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Behavioral Effects of Prenatal Exposure to Pulsed-Wave Ultrasound in Unanesthetized Rats

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ABSTRACT The present experiment examined the developmental neurotoxicity of pulsed-wave (pw) ultrasound in rats, using an exposure system designed to eliminate restraint or anesthesia from the exposure conditions. Pregnant Sprague-Dawley CD rats trained to remain immobile in a water-filled ultrasound exposure tank were scanned with 3-MHz pw ultrasound at spatial peak temporal average intensities (I_{SPTA}) of 0, 2, 20, or 30 W/cm^2 on embryonic days 4–20 for approximately 10 min/day. The data showed that such insonation produced no adverse effects on maternal weight gain or reproductive outcome, nor on the postnatal growth or survival of the offspring. No exposure-related alterations in behavioral development were observed in the offspring of rats scanned with pw ultrasound during gestation. In addition, there was no consistent evidence of an ultrasound-associated change in the adult offspring behaviors tested; i.e., no treatment effects were found on measures of locomotor activity, water maze learning, and acoustic startle reactivity. An effect on tactile startle was observed on some trials in the low exposure group male offspring, but this effect was neither dose dependent nor consistent with any other finding. Overall, these results indicate that the neurobehavioral development of rats was not altered by prenatal exposure to pw ultrasound at I_{SPTA} levels of up to 30 W/cm^2.

Ultrasonography has assumed an increasingly important role in obstetrics, with use of this diagnostic modality during pregnancy becoming nearly universal in recent years (Moore et al., '90). However, unresolved risk/benefit issues make the appropriate use of ultrasound in pregnancy a subject of continuing debate (National Institutes of Health [NIH] '84; Youngblood, '89; Ewigman et al., '93; Newnham et al., '93). There is no conclusive evidence that prenatal exposure to ultrasound in diagnostic procedures carries any risk, but a cautious approach to ultrasound use in pregnancy is dictated by the potential for biological effects demonstrated in various experimental ultrasound systems (National Institutes of Health (NIH) '84; Brent et al., '91; American Institute of Ultrasound in Medicine (AIUM) '93; American Institute of Ultrasound in Medicine [AIUM], '88). Although experimentally observed ultrasound bioeffects have generally occurred only at exposures exceeding diagnostic levels, an incomplete understanding of the dose-response relationships and mechanisms underlying these reported bioeffects limits the confidence with which the experimental findings can be applied to humans. Accordingly, further research in this area is considered integral to establishing safe exposure criteria for clinical practice (Barnett et al., '94a; Tarantal and O'Brien, '94a; O'Brien, '92a; Sikov, '86a; Schenk, '85a). A trend toward increased acoustic output levels for diagnostic ultrasound devices highlights the importance of defining threshold exposure conditions for ultrasound bioeffects (Miller, '91; Barnett et al., '94; Duck and Martin, '93).

Because the developing nervous system is considered a sensitive target for ultrasound bioeffects, the possibility of neurological or behavioral effects is of particular concern in assessing the safety of ultrasound in pregnancy (NIH, '84; Ziskin and Petitti, '88; Barnett et al., '94). However, the few epidemiological studies which have examined such long-term effects have been inconclusive. One study (Stark et al., '84) reported an increase in dyslexia among ultrasound exposed 7- and

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12-year old children compared to unexposed controls. Recently, two randomized clinical studies from Norway have followed children to 8–9 years of age. In the first (Salvesen et al., '92), no association between prenatal ultrasound exposure and dyslexia was found, and no effects on reading or spelling attainment or on measures of intelligence were obtained. In the second report (Salvesen et al., '93), this group reported a small but significant increase in non-right-hand dominance among ultrasound-exposed children at this age.

In experimental animal studies, changes in several CNS end points have been reported after in utero exposure to ultrasound (Hande et al., '93; Norton et al., '91; Tarantal and Hendrickx, '89; Sikov et al., '77; Murai et al., '75a,b). These studies suffer from a number of methodological limitations which have recently been discussed (Vorhees et al., '94). One factor which may confound the interpretation of animal studies is the use of anesthesia or forced restraint in the experimental insonation procedures, since these can themselves contribute to altered developmental outcomes (Weinstock et al., '88; Mazze et al., '85). In order to eliminate the possible effects of immobilization stress or anesthesia, we have developed a method for insonation of rats trained to remain immobile. This ultrasound exposure system has been used previously to assess the developmental effects of continuous wave (cw) ultrasound (Vorhees et al., '91, '94) and the teratologic potential of pulsed wave (pw) ultrasound (Fisher et al., '93). The purpose of the present study was to examine the possible developmental neurotoxicity of prenatal exposure to pw ultrasound. A range of ultrasound exposure levels was used in an attempt to describe a dose-response relationship for any effects which might be seen.

