exposure level for static and time-varying magnetic fields. In addition, the growth of interventional MR-guided procedures and low-field open magnetic structures will substantially increase the magnetic field exposure of physicians and medical staff (3,4). Our laboratory and several other groups (5) are concerned with the potential health effects of long-term exposure to the magnetic fields associated with high-field and interventional MRI systems.

The purpose of this study was to investigate the biological effects of long-duration, high-field (4 T) MRI exposure conditions using fetal growth of the ICR mouse as the biological end point. This model was used previously by our group to evaluate the biological effects of fetal ultrasound exposure (6,7) and 4.7 T MRI conditions both alone (spin-echo sequence TR/TE of 20/30 msec for 8 hours, specific absorption rate (SAR) of 1.5 mW/kg, dB/dt up to 0.7 T/sec) and in combination with ultrasound exposure (1 MHz unfocused, 5 W/cm^2 for 30 seconds) (8). In the present study, the ultrasound and static magnetic field conditions are significantly greater than typical continuous wave (CW) Doppler diagnostic ultrasound and MRI imaging conditions, but the radiofrequency (RF) SAR and switched-gradient fields (dB/dt) are typical of clinical MRI exposure conditions. In our previous study, we observed changes in fetal growth (decreased fetal weight and crown-rump length), neonatal health (increased incidence of postpartum deaths), and male gonadal development (a decrease in daily sperm production and adult testis size and weight) following high-field (4.7 T), long-duration, 8-hour MRI (days 9 and 12 post coitus), and sequential MRI and ultrasound (1 MHz, unfocused) exposure of pregnant female ICR mice. In this follow-up study, we repeated our basic experimental paradigm at 4.0 T using more intense magnetic field components (switched gradients and RF pulses) and increased the ultrasound frequency to 3.25 MHz (focused).

Previous researchers have addressed many of the key safety issues associated with MRI, and a consensus has emerged that, as currently used, MRI is safe and effective. However, the development of new systems at higher fields raises issues of potential health hazards that are not completely resolved (9–12). In earlier studies, a variety of animal models and exposure conditions was used to assess teratogenic effects. For example, no genetic damage was seen in Chinese hamster ovary cells exposed to low-field MRI conditions (13), and no developmental differences were found in frog embryos hatched from eggs exposed to high-field MRI conditions (14). Exposure of adult and neonatal mice to a static field of 1.89 T for an extended period (up to 624 hours over 3 months) produced no consistent differences be-

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tween exposed and control animals in gross morphology, hematocrit, and other blood chemistry parameters (15). Similarly, a study exposing pregnant mice to a static field of 6.3 T on days 7–14 of gestation resulted in no significant differences in litter size, fetal weight, intrauterine mortality rate, or external and skeletal abnormalities between control and exposed animals (16). Finally, exposure of pregnant mice to a pulsed magnetic field resulted in no adverse effects on litter size and prenatal growth of exposed litters (17).

However, not all studies have produced negative results. Heinrichs et al. (18) reported a small but statistically significant reduction in fetal crown-rump length (but no overt embryotoxicity) after prolonged midgestational exposure of mice to MRI conditions equivalent to those used in human imaging at a field strength of 0.35 T. A similar finding of decreased crown-rump length and smaller craniofacial perimeter measures was observed by Tyndall et al. (19) in the offspring of mice exposed to conventional T2-weighted spin-echo imaging conditions at 1.5 T during pregnancy. Studies by Tyndall's group have also indicated that MRI may be teratogenic for the developing eye in the mouse (20). The results of these studies suggest that more sensitive measures of teratogenic effects need to be developed in order to assess the risks presented by MRI exposure.

To address this need, we designed this study to investigate biological effects in mice following in utero exposure to high-field (4.0 T) MRI conditions. Effects on fetal growth and development of both sexes and the interaction of high-field MRI and ultrasound exposure were investigated. In addition, mice exposed prenatally to high-field MRI conditions were tested using parallel bars to examine any deficiencies in motor skills development. If there is a teratogenic effect of MRI exposure on the developing brain, it may be evident in behavior. Permanent motor deficits (particularly in balance) have been reported in response to prenatal alcohol exposure (21,22), which has also been shown to reduce brain growth, impair neuronal migration and differentiation, and increase neuronal death (23–25). As discussed in our previous study (8), alcohol, caffeine, ionizing radiation, and estrogenic compounds all exhibit adverse effects on the development of the fetus and of the mammalian testis.

MATERIALS AND METHODS

The experimental conditions used in this study are, for the most part, the same as those described fully in our previous report (8). The levels of exposure to time-varying magnetic fields (dB/dt) and RF fields (SAR) were increased as described below, while the static magnetic field was reduced from 4.7 to 4.0 T.

