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Effects of Exposure to Perinatal Ultrasound Radiation on Information Processing in the Auditory System

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EFFECTS OF EXPOSURE TO PERINATAL ULTRASOUND RADIATION ON INFORMATION PROCESSING IN THE AUDITORY SYSTEM

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GRADUATE COMMITTEE APPROVAL

of a thesis submitted by

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ABSTRACT

EFFECTS OF EXPOSURE TO PERINATAL ULTRASOUND RADIATION ON INFORMATION PROCESSING IN THE AUDITORY SYSTEM

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Ultrasound (US) has become a standard procedure used during pregnancy to document the health and development of a fetus. When ultrasound was first developed, some researchers urged caution, suggesting that the possibility of hazard should be kept under constant review. Given the routine application of fetal ultrasound imaging, any possibility of deleterious developmental effects resulting from its use is an important public health issue. Rats have a well characterized central nervous system whose neurochemical pathways and neuronal electrophysiology qualitatively correspond to those of humans. Because of this, we opted to use Wistar rats as an animal model to document effects from ultrasound exposure. We exposed one group of rats on prenatal days 15 and 20 for fifteen minutes. A control group was exposed subjected to similar conditions, however no ultrasound exposure was given. A third group was exposed for ten minutes each on post natal days (PND) 2 and 3 while a fourth control group was exposed to the same

conditions as group three with no ultrasound exposure. The rats were then watched for developmental delays. When the rats reached the appropriate age, they were given a locomotor task to test for appropriate motor responses. Acoustic startle and prepulse inhibition tests were administered to test for sensorimotor gating, hearing, and motor response. Finally, a brainstem auditory evoke potential test was given to track auditory threshold and appropriate neural firing at various auditory nuclei. Postnatally US exposed rats showed a decreased acoustic startle response and prenatally exposed rats exhibited a speeding up in components of the brainstem auditory evoked potential test.

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INTRODUCTION

Clinical use of ultrasound

Diagnostic ultrasound imaging is a valuable procedure that emerged in general medical practice in the 1960s. Since its introduction, ultrasound (US) has become a standard procedure used during pregnancy to image the fetus. Most fetuses, in fact, in developed countries are exposed *in utero* to at least one diagnostic ultrasound examination. A recent European study has shown that the mean number of ultrasound scans received by women during pregnancy was 2.6, with more than 96% of women receiving at least one scan (Whynes 2002). In the first trimester of pregnancy, ultrasound is primarily performed to evaluate vaginal bleeding, assess the age of the fetus, and confirm that the fetus is alive. In the second trimester, ultrasound is used to evaluate the fetus for anatomical or structural abnormalities. In the third trimester, ultrasound is used to evaluate the fetus' growth and to confirm its size.

Additional uses of *in utero* ultrasound include the following: 1) to guide instruments for prenatal diagnosis (as, for example, the needle used in amniocentesis) 2) to confirm pregnancy 3) to locate the baby (useful in ruling out ectopic pregnancy) 4) pregnancy dating 5) to determine whether there is more than one baby 6) to check the baby's growth 7) to evaluate movement, tone, and breathing 8) to identify sex 9) to assess the amount of amniotic fluid 10) as an adjunct to cervical cerclage or suture 11) to look for molar pregnancies 12) to determine the structure and position of the placenta (i.e., placenta previa) 13) to determine the cause of bleeding 14) for fetal surgery and 15) to confirm fetal death (Petitti, 1984).

Safety of ultrasound exposure

When US was first developed, some researchers urged caution, suggesting that the possibility of hazard should be kept under constant review, further arguing that US should never be used in the first trimester (Petitti, 1984). Given the routine application of fetal ultrasound imaging, any possibility of deleterious developmental effects resulting from its use is an important public health issue. The safety of fetal diagnostic ultrasound has been debated since its introduction as a clinical diagnostic procedure. Findings from epidemiological studies investigating the developmental effects of fetal diagnostic ultrasound have been controversial, and few firm conclusions have been drawn regarding its safety.

There is a possibility that exposure to US radiation could cause damage to the basilar membrane, a portion of the cochlea especially sensitive to sound waves. Research has shown that loud noise at any age can cause the death of the sensitive hair cells within the cochlea (Rabinowitz 2000). Other research has demonstrated that US exposure at the oval window of cats at levels that approximate clinical levels causes cochlear hair cell loss (Bouchard and Benitez, 1978). As US is a sound wave propagated into the mothers uterus, it is possible that the sound waves could affect the cochlea in its critical stages of development.

