Effects of Prenatal Ultrasound Exposure on Adult Offspring Behavior in the Wistar Rat (43937)

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Abstract. **An ultrasound exposure tank was specifically designed for experimental bioeffects studies. Thirty-six pregnant rats were anesthetized, immersed to the axilla in a water tank, and exposed on Day 15, 17, and 19 of gestation. Twelve rats were exposed to 5.0 MHz pulsed ultrasound of effective pulse duration equal to approximately 0.170 μsec, pulse repetition rate (PRF) 1 kHz, and a spatial peak, temporal peak intensity (I_{sptp}) of 500 W/cm^2, representing a clinically appropriate exposure level. The spatial peak pulse average (I_{sppa}), spatial peak temporal average (I_{spta}), and instantaneous maximum (I_m) intensities were determined to be 100 W/cm^2, 24 mW/cm^2, and 230 W/cm^2, respectively. The maximum rarefraction pressure, p_r, was measured as 12.5 × 10^5 Pa, and the total power was 2.5 mW. Twelve other rats were exposed to 1500 W/cm^2, I_{sptp}, and 12 were sham insonified. Since the focal area was about 0.05 cm^2, computer controlled stepper motors moved the rats through the ultrasound field to ensure uniform exposure of the abdominal/pelvic region. Total exposure time was 35 min. A miniature thermocouple was implanted in a few rats to verify that no significant temperature increase took place due to exposure. A total of 278 offspring were maintained until postnatal Day 60 when they were subjected to two of four behavioral tests in random order within sexes. The results indicate no consistently observed dose-related alterations in adult behavior due to prenatal fetal exposure to 5.0 MHz ultrasound below an intensity (I_{sptp}) of 1500 W/cm^2.** [P.S.E.B.M. 1995, Vol 210]

With increased clinical use of ultrasound in recent years, there has been a growing interest and concern about possible side effects of exposure to diagnostic levels of ultrasound, particularly on the developing fetus exposed *in utero*. Increased attention has also been placed on methodology for adequate characterization of ultrasound fields produced by the imaging equipment.

Ultrasound has been used as a clinical diagnostic tool to visualize internal structures of the human body for the past 25 years. With rapidly growing improvements in instrumentation technology and a lack of clear-cut adverse effects, its use has increased significantly, especially with its application in fetal imaging. It is now estimated that one out of every two children born in the United States today has been exposed to ultrasound prenatally (1), with similar increases in use occurring in European countries as well.

Damage to the central nervous system (CNS) in developing and adult mammals after exposure to high ultrasound intensities has been reviewed (2–4). In many cases, the damage reported may have been attributable to subtle biochemical alterations which occur during critical periods of brain tissue development in the fetus (5–7), resulting in lesions in the CNS. More subtle effects have been observed indirectly through the use of a battery of behavioral and reflex tests (8, 9).

Alterations in behavior may be among the most subtle abnormalities that can be observed. It has been shown that postnatal functional evaluation can be a

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more sensitive indicator of teratogenic effects than physical malformations (10, 11).

The objective of the present study was to determine if prenatal exposure of pregnant rats to ultrasound (500 or 1500 W/cm^2 I_{sptp}) at 5 MHz during the fetal period would result in dose-related postnatal alterations in behavioral test performance of adult offspring.

Materials and Methods

The ultrasound exposure unit was designed to mimic as closely as possible actual clinical conditions of a human prenatal ultrasound examination. In clinical practice, the ultrasonic source is scanned over the entire intended exposure area. The total exposure area on the subject animal averages 5 cm (horizontally) by 7 cm (vertically). Since the beam width at the point of selected I_{sptp} of the transducers used in this study averaged about 1 mm the animal had to be moved through the ultrasonic field to ensure uniform exposure of the entire uterine horn. The transducer remained stationary while the anesthetized animal was put in a holder and raster scanned through the field. The holder kept the animal in a vertical (head up) position, so the exposed area was totally submerged in water. It was not overly restrictive, since earlier studies have shown that restraint of a pregnant animal could be teratogenic (12, 13).