Magnetic Field Exposure System

The MRI exposure was performed using a 4.0 T, 31 cm diameter, clear bore imaging spectrometer [Surrey Medical Imaging Systems (SMIS), Guildford, Surrey, UK] and a superconducting magnet (Magnex Scientific, Abingdon, UK). MRI-exposed animals were placed in cylindrical plastic exposure chambers, which were 11 cm in diameter and 15 cm in length. The chambers were perforated to allow air exchange. A layer of animal bedding (ground corncobs) approximately 2 cm deep was placed in the chamber, and apple wedges were provided as a source of water and food. The exposure chamber was placed inside the SMIS volume coil (8-element birdcage, 14 cm diameter, 25 cm length). The coil and exposure chamber were positioned at the center of the 4 T superconducting magnet. The imaging parameters were set similarly to those used in our previous study (8), with the exception of the RF resonance frequency, which was 170 MHz instead of 200 MHz. A standard spin-echo imaging sequence (TR/TE 2000/30 msec, one slice) was applied for 9 hours (10 PM to 7 AM) on the chosen day of gestation. RF exposure at 170 MHz consisted of two five-lobe, sinc-modulated RF pulses, each 4 msec long with peak powers of 76 and 19 W (π and $\pi/2$ flip angles). The SAR was estimated from the total power (P) delivered to the RF coil when loaded with five mice weighing approximately 30 g each (M = 0.15 kg), from measurements of loaded and unloaded coil Q, and considering the duty factor for the 180° pulse (duration τ = 4 msec) and the TR time of 2000 msec, according the following formula (26):

$$SAR = \left(\frac{1}{M}\right)\left(\frac{\tau}{TR}\right)P\left[1 - \frac{Q(\text{loaded})}{Q(\text{empty})}\right]\text{W/kg}$$

The average SAR in mice for these exposure conditions was estimated to be 0.2 W/kg. These SAR conditions were chosen in order to maintain typical MR conditions and to avoid introducing a thermal burden on the animal; current Food and Drug Administration guidelines specify a maximum whole-body average SAR of 0.4 W/kg, with a peak SAR of 8 W/kg in any 1 g of tissue (10). Gradient exposure consisted of the readout gradient along the x- (transverse) axis and the slice-selection gradient along the z- (axial) direction of the magnet. The phase-encode gradient was turned off. The readout gradient strength was 5 G/cm with a ramp time of 500 μsec, resulting in dB/dt ranging from 0 to 5 T/sec at the center and walls of the cage, respectively, with 10 ramps applied every 2 seconds (slice, rephase, π, readout, and refocusing gradient lobes). Thus, the read gradient (Gx) was the major contributor to the dB/dt (the slice gradient Gz was small, with one slice, and the phase-encoding Gy was off).

The exposure chamber was positioned at the center of the magnet in the x- and z-directions and was approximately 4 cm below the center in the y-direction. The ambient temperature in the magnet bore was measured using an alcohol, liquid-in-glass thermometer; the mean temperature was 18°C. The mean ambient temperature of the room was 20°C. Sham MRI animals were placed in identical chambers outside the magnet at the 5 G line and remained there for the duration of the exposure. The acoustic sound level was measured (A scale weighting, peak response) at the position of the sham animals and at the bore opening of the magnet using a digital sound meter (model 01617-00, Cole-Parmer Instrument Company, Vernon Hills, IL) (27).

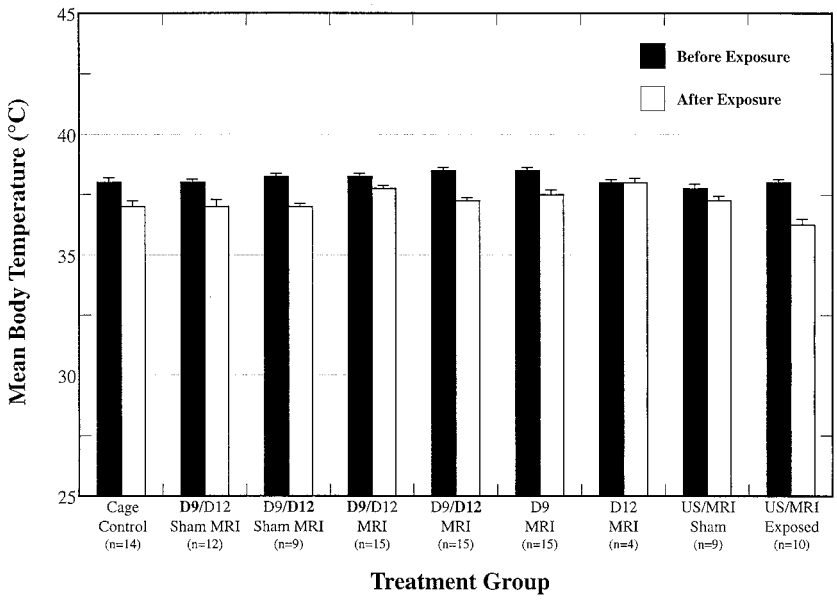

Figure 1. Mean body temperature in degrees Celsius of exposed animals measured before and after each MRI exposure. Bold type indicates exposure day for the 2-day MRI. Closed bars are pre-exposure temperatures; open bars are post-exposure temperatures. Error bars are SEM. *n* is the total number of animals in each group.

