# AN AUTOMATED ULTRASONIC EXPOSURE SYSTEM TO ASSESS THE EFFECTS OF *IN UTERO* DIAGNOSTIC ULTRASOUND

Nadine Barrie Smith[1], Charles V. Vorhees[2], Richard A. Meyer[3], and William D. O'Brien, Jr[1].

[1]Bioacoustics Research Laboratory, Department of Electrical and Computer Engineering
University of Illinois, Urbana IL. 61801
[2]Division of Basic Science and [3]Cardiology, Children's Hospital Research Foundation and
Department of Pediatrics, University of Cincinnati, Cincinnati, OH 45229-2899

## ABSTRACT

An ultrasound exposimetry system has been designed and constructed to expose rat embryos and fetuses to ultrasound. The unique feature of the exposure system is that it insonates pregnant rats which are not anesthetized or otherwise restrained. The rats are trained to float in a restricted area directly above the submerged ultrasound transducer. The water tank has been designed to confine the floating rat to a 15 x 10 cm region while being exposed by ultrasound. The ultrasound transducer operating at 3 MHz in either pulsed or CW mode is submerged below the animal and moved in a raster fashion to irradiate the entire abdominal area. Computer control of the exposure system and the ultrasound intensity levels allow for the experiments to be conducted in a blind fashion. Using a calibrated hydrophone, the pressure waveforms are obtained from which the free-field $I_{SPTA}$ and $I_{SPTP}$ are calculated at the distance of the rat's abdomen.

## I. INTRODUCTION

Of the 3.5 million babies born in the U.S., over half of them are exposed to ultrasound *in utero*. Teratology associated with insonation has been inconclusive. Malformations and defects have been reported due to ultrasound [Sikov and Hildebrand, 1976] while others have not found the same result [O'Brien et.al., 1982; Kim, et.al., 1983]. In these studies a common characteristic was that the animal were restrained or anesthetized during the exposure yet these have also been shown to produce adverse development [Mazze et.al., 1984; Weinstock et.al., 1988].

No adverse effects have been found or reported due to the clinical application of ultrasound. Yet, there are still concerns about the effects on behavioral and intellectual development. The effects of ultrasound on children should be studied because experimental data are scarce. The safety of ultrasound is reflected in the long-term, postnatal development. Studies indicate that while there does not seem to be any gross abnormalities such as birth defects, still births or miscarriages due to ultrasound, findings are inconclusive on the subtle effect, particularly behavioral effects [NIH Consensus Development Conference Report, 1984]. Therefore, a research study has been developed to examine the biobehavioral effects of ultrasound on rat embryo and fetuses without the use of restraint or anesthesia.

An ultrasound exposimetry system has been designed and assembled at the University of Illinois. The experiments using the system take place at the University of Cincinnati where the system is used to test for central nervous system effects. The unique feature of this system is that the pregnant rats are insonated without the added stress of anesthesia or forced restraint. The pregnant rats are trained to float and remain stationary in a water tank, thus providing the capability for the rat to be exposed daily to ultrasound. Ultrasound is delivered to the entire abdominal area of the floating pregnant rat by rastering a submerged transducer. After birth of the fetuses, the pups are studied for the effects of ultrasound on the central nervous system and behavioral dysfunction using reliable biobehavioral tests. The purpose of this paper is to describe the unique exposure system developed.

## II. INSTRUMENTATION

A block diagram of the ultrasound exposimentry system is shown in Figure 1.

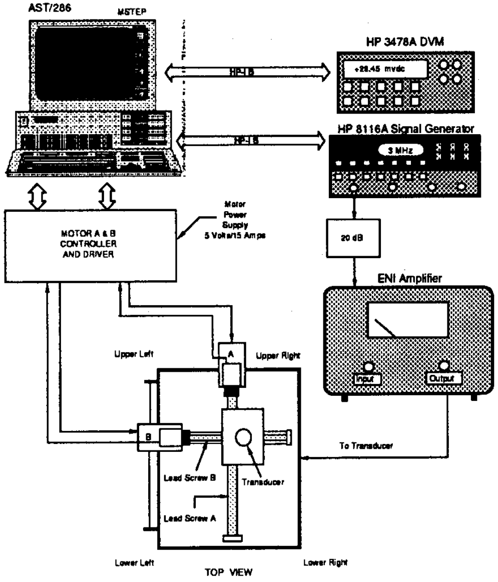

Figure 1. Block diagram of the ultrasound exposimetry system.