MATERIALS AND METHODS

Animals

Subjects were nulliparous female Sprague-Dawley CD (VAF) rats (Charles River, Portage, MI), housed according to American Association for the Accreditation of Laboratory Animal Care guidelines. Prior to breeding, immobility training was conducted in a water-filled chamber approximately the same size as that of the confinement chamber of the ultrasound exposure tank. Each female received two consecutive days of confinement for 12 min/day followed by 2 days of 10 min/day in the training tank. This repeated confinement induced conditioned immobility, i.e., an attenuation of efforts to escape. Females were housed with males on the day following the last training session. Discovery of a vaginal plug was considered embryonic (E) day 0.

On E0, dams were assigned to one of four treatment groups for pulsed-wave (pw) ultrasound exposure on a weight-matched basis and encoded on the exposimetry computer so that experimenters were blind to treatment group assignment. At least 12 dams were assigned to each group. On E3, each dam received refresher immobility training (12 min) in the exposure tank. After removal from the vessel, the abdomen of each rat was shaved and chemically depilated with Nair®.

Exposure system

A detailed description of the ultrasound exposimetry system developed specifically for these experiments has been provided previously (Vorhees et al., '91a; Smith et al., '90a). Briefly, the exposure system consisted of a water-filled, rectangular tank constructed of acrylic, with a partially focused 4.6-cm-diameter, 3-MHz, PZT-4 crystal mounted in a moveable transducer assembly platform approximately 30 cm below the water surface. Pregnant rats previously trained to remain immobile were placed in an inner confinement chamber (10 × 15 cm) directly above the ultrasound beam. The ultrasound beam was calibrated under free-field conditions with a calibrated membrane hydrophone (Marconi). For a stationary beam, at the focus that would be a location within the floating rat, the free-field 90% and 50% intensity beam widths were 1.6 cm and 3.2 cm, respectively.

Ultrasound (US) intensities were selected to span a broad range of exposures. At the focus, the pw values of the spatial peak, temporal average intensity (I_{SPTA}) were 0, 2.0, 20, and 30 W/cm^2, as calculated from the measured instantaneous pressures, all at a spatial peak, pulse average (I_{SPPA}) value of 40 W/cm^2. The lowest exposure intensity was chosen to be close to the upper limit of diagnostic intensities, while the highest intensity was calculated to produce a threshold exposure for thermal effects. Temperature calculations may be found in (Vorhees et al., '94). These pw intensity levels had previously been found to produce no effects on growth or morphological development under the same exposure conditions (Fisher et al., '93).

A method based on that described by O'Brien et al. ('82) was used to provide uniform ultrasound exposure to the floating rat's abdominal surface, which was estimated to be approximately 7 (width at the widest point) × 8 cm (xiphoid process to the prepuce) on E17. A raster scan pattern of the movable transducer assembly was set at 8 × 13.5 cm, or approximately 1 cm inside each wall of the confinement chamber, so that the entire enclosure was insonated. The raster scan sequence consisted of 16 8-cm rasters separated by 1 cm. Each rat received one raster scan per day on E4-20 for a total of 17 insonation exposures, each lasting 10 min, with 2 min of transducer positioning time at the beginning of the scanning pattern for a total of 12 min in the water per session. Based on the raster scan pattern and transducer speed, the time that a specific body site was within the 90% intensity beam width during one scanning session was determined to be 8–12 sec. For the 50% intensity beam width the exposure time ranged between 41 and 46.5 sec.

The exposure tank was refilled daily with fresh deionized water and allowed to degas overnight. Water temperature was maintained at 35°C by a proportional temperature controller (Yellow Springs model 72, Yellow Springs, OH), a temperature that was found not to alter core body temperature in awake animals.