Ultrasound Exposure System

The exposure system consisted of a Hewlett Packard (model 8660A, Palo Alto, CA) signal generator and an ENI RF power amplifier (model A150, Rochester, NY). A PZT-4, 3.25 MHz focused transducer, which has a 95% power beam width of 3 mm, was used to generate the acoustic beam. Due to the narrow beam width, a 3×3 exposure matrix was used with a center-to-center distance of 7 mm between exposure sites in order to avoid overlap of the individual exposure sites such that the focal point of the beam was 2–3 mm below the abdominal skin surface. An ultrasound exposure of 5 W/cm^2 for 30 seconds (I^2t = 750 W^2/cm^4 sec) was used to allow comparison with previous studies (6–8).

Experimental Procedures

The procedures for mating and animal care were the same as those described in our previous study. Briefly, female nulliparous ICR:HD (Harlan Sprague-Dawley, Indianapolis, IN) mice 70–100 days of age were mated to proven males, and the pregnant females were set aside for exposure on day 9 or 12 post coitus (pc). Fetal data were taken on day 18 of gestation, and data from adult animals exposed in utero were taken on day 50 post partum. Each exposure group consisted of eight pregnant dams, four for the fetal portion of the study and four for the adult portion. Nine different experimental groups of mice were used in this study (cage control, sham D9/D12 MRI, D9 MRI, D12 MRI, D9/D12 MRI, sham US/MRI, exposed US/MRI, sham D9 US, and exposed D9 US). All animals (control, shams, exposed) were housed in the same animal room and supplied with the same batch of food and bedding. The exposed and sham animals received apple slices from the same apple. Post-exposure treatment, euthanasia, and data collection and analysis were the same as for our previous study (8).

Motor Skills Evaluation

Starting on postnatal day 29, animals from each experimental group were tested on their ability to traverse a parallel-bar obstacle course, which consisted of two 0.5-cm-diameter wooden rods 1 m long and 3 cm apart, interconnecting two platforms and elevated 1 m above the floor. Time to complete the course and the number of slips were registered. Each animal ran the test 5 times a day for 7 consecutive days. Prior to the initiation of these tests, each animal was removed from its home cage and handled gently by researchers for several days to reduce anxiety in the animals and to prepare them for the subsequent behavioral tests.

RESULTS

Rectal Temperatures and Body Weights

The rectal temperatures and body weights of cage control, sham, and all MRI-exposed animals were measured sequentially in each animal before and after each 9-hour MRI exposure. For the rectal temperatures, a Bailey Instruments BAT-4 thermometer was used with an IT-18 series Teflon-coated thermocouple temperature sensor (0.6 mm diameter) at a depth of 1 cm. Approximately 15 seconds were required for each measurement, so that the entire cage of two to four mice could be measured within 1 minute. The body weights were measured using an electronic digital scale (Sartorius P600). These data are displayed in Figs. 1 and 2. Following the exposure, decreases in both rectal temperature and mean body weight were observed in the cage control, sham, and exposed groups (possibly reflecting the normal circadian pattern in the nocturnal mouse). The data were analyzed using a multi-group analysis of variance (ANOVA, Mac Systat v. 5.2.1) in order to study the differences between groups. In addition, a paired *t*-test was used to determine the statistical significance between the pre-exposure and post-

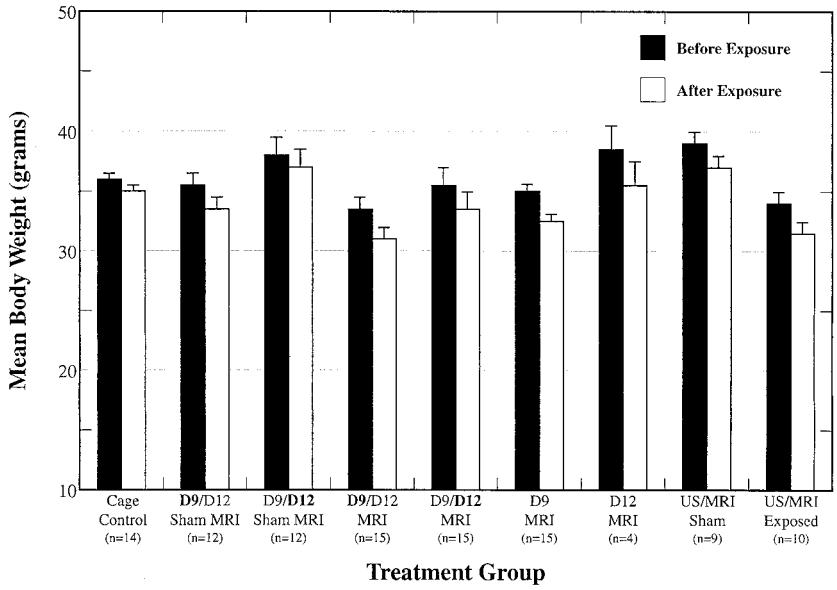

Figure 2. Mean body weight in grams of exposed animals measured before and after each MRI exposure. Bold type indicates exposure day for the 2-day MRI. Closed bars are pre-exposure weights; open bars are post-exposure weights. Error bars are SEM. *n* is the total number of animals in each group.

exposure values for each group. The ANOVA analysis showed no differences between the changes in rectal temperatures between any of the experimental groups at the $P \leq 0.10$ level.