More recently, US has become big business. In fact, commercial enterprises are appearing in malls across the United States advertising three dimensional US imaging of the fetus. Three dimensional imaging uses the same techniques as clinical US; however, the technology allows for a clearer and more human like image of the fetus that the parents can take home on video. The availability of this once clinical procedure

is now becoming commercialized and used outside the supervision of the medical professional. Since US waves vibrate at a higher frequency than normal sound and US volume can reach up to 100-db, the US procedure should me monitored by a medical professional to ensure proper parameters at maintained. In February 2004, the American Food and Drug Administration (FDA) issued the following statement warning regarding commercial US use: Persons who promote, sell or lease ultrasound equipment for making "keepsake" fetal videos should know that FDA views this as an unapproved use of a medical device. In addition, those who subject individuals to ultrasound exposure using a diagnostic ultrasound device (a prescription device) without a physician's order may be in violation of state or local laws or regulations regarding use of a prescription medical device (Rados, 2004).

Cognitive and behavioral effects of fetal ultrasound radiation in humans

In a longitudinal study that compared 123 variables at birth and again at 1 year of age in infants exposed and those not exposed to US (Scheidt *et al*., 1978), investigators found that a significantly higher proportion of US-exposed infants had an abnormal tonic neck flex but found no difference between the US-exposed and unexposed children for any of the other 122 variables. The biological importance of the abnormal reflex is uncertain, and the number of abnormal infants was small. Furthermore, because a large number of statistical tests were carried out, this difference may have been due to chance alone.

Stark and co-workers (1984) examined 425 US-exposed and 381 matched unexposed children between 7 and 12 years of age. They found no association between

US exposure *in utero* and 16 outcomes, including conductive and nerve measurements of hearing, visual acuity and color vision, cognitive function, behavior, and a complete and detailed neurological examination. However, they did find a significantly greater proportion of US-exposed children to be dyslexic based on the Gray Oral Reading Test ($p<0.01$). In their analysis, numerous statistical comparisons were made, and thus, it is possible that the difference in dyslexia between the groups was due to chance. An imbalance in factors other than US that are related to dyslexia may not have been adequately controlled and may have contributed to the finding. However, for many years, there was a concern that US exposure in pregnancy was associated with dyslexia, and the general consensus was that further research on the subject was needed (Petitti, 1984).

Another study carried out a long-term follow-up of 2161 children from two Norwegian randomized trials (Bakketeig *et al*. 1984; Eik-Nes *et al.* 1984). The main objective of the follow-up was to assess the possible association between US exposure and dyslexia. Data were collected from parents, from maternal and child-health centers, and from school teachers. Parents responded to a questionnaire with 66 questions about the child's development, handedness, hearing, vision, attention, motor control, and perception. Height and weight data were collected from health-center records of the children's visit at the ages of 3, 6 and 12 months, and at 2, 4 and 7 years. Distant visual acuity tests and pure-tone audiometry were assessed at 4 and 7 years. Neurological development during the first year of life was assessed through a short version of the Denver Development Screening Test. In the second year of primary school, 2011 children were assessed by their teachers with regard to reading aptitude, spelling,

arithmetic and overall performance. A subsample of 603 children was evaluated with specific tests for dyslexia in the third year of school. Routine US offered in weeks 19 and 32 of pregnancy did not lower school performance, as reported by teachers, among children aged 8 or 9 years, and there was no evidence of an increased prevalence of dyslexia among children whose mothers underwent routine screening with US. Routine US had no adverse effects on sensory functions, nor was there any association between US exposure and impaired neurological development. However, a significantly larger portion of the US children were classified as non-right handed compared to control children (Salvesen *et al*., 1993). This effect documents the possibility that brain function or development may in some way be altered by exposure to US.

An additional study examined the antenatal records of children with delayed speech of unknown cause and compared them with those of controls who were similar in sex, date of birth and birth order within the family. The children were similar in social class, birth weight, and length of pregnancy. The children with speech problems were twice as likely as controls to have been exposed to US *in utero* (Salvesen *et al*., 1992a; Salvesen *et al.*, 1992b; Salvesen *et al*., 1993b).