Behavioral procedures

Dams were allowed to deliver naturally. On the day of birth (P0), offspring were examined for external defects, sexed, numbered, and on P4 culled to four per sex using a random number table. On P9 offspring were individually marked in preparation for reflex testing using black indelible ink. Offspring were weaned on P28 and housed in same sex pairs, then housed individually on P42.

Reflexology. Two tests of early reflex activity and/or behavior, olfactory orientation and acoustic startle, were conducted on two male/female offspring pairs per litter (A and B). These tests have been described in detail elsewhere (Vorhees, '83). Olfactory orientation was conducted on days P9, 11, and 13 for 1 min each day by placing animals in a 12 × 38-cm runway midway between equal measured amounts of bedding and scoring movements in either direction based on line crossings marked on the floor and designated as −4 (nearest the clean bedding) to +4 (nearest the home bedding). Acoustic startle testing was conducted on days P19±1 in a San Diego Instruments (San Diego, CA) model SR startle apparatus. Each offspring received 51 trials consisting of exposure to a broad band acoustic stimulus with a predominant frequency of 4 kHz at 115 dB(A) and lasting 20 msec. There was a 70-dB background noise, an intertrial interval of 8 sec, and a 100-msec window following stimulus onset during which responses were recorded. Each session began following a 5-min chamber acclimation period. A piezoelectric force transducer converted the animal's response to a voltage that was used to quantify the maximum amplitude (V_{max}), avrage amplitude (V_{mean}), and latency to maximum response (T_{max}) on each trial.

Activity. Locomotor activity was assessed in the B and C pairs on P20 and P60 using a video tracking system described previously (Vorhees et al., '92). For each 30-min test session, computer-generated tracings of the animal's path were scored by length in different regions of the 40 × 40-cm field, under red light illumination. Activity in these regions was termed corner, side, and central activity, with the combination of corner and side activity defined as peripheral and of all regions as total activity. In addition, the number of section entries was defined as the number of transitions.

Water maze. Offspring pairs A and D were assessed for learning and memory in the Cincinnati water maze, a multiple-T maze with 9 double-ended cul-de-sacs. Rats were tested for 5 consecutive days, beginning on the nearest Monday after day P50. On the first day, rats received 4 trials in a 150 cm straight swimming channel. On the remaining days, rats received 2 trials/day in path B of the maze, using an unassisted escape test procedure. Rats were scored for errors and time to complete the maze, up to a limit of 5 min/trial. Details of the apparatus (Vorhees, '87c) and test procedures (Vorhees et al., '91b) have been described previously.

Adult Startle. Offspring pairs A and B were tested for startle on P75, using two stimulus modalities: acoustic and tactile. The acoustic startle portion was identical to that described above for preweaning assessment. The tactile test consisted of 51 additional trials in which the stimulus used was a mid-chamber air-puff of 12 psi administered for 20 msec on each trial. All other exposure and measurement parameters were as described for the preweaning startle test.

Statistical procedures

All data except mortality were analyzed using fixed-effect factorial analyses of variance, with the litter mean used to represent all the subjects within a litter, stratified by sex. For measurements involving repeated assessments of the same subjects, a split-plot analysis of variance was used with trials as a within-subjects factor in the model. Gender was also treated as a within-subject or correlated factor. In instances in which a test for sphericity of the variance-covariance matrix of split-plot analyses of variance was significant, the Greenhouse–Geisser correction of F-ratios involving the repeated measure factor was used. Mortality data were analyzed by Fisher's test for uncorrelated proportions. For all analyses of variance (ANOVA), a posteriori group comparisons were conducted using Duncan's multiple range test.

RESULTS

The reproductive outcomes of the experiment are summarized in Table 1. Ultrasound exposure had no significant effect on the number of nonparturient dams, gestation length of gravid dams, average litter size, or sex ratio within litters. There were two significant effects on newborn mortality (pups found dead when parturition was discovered), in which increases were seen in the US2 and US30 groups, but not in the US20 group. Overall, offspring viability was not different among groups.