Ultrasound Exposure System. This system was specifically designed for application in ultrasound bioeffects studies, also providing monitoring and recording of relevant acoustic intensities. The exposure arrangement was also used to scan the test animal in the ultrasonic field during exposure. Its components included: (i) A waterbath/heater system which consisted of a small tank (15 × 15 × 21 cm), lined with sound absorbing material (Sorbothane), partially submerged in a larger (24 × 18 × 18 cm) temperature controlled (37° + 0.5°C) tank. (ii) An IBM PC-XT computer, programed to calculate acoustic output and control the micropositioning system. Software developed to run this system analyzed the pressure-time waveform and automatically computed the values of I_{sptp}, I_{spta}, I_{sppa}, and I_m, both in water and *in situ* (14). (iii) A Tektronix T2430 digital oscilloscope with a sampling rate of 75 MHz was used to display the pressure-time waveform, which was then digitized and analyzed to determine factors such as peak-to-peak voltage ($V_{p\text{-}p}$), peak positive voltage (V+), peak negative voltage (V−), pressure gradient, delay time between transmitted and received pulse, and frequency of the transmitter. Use of the oscilloscope also facilitated the determination of other parameters such as pulse repetition rate (PRR) and beam profile. (iv) The transducer which generated the ultrasonic field was connected to its power source, the pulser. The pulser was also connected to the oscilloscope. (v) The hydrophones served as the receiving or detection element of the ultrasound being emitted by the transducer. The hydrophones scanned the field and recorded the point of maximum pressure-time waveform for subsequent analysis. Two types of calibrated piezoelectric hydrophones were used: a membrane-type (Sonic Technologies, Hatboro, PA) and needle-type (The Danish Institute of Biomedical Engineering, Copenhagen, Denmark). Both types employed a sensing element made of polyvinylidene fluoride (PVDF). These hydrophones were selected because of their relatively flat frequency response, adequate linearity, and sufficiently wide angular response and bandwidth. The membrane-type hydrophone consisted of a circular piezoelectric polymer film mounted on a hoop (approximately 6 cm in diameter). The thickness of the film used, 9 μm, made the hydrophone virtually transparent to ultrasound in the 1–20 MHz range. The diameter of the electrode active element was about 0.4 mm. The needle-type hydrophone consisted of a 0.5 mm diameter of piezoelectric polymer mounted on the tip of a hypodermic needle. Both hydrophones were calibrated by comparison with a standard hydrophone using time delayed spectometry (TDS). The calibration has been confirmed by an international intercomparison study carried out with national laboratories (14–16). (vi) The animal holder and micropositioning systems were used to scan the animal in the ultrasonic field during exposure. A micropositioning system was required to provide and control proper alignment of the acoustic source and hydrophones. A system with a resolution of 5 μm in the x and y directions (Velemex Inc.) was used. Scanning was completed using a computer controlled PKS Digiplan stepper motor controller to obtain a beam profile. The computer controlled micropositioning system also allowed for repeatable positioning of the hydrophones in the acoustic field. The acoustic source was positioned so that the field was generated precisely along the z direction. The plastic animal holder consisted of a removable "table" and support fixture and was strong enough to support the pregnant animal. The animal was secured by Velcro straps under the arm pits and around the feet. The holder was attached to the micropositioning system. The x axis length, y axis length, and z axis increment were adjustable and entered manually into the computer. During ultrasound exposure the animal holder moved continuously for the prescribed exposure time of 35 min.

Acoustic Power, Power Fraction, and Attenuation Measurements. The acoustic power absorbed was calculated by determining the total power in front of and behind the subject animal. Measurements of beam profiles and the power fraction absorbed were determined using "reference" rats which remained stationary during the measurement procedure. Thus,

the value of total power absorbed represented worst-case conditions, since during actual exposure time no single point would be continuously exposed. Using beam profile information, respective total powers were determined using predetermined algorithms (17).