A Canadian study (Campbell *et al.,* 1993) was set up to test a possible association between US exposure during pregnancy and delayed speech development. A matched case-control design was used with 2 controls per case. Matching variables were sex, date of birth, sibling order and associated characteristics. A speech language pathologist had established the case definition several months or years prior to the study. The study reported that the odds of suffering from delayed speech were 2.8 ($p=0.001$) times higher among children who were exposed to US at least once during

pregnancy, than among the non-exposed matched control children (Campbell *et al.*, 1993). There was no relationship between the timing of exposure and there was not a dose-response effect, but such relationships were impossible to examine, since only 3 cases and three controls had more than 1 scan during pregnancy. Also, the information on US exposure was not assessed blindly, and there is the possibility of misclassification of exposure. The design of a case-control study makes it impossible to rule out the influence of possible biases, especially related to selection of subjects and misclassification of information between cases and controls. Thus, the results from the study should be viewed cautiously.

As a result of the above study (Campbell *et al*., 1993), information on speech development that had been collected, but not assessed, in the Norwegian random follow-up study was analyzed (Salvesen *et al.*, 1994). In the Norwegian study, assessment of speech development had been performed through a parental questionnaire (three questions about speech development) and also from records collected from maternal and child-health centers. No significant differences between US and control children in speech development could be demonstrated in the parental assessment of the children. However, according to the heath-center records, US-exposed children had not been referred to a speech therapist as often as the control children (Salvesen *et al.*, 1994).

In 1994, American obstetricians published a follow-up study of children, aged 7 to 12 years, born in three different hospitals in Florida and Denver who had been exposed to US in the womb (Stark *et al.*, 1994). Compared with a control group of

children who had not been exposed, the US exposed children were more likely to have dyslexia and to have been admitted to a hospital during their childhood.

Two studies have assessed subsequent growth during childhood among children who were exposed to US *in utero* compared to unexposed children (Lyons *et al.*, 1988; Salvesen *et al.*, 1993). Lyons and co-workers found no differences in weight or height between US exposed children and controls in a cohort study ranging from birth up to 6 years of age. A similar result was found in the second study (Salvesen *et al*., 1993), in which there were no statistically significant differences in mean body weight or height between US and control children in a cross-sectional analysis of growth during childhood.

In summary, previous studies have linked prenatal US exposure in humans to dyslexia, speech problems, and non-right handedness. However, these findings should be taken cautiously due to the likelihood of error within these studies. Further research is needed to validate these effects.

Behavioral effects of ultrasound radiation in animals

There have been relatively few reports on the behavioral teratogenic potential of US exposure in animals. In one study, Murai *et al*. (1975) exposed gravid Wistar rats to Doppler US on the ninth day after conception (G9) for 5 hours to an intensity of 20 milliwatts (mW)/cm2 and at a frequency of 2.3 MHz. To expose the pregnant rats to US, they were forcibly restrained by tightly wrapping them in wire mesh. Sham-exposed and unrestrained control groups were included. A 0.3-day acceleration of eye opening was found in the exposed rats, but the effect only occurred in relation to

unrestrained controls. No effects on limb movement, hindleg movement, walking, surface righting, or cliff avoidance were found. In contrast, differences were found in grasp reflex, visual placing, and air righting behaviors. However, only the delay in the grasp reflex was significant compared to restrained controls. No effects on open-field ambulation or defecation were found. However, the authors reported that on the second and third days, a higher percentage of the insonated group vocalized than either restrained or unrestrained controls. It was also found that in a shock-avoidance paradigm, the insonated group spent more time on the unshocked portion of the testing area than unrestrained controls, but not compared to the restrained controls. Furthermore, the insonated group committed fewer crossovers from unshocked to shocked locations than either control group. A vertical verses horizontal stripe shock-escape visual cue discrimination test showed no group differences. While these data appear suggestive of US-exposure teratology, the experiment reported in these papers has numerous methodological shortcomings: 1) despite the fostering/crossfactoring conditions, fostering was ignored as a factor in the data analyses, 2) the data were analyzed by the subject without regard to litter membership, perhaps causing overestimations of the number of significant effects (Holson *et al.*, 1992), 3) the most significant differences were between insonated and unrestrained controls, which means that these effects may have been due to restraint rather than US 4) rats' abdomens were not depilated, a factor which may have resulted in an attenuated US signal and 5) the few effects which occurred between the insonated and restrained controls were small and of doubtful significance.