No significant effects were found on maternal body weights measured daily during gestation (E3–E20) or weekly (P0–P28) during lactation. Similarly, a Treatment group × Sex × Week analysis of variance (with sex and week as within-subject factors) performed on offspring preweaning body weights showed no significant treatment group main effect or interactions of treatment with any other factor. The effects of sex, week, and the sex × week interaction were significant. These effects reflected normal sexually dimorphic body weight differences with males being heavier than fe-

TABLE 1. Effects of pw ultrasound on reproductive outcome after exposure on E4–20

Variable	Group (W/cm^2) I_{SPTA} 0	2	20	30
No. sperm-positive females	13	13	16	14
No. nonparturient dams[1]	1	1	3	0
No. litters found dead	0	0	0	1
No. litters evaluated P0–P77	12	12	13	13
Gestation length (days)[2]	21.8 (0.1)	22.1 (0.1)	21.9 (0.1)	21.9 (0.1)
Litter size at birth[2]	15.7 (0.9)	15.3 (0.7)	16.3 (0.4)	16.6 (1.0)
Offspring mortality at birth	0/188	4/184*	3/212	5/216*
Offspring mortality P1–4	6/188	5/180	4/209	4/211
Offspring mortality P5–28[3]	3/96	0/96	0/104	0/104
Offspring mortality P28–77	0/93	0/95[4]	1/104	1/104

[1]Nonparturient dams were confirmed to have no implantation sites based on postmortem uterine examination.
[2]Values represent group mean ±SEM (in parentheses).
[3]Denominators cannot be derived from row above because offspring are culled to 8 per litter on P4.
[4]At weaning (P28) only 4 males and 4 females were retained. In one litter an imbalanced sex ratio resulted in 1 animal being euthanized at weaning, thereby reducing the denominator by 1.
*$P<0.05$ compared to control.

males, and the growth of all groups across weeks. A similar analysis of variance performed on postweaning offspring body weights (P35–P77) also showed no treatment group effects or interactions between group and other factors. As before, the effects of sex, week, and the sex × week interaction were significant. These effects showed the same pattern as described above for preweaning body weights.

Behavioral measures

The behavioral results are summarized in Table 2 and Figure 1. Three behavioral tests were performed on offspring prior to weaning. The first of these was olfactory orientation. Using mean score by litter, a treatment group × day ANOVA performed on the data showed no significant effect of treatment group or for the treatment group × day interaction. There was a day main effect ($P<0.05$), which reflected the progressive improvement of orientation scores across successive days of testing.

Acoustic startle was analyzed separately for each of the three dependent measures in blocks of 10 trials with the first trail analyzed separately. A treatment group × sex analysis of variance on trial 1 showed no significant effects for any dependent measure. A treatment group × sex × blocks analysis of variance for the remaining 50 trials showed no significant effect of treatment group or group-related interaction for the three dependent measures. Trial block was significant for V_{max} and V_{mean} which reflected normal startle habituation across trials. No significant effects were observed on T_{max}.

For preweaning activity, the dependent variables analyzed were distance moved and number of section transitions in 10 min intervals. Distance moved was subdivided into that occurring in the central, peripheral, corner, and side zones. Analyses of variance performed on each of these measures (treatment group × sex × interval) showed no significant effect of treatment group or treatment-related interactions. Interval was significant. The interval effect reflected normal habituation across successive time intervals. The only exception to this was for corner distance, in which the main effect of sex was also significant, because females are more active than males.

Four postweaning behavioral tests were performed. Adult activity was analyzed using the same model as for preweaning activity. No significant treatment group or treatment-related interactions were obtained on any of the dependent measures. Significant sex and interval main effects were obtained on all measures. As before, the interval effects reflected habituation and the sex effects reflected females being more active than males.

Straight channel latencies were analyzed with a treatment group × sex × trial analysis of variance (sex and trial were within-subject factors). No significant treatment group or treatment-related interactions were obtained. The trial main effects was the only significant effect, because latencies shortened across successive trials.

For the Cincinnati maze data, treatment group × sex × trial analyses of variance (sex and trial were within-subject factors) were performed on both error and latency. No significant treatment group or treatment-related interactions were obtained. On both measures, the main effect of trial was significant, attributable to progressively fewer errors and shorter latencies across trials. For latencies, there was also a significant trial × sex interaction. This was caused by the fact that males had longer latencies than females on the early trials of learning.