With regard to mean body weight, however, the observed weight losses were significantly different at the $P \leq 0.10$ level for the MRI-treated groups compared with the cage control group and for the US/MRI-exposed group compared with its sham. The pairwise *t*-test indicated that the post-exposure temperatures and mean body weights differed from the pre-exposure temperatures for each of the groups at least at the $P \leq 0.05$ level. In the case of the cage control, the mean body weight and temperature for the group prior to exposure were 36.2 g and 38.0°C, respectively. Overnight, this group lost an average of 0.9 g, and the body temperature fell an average of 0.9°C. For the sham group, which was placed outside the magnet at the 5 G line, the initial mean body weight and rectal temperature were 37.4 g and 38.1°C. At the end of the experiment, the following morning, these animals had lost an average of 1.7 g of body weight, and their body temperature had fallen an average of 1.0°C. The four MRI exposure groups used in this study all exhibited similar small decreases in body weight and rectal temperature during the exposure to MRI conditions. As an example, the day 9/day 12 MRI group, which consisted of 15 animals, showed an average drop in body weight of 2.0 g on the day 9 exposure and 2.1 g on the day 12 exposure, while the corresponding decreases in rectal temperature were 0.4°C and 0.8°C on days 9 and 12, respectively. Both sham and MRI-exposed animals had free access to food and water in the form of apple slices placed in the exposure chamber. However, the sham animals usually consumed the apple slices while the MR-exposed animals did not.

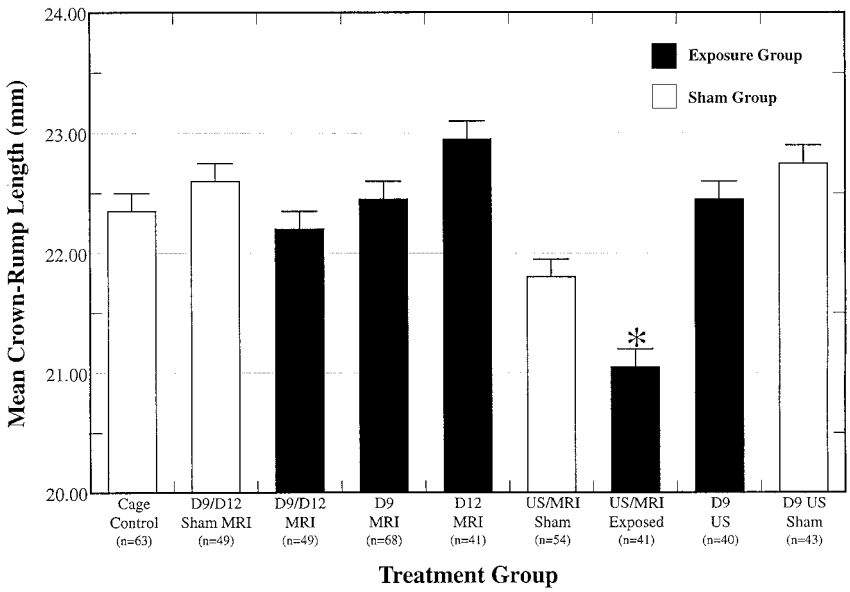

Figure 3. Mean crown-rump length in mm measured on day 18 post coitus. Closed bars are exposure groups; open bars are sham groups. Asterisk indicates group that is significantly lower ($P \leq 0.05$) than its sham group. Error bars are SEM. *n* is the total number of fetuses in each group.

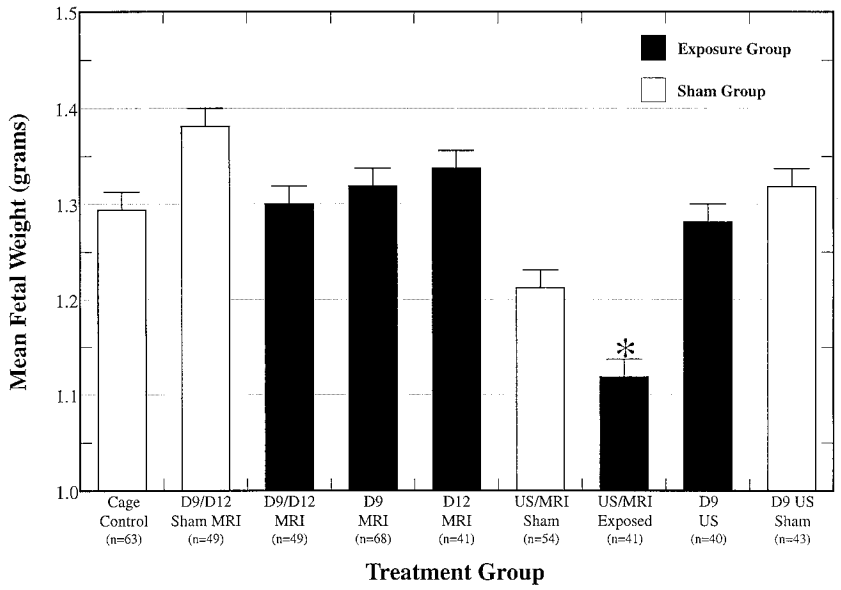

Figure 4. Mean fetal weight in grams measured on day 18 post coitus. Closed bars are exposure groups; open bars are sham groups. Asterisk indicates group that is significantly lower ($P \leq 0.05$) than its sham group. Error bars are SEM. n is the total number of fetuses in each group.