Sikov *et al.* (Sikov 1977; Sikov 1979) anesthetized gravid Wistar rats on G15, and exteriorized the uterus and exposed the fetuses to intensities of 0.01, 0.04, 0.71, 0.54, or 1.0 W/cm2 at a frequency of 0.93 MHz US for 5 minutes. They reported a delay in development of the grasp reflex on days 1 and 6, a delay in surface righting on day 6, a delay in head lifting and whole lifting on day 13, and reduced hanging from a bar on day 15. This experiment had careful characterizations of exposure parameters and used multiple groups at different US intensities. Controls were appropriately sham treated. The problem with these results, however, is that the findings are only descriptive and are reported using individual offspring as separate data points, with no allowance for litter membership. No tests of significance were provided. Group sizes were not indicated, the insonation method (direct exposure of exteriorized fetuses) was unusual, no tests of more complex functions were included, most of the findings were not dose-dependent, and no control for the separate effects of the anesthetic was included.

More recently, Norton *et al.* (1991) reported on the effects of prenatal exposure to US of 0.78 W/cm2 given for 30 minutes on day G14 at 2.5 MHz to gravid rats. Sham-exposed, anesthetic controls, and unexposed controls were included. Ultrasound-exposed offspring had significantly longer negative geotaxis times (movement of an animal using gravity for orientation) and longer reflex suspension times than either control group, but there were no differences in continuous corridor activity. On a test of gait, both the US-exposed group and the sham-exposed group had longer stride length and a smaller angle of alternate strides than untreated controls. No histological changes in cortical layers were observed.

Together, the current data suggest that some reflex delays may be attributable to US, while other effects, such as those for gait, may be more closely related to anesthesia than to US. Overall, it appears there are noticeable behavioral differences in US-exposed rats.

Non-behavioral effects of ultrasound exposure in animals

Several studies have used animal models to evaluate the effects of perinatal US exposure on non-behavioral outcomes. Several studies in rats, mice, and monkeys have found reduced fetal weight in offspring that were exposed to US *in utero* compared with unexposed (Tarantal *et al*., 1993; Murai *et al*., 1975). Clear biological effects have been reported when animals are exposed to high-intensity US radiation *in utero.* These include hyperthermia, shear stress, limb paralysis, and axonal impulse conduction block (Dunn and Fry, 1971; Young and Henneman, 1961).

Cognitive effects of ultrasound exposure in animals

Recently, Ang *et al*. (2006), showed that US disrupted neuronal migration in mice at a late stage of corticogenesis, when the migratory pathways are the longest and, thus, may be most vulnerable. In a less recent, but detailed review article, Fry (1958) described both structural and functional changes produced with exposures of the central nervous system to focused US, concluding that "by appropriate control of the dosage conditions, it is possible to produce either reversible or selective irreversible changes." Among reversible effects studied was the temporary suppression of cortical potentials in response to a flash of light (Fry, 1958). An irreversible change that Fry found was the

destruction of neural components in focal regions, thus, creating “focal lesions”; it had been shown that this could be done (by controlling dosage conditions) without interrupting blood vessels. Of particular relevance to this study, in 1987 it was demonstrated by Ellisman *et al*. (1987) that diagnostic levels of US disrupt myelination, especially at the nodes of Ranvier, the boosting stations for axonal impulse conduction in the central nervous system.