The rat was anesthetized with a subcutaneous injection of ketamine hydrochloride (Ketaset, 0.075 ml/g body wt), and the hair on the abdomen was shaved. Ketaset is not known to cause any appreciable hypothermic reaction. The animal was placed in the holder and submerged to the axilla. The hold apparatus was moved in the z direction so that the focal distance corresponding to the prescribed value of I_{sptp} lay approximately midpoint in the saggital and transverse plane of the rat. The membrane-type hydrophone was then placed directly in front of the rat and scanned in the x and y directions to find the point of maximum peak-to-peak voltage. At this point the corresponding pressure-time waveform was captured and analyzed, and the beam profile was taken.

The entire procedure was repeated directly behind the rat using a needle-type hydrophone. If there was no pressure-time waveform behind the rat, the beam profile did not need to be taken and it indicated that acoustic energy was absorbed by the rat. If the pressure-time waveform did exist behind the animal, it was recorded and analyzed.

***In Situ* Measurements; Attenuation.** Measurement of attenuation due to overlaying animal tissue was accomplished using a specially designed lead zirconate titanate (PZT) piezoelectric hydrophone probe. The PZT material was mounted at the tip of an 18-gauge hypodermic needle and the probe was calibrated by comparison with the previously calibrated PVDF membrane-type hydrophone. To determine the attenuation of the ultrasound, *in situ* measurements of the pressure-time waveform were performed using a PZT needle-type probe threaded through a 15-gauge biopsy needle. Prior to *in situ* measurements, this probe was used to find the pressure-time waveform in water at the point in the field corresponding to the desired value of I_{sptp}. This waveform, recorded in water, was subsequently compared with the waveform measured *in situ* to determine the total attenuation due to the tissue layers.

In situ measurements were taken using a "reference" rat from the group of pregnant rats being exposed that day. This rat was anesthetized and shaved, and a small incision was made in the abdominal area. A biopsy needle was inserted through the back, exiting through the incision, so the tip of the needle faced forward and was positioned on the surface of the uterine horn when retracted under the muscle layer. The incision was sutured around the tip of the needle, the rat was placed in the holder, and the hydrophone was threaded through the needle so that the hydrophone tip lay just under the muscle layer and on top of the uterine horn. Reference measurements in water were performed, and attenuation was determined in decibels per unit length. This procedure was repeated several times to establish a nonthermal exposure level.

Temperature Monitoring. Temperature was monitored during exposure by implanting miniature thermocouples (18-gauge, type T, copper/constantin needle microprobes—Sensortek, MT-30/1.5, with a time constant of 0.02 sec) into other pregnant "reference" rats used specifically for this purpose. The thermocouple diameter, 0.030 mm, was smaller than the acoustic wavelength of the transducers (0.30 diam for 5.0 MHz) by at least an order of magnitude. The probe was connected to a digital thermometer (Sensortek TH-5). Prior to temperature measurement the rat was anesthetized and shaved, and an abdominal incision was made. The thermocouple was inserted so the tip lay directly on the uterine horn and the sheathing did not disturb the ultrasound field. After suturing the rat was placed in the holder, immersed, and moved to the desired focal distance. The position of the rat was adjusted in the x and y directions so the point of maximum intensity was on the tip of the thermocouple.

The rat remained in the water until its internal body temperature equilibrated (approximately 30 min). Temperature readings were taken from the digital monitor every minute. Internal body temperature was considered stable when temperature values remained constant for 10 consecutive readings. Readings were then taken with the transducer turned on at 1 min intervals for 35 min. Because the animal was not moved through the field as it would be during ultrasound exposure, this represented worst-case exposure conditions.