For adult startle the data for the first trail were analyzed separately. No significant treatment group or

TABLE 2. Behavioral outcomes in offspring exposed prenatally to pw ultrasound[1]

Variable[2]	Sex	Group (w/cm^2) I_{SPTA} 0	2	20	30
Olfactory orientation		10.3 (0.7)	9.8 (1.2)	8.4 (0.8)	11.4 (0.5)
Preweaning startle (V_{max})	M	103.5 (5.7)	106.6 (6.4)	94.1 (8.6)	114.0 (8.9)
	F	106.9 (7.2)	109.1 (10.7)	92.8 (8.6)	104.0 (5.9)
Preweaning activity	M	1806.5 (91.8)	1642.8 (126.6)	2036.7 (168.1)	1959.0 (136.4)
	F	1977.4 (206.3)	1956.7 (189.2)	3033.4 (964.1)	2027.0 (158.7)
Straight channel latency		11.2 (0.7)	11.5 (1.2)	10.6 (0.4)	10.0 (0.5)
Cin. maze errors		10.4 (0.5)	10.2 (0.8)	10.4 (0.9)	8.5 (0.4)
Cin. maze latencies		105.5 (5.2)	105.7 (6.6)	103.8 (6.8)	92.8 (4.7)
Adult activity	M	7330.9 (327.7)	6056.6 (511.7)	6697.6 (293.3)	6761.7 (416.7)
	F	7556.9 (540.8)	7724.9 (373.5)	7433.0 (521.4)	7385.0 (610.7)

[1]Values represent mean ±SEM.
[2]Data expressed as orientation scores, startle amplitude (volts), section entries, latency (sec) and errors (per trial).