OBJECTIVES

Rationale for the study

Measuring the outcome of any intervention in pregnancy is complicated because of the numerous variables involved. Intelligence, personality, growth, sight, hearing, susceptibility to infection, allergies, and subsequent fertility are only a few issues which, if affected, could have serious long-term implications. Because a fetus grows rapidly, exposing it to US at 8 weeks can have different effects from exposure at, for example, ten, eighteen or twenty-four weeks. Further complicating the study of the effects of US exposure are the many different types of US, such as high-intensity Doppler scans, real-time imaging, triple scans, external fetal heart-rate monitors, and hand-held fetal monitors. Despite decades of ultrasonic investigation, it is still unknown whether prenatal US exposure has an adverse effect at a particular time of gestation, whether the effects are cumulative, and whether they are related to the output of a particular machine or length of examination. The mechanism by which US may affect fetal growth is also unknown. The literature review above in humans and animals underscores the woeful lack of research on the effects of diagnostic levels of ultrasound imaging and provides a reasonable rationale for the systematic evaluation of the effects of diagnostic levels of perinatal US radiation perinatally in animal models of human diagnostic US imaging. I wanted to study the effect of US-exposure on development, locomotor behavior, and auditory system functioning using rats as an animal model. Specifically, I proposed to examine the effect of prenatal (days G15 and G20) and postnatal (PND) 2 and 3 US exposure on key developmental indices, acoustic-startle

responses, locomotor activity, and brainstem auditory-evoked potentials. PNDs 2 and 3 were chosen because this time in rat brain development roughly mimics the growth spirt of the human brain that begins in gestation at the beginning of the third trimester and continues for several years after birth (Ieraci and Herrera, 2006). G15 and G20 were chosen arbitrarily to monitor effects of US exposure given *in utero.*

Hypotheses

As previous studies in rodents have failed to demonstrate any conclusive effects on developmental indices, I hypothesized that there would be no effects of prenatal or postnatal US radiation on any of our developmental indices or on gross locomotor activity. However, given the sensitivity of the basilar membrane of the cochlea to US radiation, I hypothesized that measures of acoustic sensorimotor gating (ASR) and hearing would be disrupted in US rats. Given the discrepancy between human and rodent CNS development, prenatal as well as postnatal US exposure was studied, as the former models human fetal diagnostic imaging during pregnancy and the latter models the same CNS developmental periods, approximately equivalent to the beginning of the third trimester in humans.

Proposed experiments

Experiment 1: Developmental landmarks*:* Evaluate the effects of prenatal and postnatal US exposure on developmental indices including weight, pinna detachment, righting reflex, emergence of fur, incisor development, and eye opening and compare to

sham US controls. To accomplish this, indices were monitored for the first 14 days of rat pup life and pup weight was measured for the first 30 days.

Experiment 2*: *Motor activity: Determine the effects of prenatal and postnatal US exposure on locomotor activity and motor habituation and compare to sham US controls. To accomplish this, overall motor activity was recorded with a movement transducer during five 30 min sessions.

Experiment 3: Acoustic Startle Responses: Evaluate the effects of prenatal and postnatal US exposure on acoustic startle responses (ASRs), including ASR amplitudes, ASR habituation and prepulse inhibition of the ASR and compare to sham US controls. To accomplish this, the activity of the rat during exposure to startle stimuli under various paradigms was recorded.

Experiment 4: Auditory tests: Evaluate the effects of prenatal and postnatal US exposure on brainstem auditory evoked potentials (BSAEPs) and compare to sham US controls. To accomplish this, threshold BSAEP and the typical five peaks that occur in association with a click stimulus were recorded.

METHODS

Subjects and justification for animal use

The response of neurons existing in complex neuronal circuits to the effects of a variety of experimental manipulations can only be studied and understood using the intact nervous system. The organizational aspects of neuronal networks in the intact nervous system are another reason the effects of ultrasound radiation may not be readily studied in isolated neural elements used in *in-vitro* approaches. Rats have a well characterized central nervous system whose neurochemical pathways and neuronal electrophysiology qualitatively correspond to those of humans. Their behavioral repertoires (e.g., pre-pulse inhibition) have also been well characterized and these factors make rats excellent subjects for the functional analysis of brain electrophysiology, neurochemistry and neuropathology. Compared to non-human primates, rats are also inexpensive, easily and inexpensively maintained, and can be obtained either genetically homogeneous or heterogeneous as is required for the specific hypothesis under testing.

One hundred ninety two male and female Wistar rats (4-400 g) were used in this study. All procedures were approved by the BYU IACUC board (protocol #050501). Rats were housed in temperature controlled cages (27 degrees C) under a reverse light cycle (lights ON 1800-600 hrs) and provided normal chow and tap water *ad libitum*. Rats were bred in the vivarium on the 12^{th} floor of the SWKT building. At birth, rats used for the postnatal US exposure component of the study were toe-clipped under general halothane (5%) anesthesia to ensure exact identification and handled with latex gloves to mitigate the presence of strange odors.