Exposure and Testing of Animals. Thirty-six Wistar strain female rats were maintained in a temperature- and humidity-controlled animal facility with a 12:12-hr light:dark cycle. Animals were given Purina Laboratory Chow and water *ad libitum* throughout the test period. All animals were maintained in accordance with federal guidelines (*Guide for the Care and Use of Laboratory Animals,* U.S. Department HEW, DHEW Publication No. [NIH] 85-23, 1985) and AALAC principles, as well as standards established by the Animal Welfare Act. Males and females were housed together overnight. At 9:00 AM of the morning when sperm were found in a vaginal smear females were considered to be 0 hr, 0 days pregnant. They were randomly assigned to one of three exposure groups: ultrasound exposure at 5 MHz at I_{sptp} levels of 500 or 1500 W/cm^2, or sham exposure. The 15-day pregnant rats were shaved, placed in the holder, and exposed for 35 min. This procedure was repeated on Day 17 and 19 for each rat.

The 500 W/cm^2 group of rats received a pulsed

ultrasound of effective pulse duration equal to approximately 0.170 μsec, pulse repetition rate of 1 kHz, and a spatial peak, temporal peak intensity of 500 W/cm^2, representing a clinically relevant exposure level. The I_{sppa}, I_{spta}, and I_m levels were determined to be 100 W/cm^2, 24 mW/cm^2, and 230 W/cm^2, respectively. The maximum rarefaction pressure, p_r, was measured as 12.5×10^5 Pa, and the total power was 2.5 mW. The 5-MHz acoustic source diameter was 28 mm. The 1500 W/cm^2 group was exposed to 1500 W/cm^2 (I_{sptp}) (I_{sppa}, 350 W/cm^2; I_{spta}, 58 mW/cm^2; I_m, 600 W/cm^2). Sham irradiated animals were manipulated in the same manner, but the ultrasound source was not turned on.

Mothers were allowed to deliver their offspring, at which time the litters were reduced to eight neonates per litter. A total of 278 offspring were monitored using a number of selected postnatal parameters. Postnatal weights of female and male offspring were monitored weekly for 20 weeks and were published previously. There were no significant dose-related alterations in postnatal weight or postnatal growth rates (18). Adult behavioral tests were initiated on postnatal Day 60. Each rat offspring was tested using two of four behavioral tests. The tests were randomly assigned within experimental group and sex, and were administered in a random order. The following tests were used:

Conditioned avoidance (two-way active) response. For the conditioned avoidance (two-way active) response (CAR) test, rats were placed in a computer-controlled BRS/LVE shuttle box. Each rat was given one test session per day for 4 days. Each session consisted of 100 trials. Each rat was then left undisturbed for 6 days. On the 7th day, a final test session was given. One trial consisted of a 10-sec rest period (inter-trial interval [ITI]) followed by a 10-sec (light and buzzer) warning period, after which a mild shock was administered through the flooring grid for 10 sec or until the rat crossed over to the other side of the box. A cross-over during the ITI was called a premature cross-over. A cross-over during the warning period was referred to as an avoidance, and a cross-over during the shock phase was called an escape. Data were recorded on a strip chart and automatically processed using an interfaced computer.

Water T-maze. For the water T-maze test, animals were tested in a 14-in high stainless steel tank filled with 34°–38°C water. A wire mesh platform was placed at the end of one of the short arms of the "T" (the nonpreferred side, as determined by pretesting). The rat was released at the base of the long arm, and time to choice and to platform was recorded. Criterion was five consecutive correct choices or 15 trials, if criterion was not achieved.

Open field. The rat was placed in a 3 ft × 3 ft open area with side walls 12 in high and painted flat black. The floor was divided into a gridwork of 6-in squares. During the 10-min trial period for the open field test, the following activity was recorded by an observer: squares entered, rearing, defecation, urination, grooming, jumping, and digging per min.

Activity wheel. Each animal was placed in a Wahmann activity wheel located in a sound- and light-attenuated chamber for 24 hr. The wheel was attached to a counter and to an animal cage to which the rat had free access. The number of revolutions during the 24 hr was recorded.