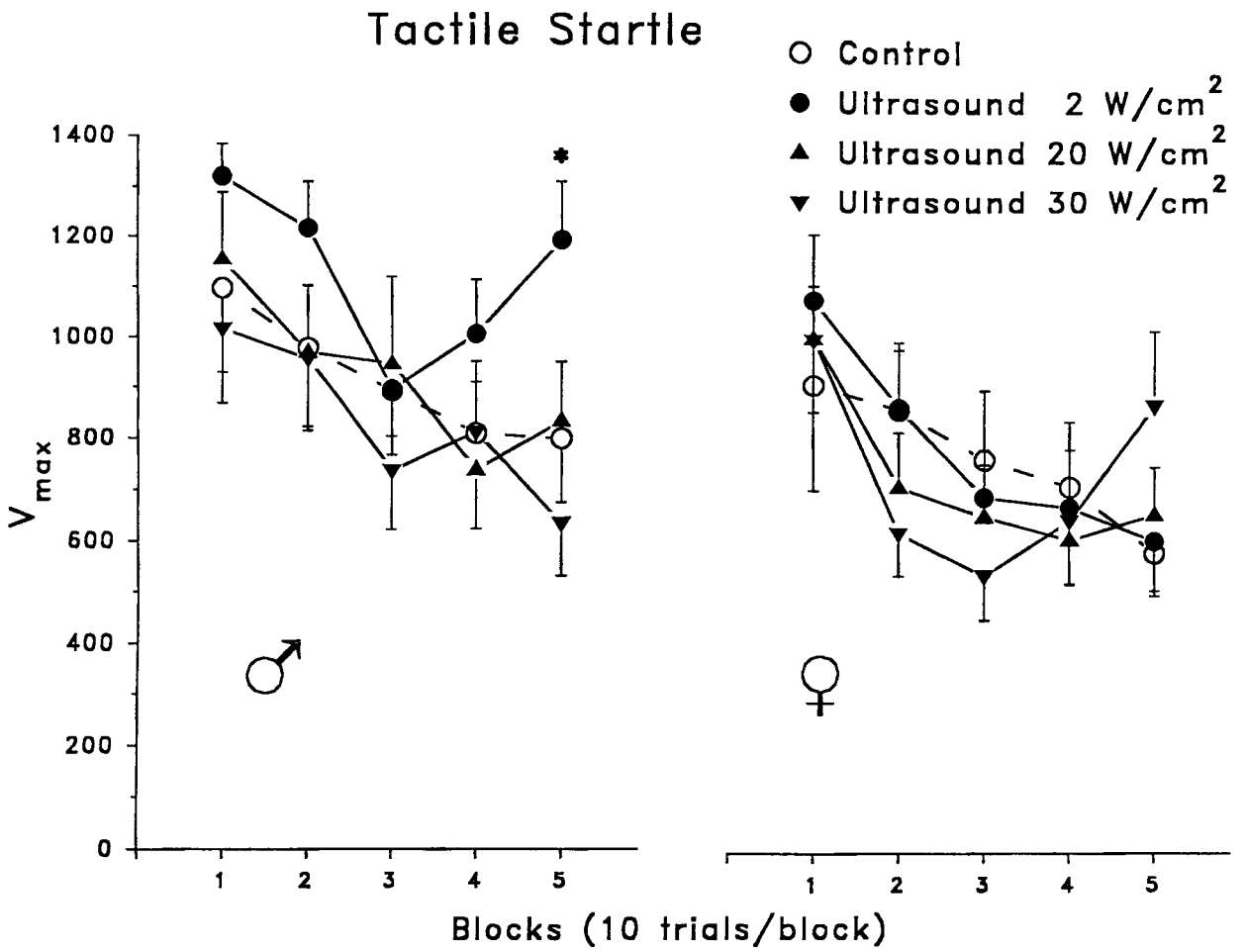

Fig. 1. Tactile startle amplitude (volts) as a function of trial block (mean ±SEM) in male and female offspring exposed prenatally to pw ultrasound. *$P<0.05$ compared to control.

treatment-related interactions were obtained. For the remaining trials, the data were analyzed in 10 trial blocks. Analyses of variance (treatment group × sex × block) were performed on each dependent measure separately for the acoustic and tactile trials. No significant treatment group or treatment-related interactions were obtained for the acoustic trials. Trial block was significant for V_{max} and V_{mean} and reflected habitua-

tion of response amplitude across successive trials. There were no significant factors found for T_{max} on acoustic trials.

A different result was obtained on the analyses of tactile startle data. While there was no significant treatment group main effect, or treatment × sex or treatment × trial interactions, there was a significant treatment group × sex × trial block interaction ($F=3.8$, $df=12/184$, $P<0.0001$) for V_{max}. In addition, the main effects of trial block and sex were significant. The trial block effect reflected habituation across trials and the sex effect reflected increased amplitude in males compared to females. The treatment group × sex × trial interaction was further analyzed using treatment group one-way ANOVAs for each sex and trial block. A significant treatment group effect was obtained only on trial block 5 for males. A posteriori Duncan group comparisons showed that, for males, the US2 group had a significantly higher startle amplitude ($P<0.05$) on the final trial block than controls (Fig. 1).

The same pattern was found for the analysis of V_{mean}. There was a significant treatment group × sex × trial block interaction ($F=3.3$, $df=12/184$, $P<0.0005$). Further analyses showed that the effect was the same as for V_{max}; i.e., in males, the US2 group showed higher startle amplitude on the last trial block than controls (not shown). As before, the main effects of trial block and sex were also significant reflecting habituation across trial blocks and higher startle amplitude in males than in females. For T_{max}, the only significant factor was the main effect of sex. Males had shorter latencies than those of females.

DISCUSSION

We employed a conditioned immobilization procedure to examine the developmental effects of gestational pw ultrasound exposure in rats without the use of anesthesia or forced restraint. The results of this study indicate that insonation produced no effects on maternal reproductive characteristics or offspring growth, survival, or behavioral development. No effects were found on most of the neurobehavioral dependent variables measured in the offspring as adults, including measures of locomotor activity, water maze learning, and acoustic startle reactivity. An effect on postweaning tactile startle amplitude was observed on some trials in the low exposure group (US2) male offspring, but was not seen in either of the higher exposure groups in males or females (US20 or US30). In the absence of other evidence of a nonlinear dose-response relationship for ultrasound effects, the pattern of this effect does not support the view that it was treatment related. The tests which were used here have revealed treatment effects after prenatal exposure to cw ultrasound (Vorhees et al., '94) and other behavioral teratogens, such as phenytoin (Weisenburger et al., '90; Vorhees and Minck, '89; Vorhees, '83, '87), trimethadione (Vorhees, '83), valproic acid (Fisher et al., '94; Vorhees, '87), ethanol (Vorhees, '89; Vorhees and Fernandez, '86), methamphetamine (Acuff-Smith et al., '95; Vorhees and Fernandez, '86), and most recently cocaine (Vorhees et al., '95); the group sizes in this study (12–13 litters/group) were comparable to those used in the studies cited above. Accordingly, the negative results of the present experiment can reasonably be attributed to the inability of pw ultrasound to induce long-term behavioral effects within the range of exposures evaluated rather than to the insensitivity of the tests or a lack of statistical power.