General Experimental Plan: Group design: Ultrasound treatment

A battery of developmental, behavioral and physiological tests were performed to evaluate developmental and neurological landmarks in prenatal and postnatal US-exposed and control rats. Anatomical development such as weight and sex, as well as basic milestones such as pinna detachment, eye opening, righting reflex, incisor development, and fur appearance were recorded. Rats were evaluated in a test of acoustic startle and pre-pulse inhibition, methods of evaluating sensorimotor information processing independent of learning that provide important information about brain function in animals (Faraday *et al.*, 1999) and humans (Braff *et al.*, 2001). We also studied BSAEPs in the animals to physiologically probe every stage of neural processing in this system. Finally, following the experimental tests, rats were euthanized by fatal inhalation of isoflurane (5%).

Rats were ultrasounded with an Ausonics Opus 1 (model 040-530) 7.5 MHz ultrasound imaging instrument. The focal length was 4 mm and the intensity (special speak temp average (Ispta) = 23 mW/cm^2; peak pulse average (Isppa) = 32 mW/cm2; max intensity (Im) = 48 W/cm^2). This level of US radiation is commonly used in animal and human fetal diagnostic imaging. To study the effects of US radiation on developmental, behavioral and auditory indices, rats were divided into 4 groups according to time of US exposure and their sham US controls: group 1 rats were exposed twice *in utero* to US at G15 and G20 by application of the US to the dams for 15 min; Group 2 rats were the prenatal US sham controls; Group 3 rats were exposed twice postnatally at PND2 and PND4 with US; and Group 4 rats were the postnatal US

sham controls from the same litters. In order to determine gestational day, conception was ascertained by the appearance of a sperm plug at the bottom of the breeding pair cage, signifying day G0. In order to accomplish the prenatal exposure to US and to effectively model average human fetal US exposure, groups 1 and 2 dams were placed under halothane anesthesia (5%). The mother was positioned on her back over a temperature-regulated heating pad (37 degrees C) and her stomach shaved and covered in ultrasound gel (Scan ultrasound gel, Parker Laboratories, Inc.). The transducer was systematically moved around the mothers' stomach for 15 minutes. In group 2 rats, the transducer was not turned on; otherwise the rats were handled identically to those in group 1.

In order to accomplish postnatal exposure to US and to model human fetal US exposure at analogous brain developmental periods, Group 3 rats were exposed twice to US radiation postnatally on post-natal days PND2 and PND3. The US exposure at PND 2 and 3 models similar stages of neural development between humans and rodents. For example, 2-5 day-old rats have approximately the same time course of myelination as the human fetus at in the last trimester. The rat pups in Group 3 were placed on a gel pad (stand-off gel pad) that was attached to a ringed platform approximately 8 inches above a table. The top of the gel pad was coated with ultrasound gel and the rat's head was secured on the top side of the pad with transparent tape above the US transducer, which was positioned to the underside of the pad directly beneath the head of the rat pup. The rat was subsequently exposed to 10 min of US radiation. Group 4 rats were placed on the pad and secured in the same manner, but no ultrasound was administered. Rats in each of the litters were weaned at PND25, separated by sex, and culled in

groups of 3 to a cage for males and 4 to a cage for females. Monitoring of developmental indices began on PND2 and behavioral and hearing tests were initiated on PND30.

Developmental indices

Often, delays in basic developmental landmarks for rats can be a sign of developmental delays that later appear in cognitive, behavioral, or physical form, suggesting that the rat was exposed to an environmental stimulus with teratogenic effects. (Wood *et al*., 1994). Common developmental markers that are monitored postnatally are weight, pinna detachment, righting reflex (ability for the rat to return to its feet when placed on its back), emergence of fur, emergence of incisors, and the date of the eye opening. We monitored on a daily basis until PND14 the onset of 5 specific developmental indices in the prenatal and postnatal US-exposed and control rats: pinna detachment, righting reflex, emergence of fur, protrusion of incisors, and the onset of eye opening. We also recorded the body weights of perinatal US rats and their sham controls at PND30.