### (a) Computer System

Computer control of the exposure system is performed via the AST/286, shown in Figure 1. Within the AST/286's slots 3 and 5, respectively, are the MSTEP-5 motor controller board and the Hewlett-Packard Interface Bus (HP-IB) card [MSTEP-5 Manual, 1987- HP-IB Command Library for MS-DOS, 1986]. The MSTEP-5 is an incremental shaft encoder motion and stepper motor control board with a standard 50-pin mass termination. The HP-IB card is an IEEE-488 instrument control card for communication to HP-IB devices.

### (b) Electrical and Acoustic Equipment

A continuous wave 3 MHz low level, RF signal is supplied by the HP 8116A pulse/function generator, a multiwave programmable 50 MHz generator with a minimum amplitude of 10 mV zero-to-peak. Operation of the HP 8116A can be regulated manually or by the HP-IB computer control. The signal from the HP 8116A is attenuated 20 dB to achieve signal levels below the signal generator's miniumum signal of 10 mV zero-to-peak.

Signal amplification is performed by the 55 dB gain, RF power amplifier which operates over a frequency range of 0.3 to 35 MHz with a maximum power output of 150 watts [Broadband Power Amplifier Instruction Manual Model A150, 1987, ENI, Rochester, NY]. The amplifier is not programmable.

Ultrasound is produced by a PZT-4 crystal (Valprey-Fischer, Stamford, CT) with a 33.02 cm radius of curvature and crystal diameter of 5 cm. The transducer plating consists of an electrode on the concave surface that wraps around to the back. The back electrode having a 4 cm diameter is separated by a gap of 3 mm from the wrapped-around electrode and is centered on the crystal. The crystal is housed in a watertight, machined stainless steel metal casing equipped with a coaxial cable that terminates in a BNC connector referred to as the transducer assembly shown in Figure 2. The transducer assembly is positioned on a moveable platform, shown in Figure 1, with the ultrasound beam directed towards the surface of the water in the tank.

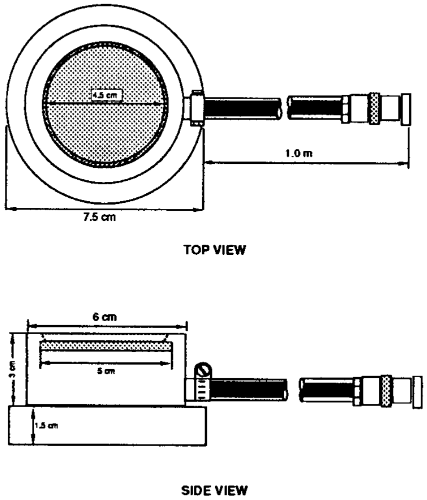

Figure 2. Transducer assembly.

(c) Mechanical Equipment

For the transducer to be mobile, motors and lead screws are used as a rotor to linear actuator. The tank and mechanical equipment used to move the transducer assembly are described in this section.

Plexiglas®, 1.5 cm in thickness, is used for the 38 x 51.5 x 62 cm watertight tank shown in Figure 3. The top view of the tank in Figure 1 designates the maneuver area of the transducer. Transverse motion is defined as right and left and longitudinal motion is defined as upper and lower with respect to the tank's top view as designated in Figure 1.

Slots along the inside of the tank hold opaque, slide-in Plexiglas® of 2 mm thickness to confine the animal to a specific exposure region. The animals are trained to float on the water and remain stationary by pressing their paws against the side walls of the exposure region. Rats are gently placed in and removed from the exposure region by hand. At the end of the exposure the rat is gently lifted out of the confinement tank.

The coupling medium for sound transmission is tap water. At the bottom of the tank is a hose which allows for a daily water change. Fresh water is placed in the tank over night to allow it to degass before an exposure experiment the next day. The water level is dependent on the location of the ultrasound beam focus and is generally set to be 3 cm above the focus.

The transducer scans in a raster fashion and exposes from below the underside of the rat in a raster fashion due to two lead screws connected to computer controlled motors. A mechanical system was built to control the movement of the transducer assembly in a plane parallel to the water surface at a depth of approximately 30 cm. Each motor has an encoder for closed-loop control. Lead screw A and B are connected to two independent motor and encoder teams (Figure 1). Motor A and encoder A are permanently attached to the top of the tank. Motor B and encoder B are located on the left side of the tank and move along a stainless steel rail. Lead screw A moves both lead screw B and the transducer platform in the longitudinal direction and lead screw B moves only the transducer platform in the transverse direction.