In a previous study (Vorhees et al., '94), we found evidence of behavioral effects after prenatal exposure of rats to cw ultrasound at an intensity level of 30 W/cm^2, equivalent to the highest exposure in the present study in terms of spatial peak, temporal average intensity. The failure to detect similar effects in the current study may reflect differences in the biophysical properties of cw and pw ultrasound (Carstensen, '87; Sarvazyan et al., '82). For a thermal mechanism of action, pw ultrasound would be expected to have the same biological effects as cw ultrasound at the same time-averaged intensity; however, additional exposure parameters may be important for the production of effects by cavitation or other nonthermal mechanisms (Barnett and Kossoff, '84; Child et al., '81; Barnett et al., '94; National Council on Radiation Protection and Measurements [NCRP] '83). Although the temporal average intensity levels used in our study were much higher than typical clinical exposure levels from pw devices, the pulse average intensity of 40 W/cm^2 used for all three exposures is within the range of present commercial instrumentation. Some diagnostic pw devices have pulse intensities as high as 1000 W/cm^2, and the FDA approval process for diagnostic ultrasound devices allows for pulse average intensities as high as 190 W/cm^2 (Food and Drug Administration [FDA] '85; Miller, '91). Hence, while the absence of effects in this study indicates a large margin of safety for this type of exposure, it is possible that pw ultrasound with different physical characteristics could produce adverse effects on embryonic development at clinically relevant exposure levels. However, based on a review of previous developmental neurotoxicity studies of ultrasound (see discussion in Vorhees et al., '94; also Jensh et al., '94, '95) and the results of the present study with pw ultrasound, we conclude that the rodent models of intrauterine ultrasound exposure generally show no evidence of developmental neurotoxicity at exposure intensities in the diagnostic range. The behavioral effects that have been noted have occurred at exposure levels above those used clinically.

One difference between our approach to ultrasound exposure and that of all previous investigations, is that we used conditioned immobility to maintain the test animals' position during exposure rather than relying upon forced restraint or anesthesia. The advantage of

this approach is that it avoids the introduction of additional procedures which either by themselves or through some interaction with ultrasound might attenuate or exacerbate ultrasound's bioeffects. From this perspective, the present data and those from our previous experiments using this approach (Vorhees et al., '91, '94; Fisher et al., '93) and other recent data (Jensh et al., '94, '95), using a different exposure system, may be viewed as reassuring in that they suggest that no new teratogenic or behavioral teratogenic effects occur at the moderate and lower intensity levels. However, the conditioned immobility exposure approach is not without its own disadvantages. Primary among these is that what was gained in reduced confounder variables may be offset by exposure imprecision. Loss of exposure precision occurs because conditioned animals are not completely immobile. Rats periodically shift their position during insonation. These adjustments may be minor, such as moving their leg or turning their head, or major, such as turning 180° to face the opposite direction. The animals also do not maintain their body attitude at a precise perpendicular angle to the incident ultrasound beam. This produces two types of exposure discrepancies: (1) because the caudal body regions are held deeper in the water, the incident angle to the ultrasound beam is neither 90° (it is closer to 45°), nor is this angle uniform across the abdomen, but rather varies continuously because the animal typically assumes a C-shaped posture; and (2) the caudal body regions are a different distance from the ultrasound source, which translates to differential exposures for vaginally positioned embryos in the uterine horns nearer the margin of the depth of focus field compared to those embryos at the ovarian end of the uterine horns. In the case of cw ultrasound, we have estimated that these two effects, when combined with calculations of tissue attenuation factors, result in a total signal attenuation of 65–87% of the incident intensity. Given this, the data suggest that the absorbed intensities we have used are actually much lower than the nominal values. This implies that our exposures are closer to those obtained in humans than might be anticipated based on the free field measured values.

Taken as a whole, the four experiments in this series (morphological and behavioral teratologic conducted with both cw and pw ultrasound), each using the same exposure system and comparable ultrasound intensities, provide no evidence from rats that ultrasound exposures in the diagnostic range of exposure intensities induces either teratogenicity or in utero neurotoxicity.

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