Locomotor activity

A motor habituation task enables examination of a rat's ability to adjust to a new environment. Under normal conditions, a rat in a new environment forages around the area, which results in a high level of movement. Once the rat has been in the new area for awhile, motor activity declines at a fairly steady rate. If a rat does not exhibit this behavior, it could be an indication of deficits in motor function. Normal rats show

habituation in this paradigm within each session, with increasing habituation in subsequent sessions (Sousa, 2006). We placed rats in a 24 inch by 24 inch by 24 inch sound-attenuated chamber with a piezoelectric transducer mounted to the underside of the suspended floor of the chamber. The piezoelectric device was sensitive to movements on the order of whisker-movement amplitudes and could effectively resolve movement frequencies of 1-100 Hz (Seaman, 1996). The piezoelectric signal from each of four chambers was amplified 10X with an Axon Instruments CyberAMP amplifier (Foster City, CA), filtered at 100 Hz and digitized at 200 samples/sec with a National Instruments PCI-MIO 16 channel A/D converter and processed off-line with root-mean-square digital signal processing algorithm using Igor Pro Software (Lake Oswego, OR). The amplitude of the piezoelectric signal was proportional to the overall movement of the animal.

Acoustic startle responses, startle habituation and prepulse inhibition

Presentation of a high intensity auditory stimulus evokes an acoustic startle response (ASR). Differences in this task indicate deficits in one or more of the three areas: defects in cognitive processing, deficits in motor tasks, or abnormalities in the auditory system. The ASR may be considered as a test of hearing, sensorimotor gating and memory depending on the component of the ASR that is tested. The ASR itself is a gross determination of hearing. Startle habituation accrues to non-random presentations of the ASR, and depends on memory. The ASR can be inhibited by a prepulse occurring 100-500 msec before the ASR. Acoustic startle with prepulse is currently used in human subjects to test for neurobiological abnormalities in neuropsychiatric

disorders such as schizophrenia (Hagen *et al.*, 2005). Prepulse inhibition of the ASR is independent of memory and is considered to be a reliable measure of sensorimotor gating, thought by many to be pre-attentive (Hagen *et al.*, 2005). We performed all 3 components of the ASR test; mainly, non-random ASR habituation and prepulse inhibition of the ASR. These tests were performed in separate sessions on separate days. Each rat was placed in a 24 inch by 24 inch by 24 inch sound-attenuated chamber with a loudspeaker that produced a 120-dB startle tone. The same piezoelectric transducer used in the locomotor studies was used in the ASR studies (see above). For the startle-habituation test, startle tones were given at set intervals (e.g., 60 sec). We measured the amplitude of the response for each of 12 startle tones. For this test, no averaging was done in order to determine if habituation of ASR waveform was occurring. Waveforms were captured 100 msec before the presentation of the 120-dB tone stimulus (20-msecduration) and were followed for 500 msec after the stimulus. For the pre-pulse inhibition of the ASR experiments, a 68-dB prepulse was administered 100 msec prior to the ASR. The ASR was randomly presented at 30-60 sec intervals and randomly presented with epochs of prepulse stimuli. The startle ASR waveforms were averaged (12 trials within a session—randomized) separately from the prepulse startle ASR waveforms (also 12 trials with a session) by an Igor Pro waveform-averaging algorithm. The ASR peak amplitude was determined by manually adjusting cursors before the presentation of the acoustic stimulus and at the peak of the ASR.

Brainstem auditory evoked potentials.

Brainstem auditory evoked potentials (BSAEPs) can be used to assess the normal physiology of the neuroaxis from the peripheral auditory nervous system structures to cortical auditory areas. They have also been used to assess myelination along each of the central pathways. By presenting a sound to the rat, a BSAEP can track the flow of the neural message, with latencies in the pathway indicating whether a specific portion of the pathway has been damaged (Kadner, 2006).

In the human auditory system, the peaks of an BSAEP are the firings of neurons that begin after the cochlear nerve leaves the internal auditory meatus and terminates on the dorsal and ventral cochlear nuclei. These are the first and second peaks, respectively. Neurons arising from the cochlear nuclei take one of four pathways. One pathway travels ipsilaterally from the anteroventral cochlear nucleus to the medial and lateral superior olivary nuclei. The other three pathways form the dorsal, intermediate, and ventral acoustic striae. Some fibers from the anteroventral cochlear nucleus form the trapezoid body, which in turn project to and terminate contralaterally in one of three areas: the medial nucleus of the trapezoid body, which then terminates on the lateral superior olivary nucleus, the medial superior olivary nucleus, or the dorsal nucleus of the lateral lemniscus and the inferior colliculus. The superior olivary complex is the third peak in the BSAEP and is important in sound localization and intensity. The fourth peak is the firing of the lateral lemniscus pathway, which arises from neurons in the dorsal and ventral cochlear nuclei as well as from the superior olivary nuclei. The fifth peak in the BSAEP is the firing of neurons in the inferior colliculus. This structure receives afferent inputs from the cochlear nuclei, the superior olivary complex, and