Fetal Study

The crown-rump length (CRL) and fetal weight were measured on day 18 pc; these data are shown in Figs. 3 and 4. No significant differences were observed between the cage control group (or sham-exposed groups) and the experimental treatment groups exposed to ultrasound or MRI alone. Statistically significant ($P \leq 0.05$) reductions in fetal weight and CRL were observed for the experimental group exposed to both ultrasound and MRI when compared with the sham-exposed animals. Litters from four dams were analyzed for each treatment group, resulting in an average of 50 fetuses per group. The sham MRI/ultrasound group also showed weight and CRL reductions when compared with the cage control group, suggesting the influence of animal handling procedures on this combined exposure group. No significant differences between treatment groups were observed for litter size or the number of fetal deaths (Table 1). However, the number of resorptions and stillbirths was increased for the day 12 MRI-exposed group (statistically significant at the $P \leq 0.05$ level) and the day 9/day 12 MRI-exposed group (difference *not* statistically significant when compared with the sham group). The ratio of males to females per litter was also not significantly different between treatment groups (data not shown).

Adult Study

The mean weights for each treatment group at birth, weaning, and euthanasia on day 50 are shown in Table 2. Significant reductions in weight at birth, weaning, and sacrifice were observed for the group exposed to ultrasound on day 9 and MRI on day 12 of gestation compared with its sham group. No statistically significant changes in weight were observed for the other treatment groups when compared with the shams or the cage controls.

The incidence of post-partum deaths was significantly higher for the day 12 MRI-exposed group than for any of the other treated groups (Fig. 5). This group also had a significantly higher incidence of resorptions and stillbirths in the fetal portion of the study (Table 1). There were no significant differences in spleen weights between treatment groups for either males or females (Table 3). There were also no significant differences in testis weight or seminal vesicle weight (Table 3) between the exposure groups and the sham or cage control groups.

Table 1
Mean Litter Size and Number of Stillbirths and Resorptions of Treatment Groups

Treatment group (n = 4)	Litter size	Stillbirths	Resorptions	SB + Res	(SB + Res)/N
Cage control	12.6 ± 1.5	0	0	0	0.00
D9/D12 MRI sham	12.2 ± 1.7	0	1	1	0.25 ± 0.25
D9/D12 MRI	12.2 ± 1.7	0	5	5	1.25 ± 0.75
D9 MRI	11.5 ± 1.4	0	2	2	0.50 ± 0.28
D12 MRI	10.2 ± 1.7	1	6	7	1.75 ± 1.03*
D9 US/D12 MRI sham	13.5 ± 1.7	0	2	2	0.50 ± 0.50
D9 US/D12 MRI	13.7 ± 1.9	0	0	0	0.00
D9 US	10.0 ± 1.7	0	0	0	0.00
D9 US sham	10.7 ± 1.7	0	2	2	0.50 ± 0.50

*Indicates mean that is significantly different ($P \leq 0.05$) from the sham group.

Table 2
Mean Weights (g) of Treatment Groups at Birth, Weaning (Day 21), and Day 50

Treatment group	Birthweight (D0)	Weaning weight (D21)	Male weight at D50	Female weight at D50
Cage control	1.50 ± 0.02	7.6 ± 0.2	27.0 ± 0.4	21.8 ± 0.3
D9/D12 MRI sham	1.48 ± 0.03	7.9 ± 0.3	27.6 ± 0.6	22.6 ± 0.5
D9/D12 MRI	1.50 ± 0.02	8.0 ± 0.2	28.8 ± 0.4	22.9 ± 0.4
D9 MRI	1.56 ± 0.03	7.6 ± 0.3	27.7 ± 0.5	22.0 ± 0.4
D12 MRI	1.61 ± 0.03	9.4 ± 0.3	26.6 ± 0.5	23.8 ± 0.4
D9 US/D12 MRI sham	1.52 ± 0.02	9.0 ± 0.3	28.4 ± 0.5	23.2 ± 0.4
D9 US/D12 MRI	1.32 ± 0.02*	6.9 ± 0.3*	25.1 ± 0.5*	20.9 ± 0.5*
D9 US	1.45 ± 0.02	9.6 ± 0.3	26.5 ± 0.8	21.9 ± 0.4
D9 US sham	1.54 ± 0.03	8.9 ± 0.4	26.3 ± 0.5	21.6 ± 0.4

*Indicates means that are significantly different ($P \leq 0.05$) from the sham group.

The results of the analysis of daily sperm production (DSP) and efficiency (DSP/gram of testis) are shown in Table 4. The day 9/day 12 MRI-exposed group and its sham both had statistically significantly lower values than the cage control group. However, compared with its sham, no other treatment group exhibited a statistically significant difference in daily sperm production.