Data were statistically analyzed using the Kruskal-Wallis and Mann-Whitney test procedures. Kruskal-Wallis is a nonparametric test which examines for differences in location across "k" groups. Mann-Whitney is the analogous nonparametric procedure for two groups. Means and standard deviations are presented for descriptive purposes. Due to severe right and left skewing in the adult measurements, results of nonparametric analyses within sexes are reported. All variables exhibiting a dose-response gradient across sexes were analyzed further using the classical nested analysis of variance (ANOVA) model. Variables exhibiting extreme location-spread problems were examined with a nested ANOVA analysis following appropriate variance stabilizing transformations. No additional statistically significant ($P < 0.05$) effects were found with the nested ANOVA approach.

Results

Results of absorption analysis indicated that, since no pressure-time waveform was observed directly behind the subject rat, most of the power measured was absorbed. A small fraction of the power, however, may have been reflected and/or scattered as it propagated through the water and/or animal. Since no reflections were observed, any reflected fraction of power was considered negligible. Average attenuation of ultrasound in rat tissue was calculated to be 1.567 ± 0.175 dB/cm. This value agrees with values listed in the literature (19). The variability of 11% between each measurement is most likely due to tissue variability, since a different rat was used for each attenuation measurement.

Results of intrauterine temperature monitoring plots confirmed that there was no instance where the temperature rose more than 1°C during ultrasound exposure. Some rats did exhibit slight temperature variations, most likely due to slight muscle movements during respiration, which could cause the thermocouple to move slightly and affect temperature measurements.

Analysis of the results of the open field test for number of rearings and number of squares entered for male and female offspring indicated no significant ($P < 0.05$) dose related differences among the groups. Within groups, females were consistently more active than males for both parameters (Table I).

Table I. Open Field Test Results

Group	*n*	Number of squares entered	Number of rearings
Male:			
0 mW/cm^2	46	250 ± 66	59 ± 27
500 mW/cm^2	47	256 ± 72	55 ± 21
1500 mW/cm^2	42	270 ± 67	56 ± 27
Female:			
0 mW/cm^2	47	287 ± 74	68 ± 22
500 mW/cm^2	42	326 ± 71	76 ± 22
1500 mW/cm^2	49	299 ± 67	72 ± 25

Note. Values are expressed as mean ± SD.

Water T-maze data for male and female offspring were analyzed for four parameters: time to choice, five correct trials; time to platform, five correct trials; time to choice, all trials; time to platform, all trials. There were no statistically significant differences ($P > 0.05$) within the sexes for these parameters due to ultrasound exposure (Table II).

Activity wheel data were analyzed for each sex during the 12-hr light cycle, the 12-hr dark cycle, and the total 24-hr period. Only male offspring exhibited a dose-related decrease in activity, and only during the light cycle ($P < 0.01$). Within groups, females were consistently significantly ($P < 0.05$) more active than males (Table III).

CAR data were analyzed for the number of premature crossings, the number of avoidances, the number of escapes, the mean avoidance time, and the mean escape time. Two types of retention score were created, one comparing the retest score with the 4th day score and one comparing the mean of the scores from the first 4 days with the retest score. There were no significant ($P > 0.05$) dose-related differences for any parameter for either male or female adult offspring (Table IV–VI).

Discussion

The ultrasound exposure system used in the present study provided quantitative measurement of relevant acoustic output parameters of the ultrasonic source as well as exposure parameters of subject animals, including continuous monitoring and adjustment of desired exposure parameters during the bioeffects experiment. Therefore, subject animals could be exposed to precisely prescribed field parameters. The raster scanning mechanism ensured an exposure procedure that closely mimicked clinical ultrasound examination conditions.

Overall uncertainty in derived intensity parameters averaged ±20%. These values are of similar magnitude to those determined by another laboratory (15, 16). Since systematic errors were generally due to hydrophone performance characteristics, it is likely they can be minimized by improving design and performance of the hydrophone itself.

Errors in determining the beam profile and, subsequently, total acoustic power were influenced by the effective diameter of the hydrophone. Maximum errors due to spatial averaging effects were approximately 12.5%. Beam profile measurements can also be influenced by the parameters used for their determination. In this study, peak negative pressure was used for beam profile determination. For distorted waveforms such as those generated by the transducers used here, this measurement technique may have slightly increased the overall error in the beam profile measurement (20).