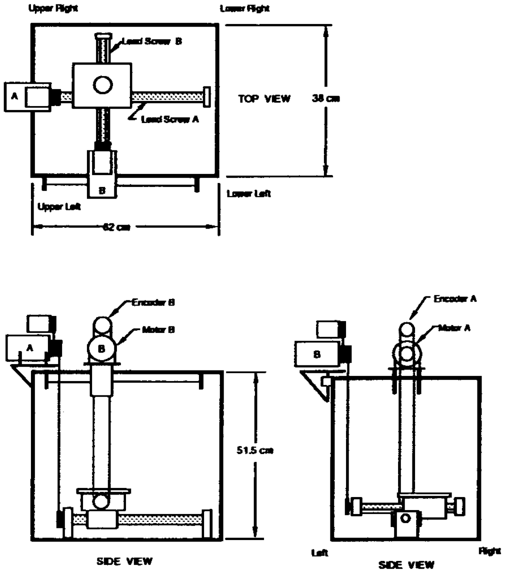

Figure 3. Three dimensional view of exposimetry tank.

Both stepping motors are identical and have a resolution of 1.8° per step, that is, 200 steps per revolution [Mosel and Barrett, 1980]. Lead screws A and B are rotation-to-linear actuators which cause the transducer assembly to move with the steps of the motors. The thread size of lead screws A and B are 8 and 10 threads/inch, respectively.

The two motors and shaft encoders are controlled from the computer using two motor drivers located with the 5 volt/15 amp motor power supplies shown in Figure 1. The speed of the motors varies depending on instructions from the software [MSTEP-5 Manual, 1987]. The motor step rate of the motors is defined by

$$\text{Rate} = 12{,}500 \,/\, D\%(1)\ ;\ \text{steps/second} \qquad (1)$$

The speed variable D%(1), which is set from the computer software, has a valid range from 20 to 255, fastest and slowest speed, respectively. Motor A is set at the slowest rate (or greatest torque) to assure minimum linear speed error, which occurs from frictional drag in lead screw A. The linear speeds for lead screw A and B are 0.08 cm/sec and 0.2 cm/sec, respectively.

A 15 cm x 9 cm area is exposed with a raster movement at the transducer assembly. The movement can be operated in two modes. The first mode involves a pattern where the sound is pulsed for 5 seconds while the transducer is stationary; the transducer is then moved 1 cm and pulsed again. This pattern continues until the entire area is covered. The second mode is a continuous scan where the sound is on during the entire raster pattern. The former method takes approximately 29 minutes and the latter takes 19 minutes to complete an exposure.

(d) Temperature Control

For a constant temperature to be maintained in the tank, immersion heaters formed along the tank walls are used (OMEGA Series 355a, Stamford CT). The heaters are connected to the proportional controller which is connected to a thermistor probe immersed in the water to give temperature feedback information. (YSI Model 72, Yellow Springs, OH). To assist the circulation of the heated water, a variable speed stirrer is mounted on the side of the tank (Cole Parmer J-4330-00, Chicago, IL).

### (e) Software

To insure the integrity of the study, the daily exposures are performed in a blind fashion. Therefore, the exposimetry system is designed for use by two primary people, the Principal Investigator and the Investigator. While the Principal Investigator knows the exposure intensity for each animal, the Investigator does not. The responsibility of the Investigator is to perform the actual exposures on each rat. The Principal Investigator enters exposure conditions and animal data into a password protected file not accessible by the Investigator.

The system is designed so that the Investigator can concentrate on the animal during the experiment and not be concerned about the electrical and mechanical equipment. Programs accessible to the Investigator are chained together by one main server menu. Menu driven software enables the Investigator to move from one program to another without having to return to DOS to execute another program. The Investigator need only to enter an animal identification number to the computer to begin the preprogrammed exposure. When the exposure is complete, the computer signals the Investigator. The software that controls the mechanical, electrical and acoustical equipment is menu driven and user-friendly, since the Investigator is assumed to have little knowledge of equipment. Exposure and animal data are stored and updated by the software in files for a complete record of the experiment.

### (f) Error Warnings Due to System Failure

Built into the software that runs the equipment are error codes and warnings, signaling the Investigator when the equipment is not operating correctly. When the system fails to complete a instruction given from the menu driven software by the Investigator, the computer beeps and displays a notice specifying type of failure. For example, if a motor is instructed to move 1.0 cm but fails, then the computer would beep and display the message `MOTOR A FAILURE, ACTUAL MOVEMENT 0.5 cm, PLEASE CHECK`. The Investigator is then instructed to see if the error message is due to an object physically blocking lead screw movement or electrical failure. Another example would be if the Investigator tried to enter an animal identification number that was not in the animal exposure data base. The computer would display that the animal is not in the data base and not allow the ultrasound scanning to take place.