Motor Skills Evaluation

A two-way ANOVA with repeated measures (Mac Systat v. 5.2.1) was used to distinguish between the experimental groups as a function of treatment and day of test. The statistical evaluation showed that significant differences in motor skills at the $P \leq 0.01$ level occurred between the sham treatment group and both the day 9 MRI-exposed and day 12 MRI-exposed groups (Fig. 6). Differences in the time to complete the obstacle course were observed between the day 9/day 12 MRI-exposed group and its sham at the $P \leq 0.025$ level and between the day 9 US/day 12 MRI-exposed group and its sham at the $P \leq 0.10$ level.

Acoustic Noise

The read gradient was identified as the principal audible noise source, corresponding with the 2 second TR time, and was manifest as a repetitive thump-thump sound. During the MR sequence the sham animal noise level was 70 dB (ambient 55 dB), and the sound level at the magnet bore opening was 85 dB. From the rate of increase in the noise level in the axial direction, it is estimated that the noise level at the position of the mice was between 90 and 100 dB. The measured acoustic noise level is near the OSHA standard for long-term exposure (90–105 dB) (27).

DISCUSSION

The long-duration exposure conditions used in this study constitute a stress to the animal and as such can be expected to produce effects on sensitive biological systems. Stress administered during pregnancy is well known to influence fetal development, learning, and subsequent physiological functions in the adult (28–34). Thus, experimental studies of pregnant animals (typically rats or mice) provide direct evidence for assaying the risks associated with new technology (35,36). In studies of the biological effects of electric or magnetic fields, the sham exposure conditions are difficult to design in a manner such that all contributing environmental factors (eg, temperature, air flow, acoustic noise, etc.) are well controlled. Considerable litera-

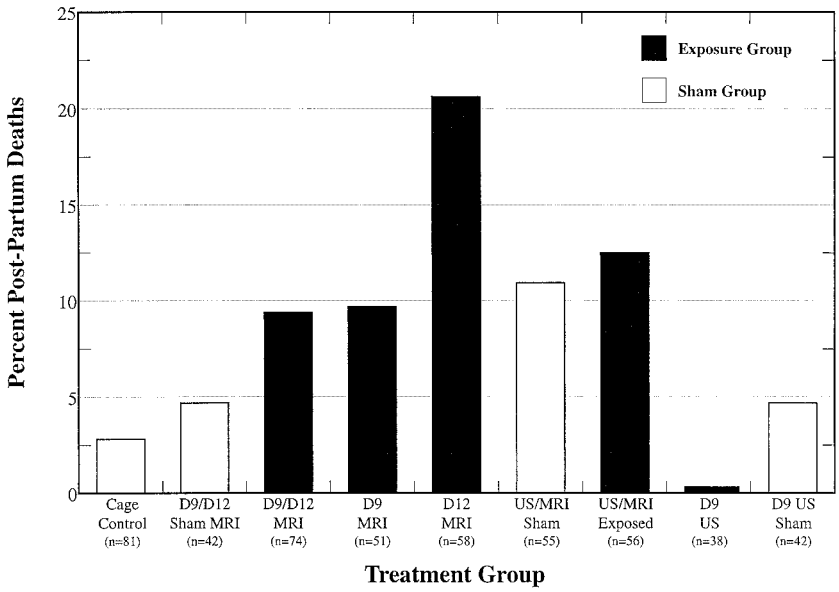

Figure 5. Percent post-partum deaths for each treatment group. Closed bars are exposure groups; open bars are sham groups. *n* is the total number of animals born in each group.

Table 3
Spleen, Testis, and Seminal Vesicle Weights (mg) for Treatment Groups on Day 50

Treatment group	Female spleen weight	Male spleen weight	Testis weight	Seminal vesicle weight
Cage control	95 ± 8	102 ± 8	97 ± 2	96 ± 4
D9/D12 MRI sham	97 ± 11	102 ± 13	96 ± 4	93 ± 7
D9/D12 MRI	96 ± 10	132 ± 8	103 ± 2	112 ± 4
D9 MRI	104 ± 10	108 ± 10	98 ± 3	95 ± 5
D12 MRI	112 ± 10	97 ± 10	98 ± 3	114 ± 5
D9 US/D12 MRI sham	116 ± 9	117 ± 11	98 ± 3	103 ± 6
D9 US/D12 MRI	91 ± 11	82 ± 11	101 ± 3	103 ± 6
D9 US	99 ± 10	97 ± 16	102 ± 5	102 ± 9
D9 US sham	92 ± 10	94 ± 10	91 ± 3	92 ± 5

ture exists concerning animal conditioning, particularly with respect to electromagnetic field exposure (37); in our experiments, however, the timed nature of the exposures to specific stages of fetal development restricted the opportunity for animal conditioning. In future studies, we hope to conduct experiments with groups of animals exposed to individual field components alone (ie, static magnetic field only, switched-gradient field only, RF field only) and to include for each group a suitable period for their adjustment to the experimental paradigms; however, in this study, we chose to rely on cage control animals and sham-exposed animals for comparison of effects.

The cage, temperature, and lighting conditions were identical for the sham and the exposed animals. The acoustic noise level for the sham (at the 5 Gauss line) was approximately 15 dB above ambient and 25 dB below that estimated for the exposed animals. Thus both groups are exposed to a loud "thump-thump" sound (TR 2 seconds), but the exposed mice experienced a sound level (90–100 dB) intensity. Neither groups of mice were observed to exhibit a startle reflex or to jump when the imaging sequence began.