Restraint stress, a well known teratogen, could be a confounding variable in the present study (12, 13, 21). There is no evidence, however, that restraint of unconscious mothers causes significant alterations in pregnancy outcome or postnatal behavior. The present study controlled for possible restraint effects by including this variable in the sham-exposed group.

One of the first reported and best understood interactions of ultrasound and biological tissue is the production of heat (22). This phenomenon is most likely to occur during ultrasound applications of high-intensity, uniform, continuous-waves (cw) for a considerable period of time. While useful in therapeutic applications, heat production is undesirable in diagnostic applications of ultrasound. This is especially

Table II. Water T-Maze Test Results

Group	*n*	Five correct trials to choice	Five correct trials to platform	All trials to choice	All trials to platform
Male:					
0 mW/cm^2	45	15.4 ± 11.4	18.1 ± 10.8	15.3 ± 8.8	23.0 ± 14.1
500 mW/cm^2	42	14.1 ± 12.2	16.3 ± 13.1	14.2 ± 7.3	21.1 ± 11.6
1500 mW/cm^2	40	13.1 ± 6.0	15.5 ± 7.2	13.9 ± 5.1	21.0 ± 8.7
Female:					
0 mW/cm^2	44	12.6 ± 6.7	17.1 ± 12.6	13.4 ± 5.7	24.3 ± 20.7
500 mW/cm^2	41	13.8 ± 10.0	16.4 ± 11.1	13.3 ± 5.9	20.3 ± 10.5
1500 mW/cm^2	47	12.2 ± 5.6	15.2 ± 7.0	12.6 ± 4.5	20.9 ± 13.8

Note. Values are expressed as mean time ± 1 SD.

Table III. Activity Wheel Test Results

Group	*n*	12-hr light cycle	12-hr dark cycle
Male:			
0 mW/cm^2	40	170 ± 100	298 ± 189
500 mW/cm^2	40	164 ± 113	328 ± 279
1500 mW/cm^2	44	106 ± 117	243 ± 225
Female:			
0 mW/cm^2	45	578 ± 366	620 ± 348
500 mW/cm^2	35	672 ± 408	778 ± 340
1500 mW/cm^2	46	503 ± 264	624 ± 401

Note. Values are expressed as mean number of revolutions ± 1 SD.

true during fetal imaging, since hyperthermia is a well-known teratogen (23–26). Brent *et al.* (4), in a review of the reproductive effects of ultrasound exposure, indicated that temperatures of at least 2.5°C above normal at an appropriate stage in human pregnancy were necessary to elicit teratogenic activity. Ziskin (27) also stated that a 2.5°C increase above normal is necessary to induce microcephaly and other morphologic abnormalities, dependent upon the gestational stage at the time of exposure.

Another mechanism of interaction between biological material and ultrasound is ascribed to cavitation. For cavitation to occur during ultrasound exposure, gas nuclei must be present in the form of small bubbles or pockets with dimensions of micrometers or smaller. Two types of cavitation are found to exist: stable and transient. Stable cavitation is described as volume oscillation, or vibration of gas bubbles (19). These vibrating gaseous bodies may lead to microstreaming in the liquid-like media adjacent to the bubbles. Microstreaming may then produce mechanical stresses sufficient to disrupt the cell membrane (28). Until recently, it was believed that diagnostic ultrasound pulses are too short to produce cavitation. Recent studies, however, suggest that the microsecond-length pulses of ultrasound can cause transient cavitation (29). While this type of cavitation was observed to be produced by pulsed ultrasound *in vitro*, it remains to be seen whether it can be produced at diagnostic levels *in vivo* (30). There is no experimental evidence for transient cavitation to occur *in vivo* at diagnostic ultrasound levels.