## III. EXPERIMENTAL CONDITIONS

To provide a uniform ultrasound exposure to the floating rat's abdominal surface, a modified approach to that used by O'Brien et al. (1982) was used. The rats were trained to remain relatively immobile and placed in an inner confinement chamber 11 x 16 cm constructed of black acrylic. The immobile rat's surface area was estimated to be approximately 7 (width at the widest point) x 8 cm (xiphoid process to the prepuce) on embryonic (E) day 17, E17, (width varying from 6-8 cm from E4-E19), but the rat did not necessarily remain completely immobile. Therefore, a raster scan pattern of the moveable transducer assembly was set at 9 x 15 cm or approximately 1 cm inside each wall, as shown in Figure 1, in order to assure that the entire confinement chamber was insonated. This was done since dams spread out their extremities to the side walls in order to prop themselves during the confinement period, but sometimes they rotate 180° within the region during the scanning protocol.

## IV. TRANSDUCER CALIBRATION

The transducer was calibrated at the Bioacoustics Research Lab using an automated system set up according to the AIUM Acoustic Output Measurement and Labeling Standard for Diagnostic Ultrasound Equipment [AIUM, 1988]. Using this system, a hydrophone can be moved through the sound field with a precision of 1 $\mu$m movements (Daedal Positioning, Harrisburg, PA). The ultrasound field was calibrated under free-field conditions with a calibrated (at NPL) membrane hydrophone (Marconi model Y-33-7611). A DFT of the recorded CW waveform was performed. The waveform was found to have a center frequency of 2.97 MHz and a 3dB beamwidth of 0.97 MHz.

Intensity time average (ITA) data for the axial plot was recorded every $\lambda/2$ steps. The hydrophone was rotated 10° to reduce standing waves between the transducer and the hydrophone, and the derating factor due to the rotation was recorded. The transducer was weakly focused with a measured focal length of 28.9 cm and 3 dB axial depth of focus of 11.2 cm as shown in Figure 4.

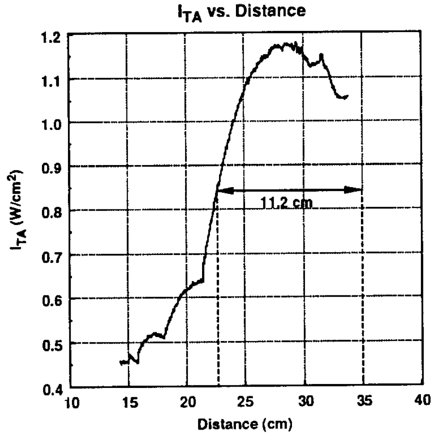

Figure 4. Axial plot of ultrasound field. Focal point located at 28.9 cm.

The ultrasound beam is directed upward towards the floating rat. Figure 5 shows a 3-dimensional lateral view at the beam focus of the ITA. Data for the lateral plot was recorded every 1 mm steps. For a stationary beam at the focus which would be at a location within the floating rat, the free-field 90 and 50 percent intensity beam widths were 1.6 cm and 3.2 cm, respectively.

Under stationary beam conditions, at the focus, the four continuous wave values of the spatial peak, temporal average intensity ($I_{SPTA}$) used in this study were 0 (control), 0.1, 2 and 30 W/cm$^2$, as calculated from the measured instantaneous pressure or the voltage R.M.S.$^2$ ($V_{RMS}{}^{2}$) waveform. Figure 6 shows the recorded $V_{RMS}{}^2$ from the hydrophone and the corresponding $I_{SPTA}$. Over the intensity range of 0.0-50.0 W/cm$^2$, the correlation coefficient between the intensity and the voltage is 0.99.

## V. EXPOSIMETRY

Figure 7 shows a top view of the confinement chamber and the pattern of a single raster scan sequence which consists of 16-9 cm rasters separated by 1 cm. The transducer assembly platform is positioned at the center of the chamber when the rat is placed in it with the ultrasound turned off. The platform as actuated by two lead screws connected to stepping motors which are under computer control. At the initiation of the exposure protocol, the transducer assembly is moved to the upper left corner, which takes 116 sec, at which time the ultrasound is turned on, and the beam rasters across at rate of 0.2 cm/sec, steps down 1 cm at a rate of 0.08 cm/sec to the next raster line and rasters across. This is continued for 16 raster lines, to the lower left when the ultrasound is turned off and the transducer assembly returns to its starting position.