The day of exposure (day 9 or day 12) was selected based on previous studies that indicate critical developmental changes in the mouse (eg, day 9—neural tube growth; day 12—reproductive system development). Our previous ultrasound studies (6) and our ultrasound/MRI study (8) confirm that fetal development in the mouse is sensitive to perturbation by ultrasound and magnetic fields plus the exposed mice experienced a higher sound level intensity on these days.

This study can be divided into two parts (fetal and adult). The results of the fetal study are consistent with our previous findings (8). A significant ($P \leq 0.05$) decrease in fetal weight and CRL was observed in fetuses exposed to both MRI and ultrasound (when compared with the cage control and sham MRI/ultrasound groups). However, the sham MRI/ultrasound group also showed weight and CRL reduction compared with the cage control group, suggesting that the experimental and environmental conditions are a contributing factor. There was no difference in litter size between groups, but there was a significant increase in the number of stillbirths and resorptions in the day 12 MRI-exposed animals. The data from the adult group, unlike that obtained in our previous study, showed a significant decrease in birthweight of the pups in the combined MRI/ultrasound exposed group. The weaning weight for this group was also significantly lower than that of the MRI/ultrasound sham group. In addition, the day 12 MRI-exposed pups showed increases in weight compared with the sham and cage control groups, a pattern consistent with this group in our previous study.

The adult weights on day 50 were reduced following exposure to ultrasound on day 9 of gestation and MRI (4.0 T) on day 12. These results correspond to similar reductions in birthweight and weaning weight for animals in this group seen in our previous study. These results differ from the 4.7 T study, in which the weight reduction for the ultrasound/MRI group was found only in the fetal animals and was not seen after birth. The incidence of post-partum deaths was significantly higher for the day 12 MRI-exposed group than for any of the other treated groups. This same group also had a significantly higher incidence of resorptions and stillbirths in the fetal portion of the study. The post-partum death rate was 16.1%. There were no significant differences in spleen weights between treatment groups for either the males or females (Table 3). There were also no significant differences with respect to seminal vesicle or testis weight (Table 3) for the males. Analysis of DSP and efficiency (DSP/gram of testis) shows that the 9/12 day MRI exposure and the sham had values significantly lower than the cage control group. Additionally, the other groups that either received MRI exposure or

Table 4
Mean Daily Sperm Production (DSP) for Each Treatment Group at Day 50

Treatment group	No.	DSP ($\times 10^6$)	DSP/g ($\times 10^7$)
Cage control	37	2.03 ± 0.09	1.97 ± 0.08
D9/D12 MRI sham	13	1.47 ± 0.14*	1.44 ± 0.14*
D9/D12 MRI	34	1.31 ± 0.09*	1.20 ± 0.09*
D9 MRI	23	1.72 ± 0.11	1.74 ± 0.11
D12 MRI	24	1.79 ± 0.11	1.79 ± 0.10
D9 US/D12 MRI sham	20	1.60 ± 0.12	1.61 ± 0.11
D9 US/D12 MRI	18	1.81 ± 0.12	1.85 ± 0.12
D9 US	9	2.01 ± 0.17	1.90 ± 0.17
D9 US sham	23	2.00 ± 0.11	2.10 ± 0.11

*Indicates values that are significantly different ($P \leq 0.05$) from the cage control group.

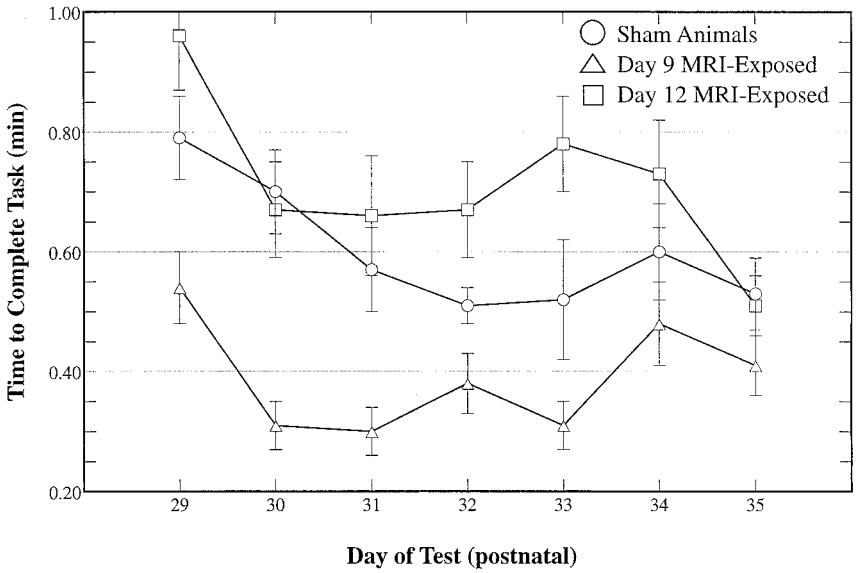

Figure 6. Daily latencies to complete the parallel bars motor test. Comparison of the time in minutes required to complete the parallel bar obstacle course for the day 9 MRI-exposed group (n = 16), the day 12 MRI-exposed group (n = 15), and their sham group (n = 15). Circles represent sham values; triangles and squares are day 9 MRI-exposed and day 12 MRI-exposed values, respectively. Error bars are SEM.

were placed in close proximity to the MRI device (shams) had lowered DSP and DSP/g values. This suggests that a form of environmental stress, quite likely acoustic noise, may be contributing to the decrease in DSP.