A number of studies have shown that fetal exposure to ultrasound can cause alterations in pregnancy outcome, while others have not observed significant exposure-induced changes. O'Brien and Stratmeyer (31) observed significant postnatal weight reduction at 8 weeks in mice exposed to ultrasound for 2 min on Day 13 of gestation. McClain *et al.* (32) exposed rats to 10 mW/cm^2 (I_{sata}), 2.5 MHz cw for 30 min or 2 hr on Day 8–13 or 11–13 and examined the fetuses on Day 20 of gestation. They did not observe any significant changes in mortality or abnormality rates.

Sikov and Pappas (33) exposed rats on Day 9, 10, 12, or 15 of gestation to 0.8 MHz cw ultrasound at intensity levels of 0–20 W/cm^2, I_{sata}. Embryolethality rates were similar for exposures on any of the first 3 days, but less so from exposure on Day 15. They also observed a difference in the type of malformations observed, with cardiovascular and CNS malformations corresponding to earlier exposure times and skeletal and limb defects to later times. Kimmel *et al.* (34, 35) did not observe significant teratogenic activity in mice on Day 17 due to ultrasound exposure (1.0 MHz cw) on Day 8 of gestation for 10 min at intensities of 0–1.0 W/cm^2, I_{sata}.

The adult behavior tests used in this study are well established parameters (36). These tests have also been shown to be sensitive indicators in other studies completed by this laboratory (37, 38).

Vorhees *et al.* (39) used a unique approach to study possible teratogenic effects of ultrasound exposure. Rats were trained to remain immobile while exposed to ultrasound, thus obviating the need for anesthesia or possible complications resulting from forced restraint. The animals were exposed to 0.1, 2.0, or 30.0 W/cm^2 (I_{spta}), 3.0 MHz cw, for about 15 min/day on Day 4–19 of gestation. There were no dose-dependent changes in a variety of maternal parameters, the incidence of malformations, or fetal weights. Vorhees *et*

Table IV. Results of CAR Test—Day 4

Group	*n*	Premature crossovers	Number of avoidances	Number of escapes	Mean avoid time	Mean escape time
Male:						
0 mW/cm^2	44	10.5 ± 8.5	96.0 ± 10.0	3.7 ± 9.5	2.9 ± 1.2	1.0 ± 1.6
500 mW/cm^2	43	7.2 ± 5.4	94.0 ± 16.4	5.8 ± 16.4	3.5 ± 1.1	0.8 ± 0.7
1500 mW/cm^2	41	11.4 ± 9.3	91.1 ± 17.5	7.2 ± 13.0	3.0 ± 1.3	0.7 ± 0.7
Female:						
0 mW/cm^2	45	13.2 ± 8.0	97.8 ± 2.5	1.7 ± 1.9	3.4 ± 0.8	0.6 ± 0.6
500 mW/cm^2	40	9.9 ± 6.8	97.1 ± 5.5	2.8 ± 5.4	3.6 ± 0.9	1.0 ± 1.2
1500 mW/cm^2	49	16.1 ± 9.3	95.2 ± 12.0	3.9 ± 9.1	3.5 ± 1.1	0.7 ± 0.6

Note. Values are expressed as mean number ± 1 SD.

Table V. Results of CAR Test—Retest Day

Group	*n*	Premature crossovers	Number of avoidances	Number of escapes	Mean avoid time	Mean escape time
Male:						
0 mW/cm^2	44	10.3 ± 7.0	96.6 ± 7.8	2.6 ± 6.1	3.0 ± 1.0	0.6 ± 0.9
500 mW/cm^2	43	9.6 ± 6.8	93.3 ± 16.0	6.4 ± 15.9	3.4 ± 1.2	0.7 ± 0.6
1500 mW/cm^2	41	13.0 ± 8.0	95.2 ± 10.2	3.9 ± 8.0	3.1 ± 1.0	0.7 ± 0.9
Female:						
0 mW/cm^2	45	16.8 ± 9.2	97.3 ± 3.4	2.4 ± 3.0	3.4 ± 1.0	0.7 ± 0.5
500 mW/cm^2	40	13.4 ± 8.0	97.6 ± 4.6	2.2 ± 4.5	3.3 ± 0.8	1.2 ± 2.1
1500 mW/cm^2	49	17.4 ± 9.6	96.6 ± 5.9	3.2 ± 5.9	3.5 ± 0.9	0.8 ± 0.8

Note. Values are expressed as mean number ± 1 SD.

al. (40) also exposed rats to these levels of ultrasound daily for 10 min on gestational Day 4–20. Although they did not observe exposure-related alterations in maternal parameters, offspring survival and growth, or neonatal psychophysiologic parameters, changes did occur in offspring adult behavior in locomotor activity and in two measures of the multiple-T water maze test performance at the highest dosage level.