Exposure time can be estimated by evaluating, from the raster scan pattern, the time that a fixed point on an immobile rat's abdominal surface received ultrasound. Assume that the fixed point is on one of the raster lines (point "a" in Fig. 7) and that the beam width

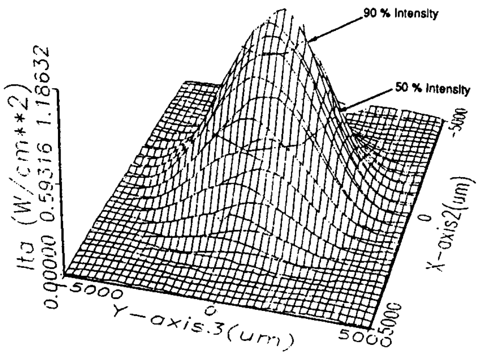

Figure 5. Lateral intensity plot at beam focus. Shown in circles on the plot are the 90 % and 50 % intensities.

on the rat's abdominal surface is 1.6 cm, that is, the 90 percent intensity beam width. The time that the fixed point is within the 90 percent intensity beam width is determined from a single raster scan to be (1.6 cm)/(0.2 cm/sec) = 8.0 sec. For this case, if the fixed point is midway between two raster scans (point "b" in Fig. 7), it is exposed twice and the exposure time equals 12.0 sec. For the 50 percent intensity beam width, the total exposure time ranges between 41.0 and 46.5 sec.

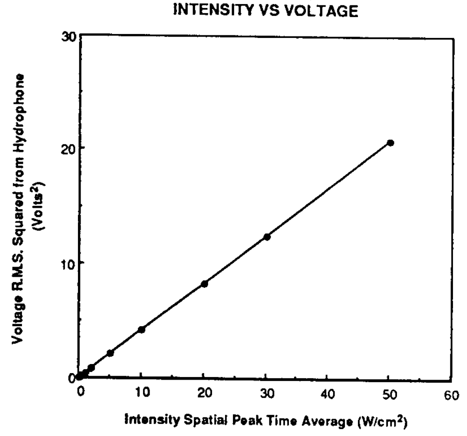

Figure 6. Voltage vs. Intensity data at the spatial peak.

Each rat received one raster scan pattern per day on E4-19 for a total of 16 exposures, each lasting 15 min, 7 sec with 3 min, 52 sec transducer positioning time at each end of the scanning pattern for a total of 19 min per session.

## CONCLUSIONS

The exposure system provides a unique method for assessing the effects of *in utero* diagnostic ultrasound by exposing pregnant animals to varying levels of ultrasound intensity while neither using physical restraint or anesthesia. The automated, user-friendly system has been used on a daily basis at the University of Cincinnati to conduct the exposure experiments. Electrical, mechanical and computer are working according to expectations and design criteria. Thus, intrauterine exposure to continuous wave ultrasound at daily exposure levels of 0.1 to 30 W/cm$^2$I$_{SPTA}$ are being performed to test for embryotoxic results.

Further experiments using the exposure system will involve using higher intensity pulsed wave ultrasound to determine whether gross abnormalities can be found at these levels.

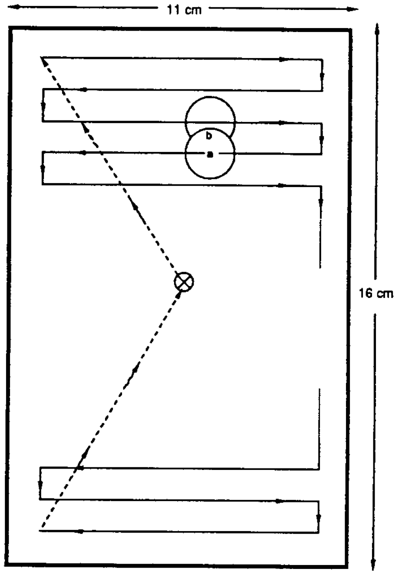

Figure 7. Top view of the 11 x 16 cm confinement chamber showing the 16-9 cm rasters. The encircled x denotes the starting position. The dashed lines indicate the ultrasound "off" condition and the solid lines indicate the ultrasound "on" condition. The two 90 percent beam widths demonstrate the exposure time for the two separate fixed positions. Position "a" is exposed once to the raster beam, whereas position "b" is exposed twice.

## VII. ACKNOWLEDGEMENTS

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