Since it appears that fetal development is particularly sensitive to combined ultrasound and MRI exposure, we think it is appropriate to place these results in the context of recent studies of MRI-associated bioeffects. Although a number of previous MRI bioeffects studies have investigated nonmammalian and in vitro cellular systems (13,14,38–44), our focus is on mammalian systems due to their similarity to the human. We are aware of 11 studies investigating reproductive and developmental effects in mice exposed in utero to high-intensity static magnetic fields (15,16,19,45–49) and MRI-associated magnetic fields (17,18,50). These studies employed static magnetic field strengths from 0.03 to 6.3 T and exposure durations from 36 minutes to 90 days, with most studies focusing on days 7–14 pc. Six of these studies found no effect on the developmental parameters monitored, while five reported changes in fetal weight, CRL, fetal resorptions, and litter size. No correlation is evident in these results with respect to either static magnetic field strength or exposure duration. The lack of consensus is typical, in general, of electromagnetic field-induced bioeffects studies in which a variety of exposure chambers, environmental conditions, animal strains, and experimental endpoints are used.

However, three studies using MRI exposure conditions comparable to those used in this study have observed effects similar to those reported here. In particular, Heinrichs et al (18) found a small but significant reduction in fetal CRL after exposure of fetal mice to MRI conditions equivalent to those used in human MRI at a field strength of 0.35 T. Exposures in this study were on the same day of gestation (day 9) as ours, but were 16 hours in duration. The study of Heinrichs et al. (18) like ours, showed that fetal weights were significantly lower in the MRI-exposed animals despite reduced litter size. Fetal weight in mice is normally inversely related to litter size; therefore, in both our study and that of Heinrichs et al., the fetuses from the smaller litters should have been greater in weight. A similar result of decreased CRL (and also smaller craniofacial perimeter measures) was observed by Tyndall (19) in the offspring of mice exposed to a conventional T2-weighted spin-echo imaging sequence (36 minutes' duration) at 1.5 T on day 7 of pregnancy.

In a different study by Tyndall and Sulik (50) using the same MRI exposure conditions, these investigators observed an increased fetal resorption rate and a significant increase in eye malformation. These effects are consistent with reports of a decrease in fetal weight at birth and increases in the number of fetal resorptions following exposure of mice (45) and rats (51) to static magnetic fields alone. In a mouse study by Mevissen et al (45), the mice were exposed from day 1 to day 20 of pregnancy to a magnetic field of 0.03 T, while mice in the Barnothy (46) study were exposed for 18 days to a magnetic field of 0.3–0.6 T. In the rat study by Lax (51), the animals were exposed for 8 hours a day from day 12 to day 16 pc in a 10 T field. When taken as a group, these studies suggest that long-duration exposure of rodents to MRI-associated fields can alter developmental endpoints if applied at sensitive stages in fetal growth. The exposure thresholds and mechanisms of such effects need further study before the safety risks associated with long-term exposure of humans to high-field MRI systems can be addressed.

The literature on the combined bioeffects of physical and chemical agents describes many examples in which the combined actions of two agents is much greater than the individual effects. Many enzymatic or developmental pathways can compensate for a single defect or perturbation whereas two defects completely inhibit the process, generating a non-linear response. Examination of the sham results shows that the combined ultrasound/MRI group is stressed by the sham treatments, resulting in a reduced mean CRL compared with day 9 ultrasound or day 12 MR exposure. The observed weight loss and rectal temperature decrease

in the combined exposure group (although not statistically significant) also indicates that this combined treatment perturbs the basic metabolism of the animals. These results suggest that while MRI alone may not be perturb fetal development, if combined with another chemical or physical stressor, the sum could possibly be greater than expected.

Recent studies of neural development in the mouse report that by day 9 higher cognitive centers are present but that no extensive neural interconnections occur until day 12. Thus, our motor learning study's results are consistent with an effect on this later development stage.

The results obtained in this study show that in utero exposure of mice to high-field (4.0 T), long-duration (9 hour) MRI conditions decreases daily sperm production while increasing fetal resorptions, the incidence of postpartum death, and the time required to acquire motor learning skills. In combination with ultrasound exposure (3.25 MHz, CW, 5 W/cm^2 focused), high-field MRI exposure reduces mean CRL at birth and mean weight throughout fetal development and maturation when it occurs during sensitive times (day 9 and/or day 12) of the developmental cycle. The mechanism responsible for these results could involve one or more of the applied fields interacting with a sensitive stage of fetal development. For example, a hypothesis that gonadal development can be altered by changes in hormone level that adversely affect Sertoli cell number and hence gonadal development was presented in our previous publication (6,8). This research, which employs substantially greater magnetic fields, field gradients, and durations of exposure than are currently in general clinical use, is an important step in insuring safe operating conditions for developing NMR technology.

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