Norton *et al.* (41) did observe histologic changes 24 hr after exposure of Day 15 rat fetuses to 2.5 MHz pulsed ultrasound at an intensity level of 0.78 W/cm^2 (I_{spta}) with a PRR of 50 KHz for 30 min. Average peak temperature rose to 40.1°C. They observed an increase in the number of pyknotic cells and macrophages, and a decrease in mitotic figures in the telencephalon. They also observed changes in several neonatal and adult psychophysiologic parameters (9). They stated it was possible the results might have been due entirely or in part to thermal activity.

The majority of studies on the effects of ultrasound on behavior have been performed using continuous wave ultrasound. In most of these studies, exposure was limited to the period of major organogenesis. Day 15, 17, and 19 of gestation were chosen for the present study since they correspond to a critical period in the development of portions of the CNS where there may be sensitivity to ultrasound disruption (7). These days also correspond to the time during human fetal development when diagnostic ultrasound examinations are commonly performed. The ultrasound exposure was performed on 3 days, instead of a single exposure, to maximize the likelihood of a bioeffect.

Table VI. CAR Test Results

Group	*n*	Retention score	
		Retest vs 4th day score	Retest vs mean of scores from 4 days
Male:			
0 mW/cm^2	44	1.1 ± 6.6	27.3 ± 51.5
500 mW/cm^2	43	2.0 ± 18.8	8.4 ± 12.8
1500 mW/cm^2	41	9.6 ± 29.5	23.7 ± 42.8
Female:			
0 mW/cm^2	44	−0.5 ± 4.6	11.9 ± 12.0
500 mW/cm^2	39	1.0 ± 9.6	7.5 ± 9.5
1500 mW/cm^2	51	7.4 ± 51.4	10.7 ± 18.6

Note. Values are expressed as mean ± 1 SD.

Murai *et al.* (42) exposed pregnant rats to cw ultrasound with spatial average, temporal average intensity of 20 mW/cm^2 for a total of 300 min. Results showed some possible delay in the development of the neuromotor reflex in the offspring. A similar study performed by the same group of researchers under the same exposure conditions resulted in altered emotional behavior in the adult rat. Sikov *et al.* (43) found delayed neuromuscular development in the rat exposed for 5 min prenatally to cw ultrasound.

Tarantal and Hendrickx (44, 45) exposed cynomolgus macaques five times weekly to 7.5 MHz ultrasound (I_{spta}, 12 mW/cm^2) on Day 21–35, three times per week on day 36–60, and once a week on Day 61–150 of pregnancy. Most of the numerous morphologic parameters measured did not change significantly, but there were alterations in birth weight and crown-rump length, and in white blood cell counts. There were no exposure-induced changes in behavior, but a significant increase in muscle tone was observed among the exposed offspring.

Results of the present study indicate no significant dose-related alterations in adult behavior due to prenatal fetal exposure to 5.0 MHz pulse echo ultrasound below an intensity level (I_{sptp}) of 1500 W/cm^2. Possible exposure-related effects for several parameters were observed at the high dosage level (three times the clinically relevant exposure). There were provocative trends for a few measures of activity, however they were of insignificant magnitude and, therefore, preclude any meaningful association with ultrasound exposure. The addition of a group exposed at 5000 W/cm^2 (I_{sptp}) will aid in determining if any significance can be attached to these observations, and in the establishment of dose-response relationships for all of the neonatal and adult parameters.

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