nuclei of the lateral lemniscus, all of which are traveling up the lateral lemniscus pathway. It is involved with sound localization (Patestas *et al*., 2006).

BSAEP can also be used to assess hearing function in rats, with peaks correlating to the homologous structures in humans. Because the present study focuses on the effect of US exposure on development of the auditory system, it is important to note when structures that can be assessed with BSAEP develop in the rat. The first portion of the rat auditory pathway to develop (that can be monitored by BSAEPs) is the vestibulocochlear nerve. The vestibular portion of the vestibulocochlear nerve begins to appear at approximately day G11 while the cochlear nuclei neuroepithelium appears at day G12. Following this, on G15, the inferior colliculi appears. On day G16, the superior olivary nucleus appears in the posterior portion of the pons followed by the lateral lemniscus, which appears on G18 next to the fourth ventricle (Altman *et al.*, 1995).

To record BSAEPs, each rat was anesthetized with 2% isoflurane gas, and body temperature was maintained with the help of a feedback-regulated heating pad. Stainless-steel electrodes were inserted under the skin at the vertex (active electrode) and mastoids (reference) and recorded differentially with a Cadwell 5200A signal processor. Monopolar clicks from a speaker of 1 msec duration, 22.2 Hz rate, and variable intensity (10dB-90dB) were delivered via hollow tubes controlled by the Cadwell 5200A. The speakers were calibrated with a sound level meter. The signal measured by the electrodes was amplified 1000X, filtered between 10-2000 Hz and sampled at 50 kHz. For any particular sound intensity, the average of 500 responses, each measured from 0 to 10 msec after the click onset, were determined. The average

waveforms generated as the sound pressure level were lowered in 10-dB and then 5-dB steps and were compared to estimate visually the threshold for which a BSAEP could be observed with a 2/1 signal to noise ratio. Threshold was defined as the intensity level at which a BSAEP wave component I with an amplitude of 0.05 µV will be seen in 2 averaged runs.

RESULTS

Developmental indices

There were no significant developmental differences between US-exposed rats and sham-exposed rats in any of the measured developmental indices (**Table 1**).

Developmental landmark	*Prenatal Ultrasound*	*Sham Control*	*Postnatal Ultrasound*	*Sham Control*
1) Pinna detachment	***PND2***	***PND2***	***PND2***	***PND2***
2) Righting reflex	***PND3***	***PND3***	***PND3***	***PND3***
3) Emergence of fur	***PND5***	***PND5***	***PND5***	***PND5***
4) Incisors	***PND6***	***PND6***	***PND6***	***PND6***
5) Eye opening	***PND14***	***PND14***	***PND14***	***PND14***

Table 1. Perinatal ultrasound does not affect select developmental indices. The day of pinna detachment, righting reflex, emergence of fur, incisor eruption, or eye opening did not differ in prenatal or postnatal US versus sham US rats (n=30 each).

Furthermore, there were no significant differences in body weights in prenatal or postnatal US rats compared to sham controls (prenatal US male mean weight = 141 ± 3 grams versus sham male mean weight = 138 ± 3 grams; prenatal US female mean weight = 115 ± 3 grams versus sham female mean weight = 117 ± 4 gms; postnatal US male mean weight = 138 ± 2 grams versus sham male mean weight = 143 ± 4 grams; postnatal US female mean weight = 111 ± 4 grams versus sham female mean weight = 115 ± 3 grams; n=24 each; $P>0.05$).

Locomotor activity

Figure 1 shows the effects the overall motor activity in a single session in prenatal and postnatal US vs sham rats. There was no difference between groups for either of the perinatal US exposures within the first session (P>0.05; n=24 each; Session 1) or in the habituation between subsequent sessions (P>0.05; n=24 each; Session V)

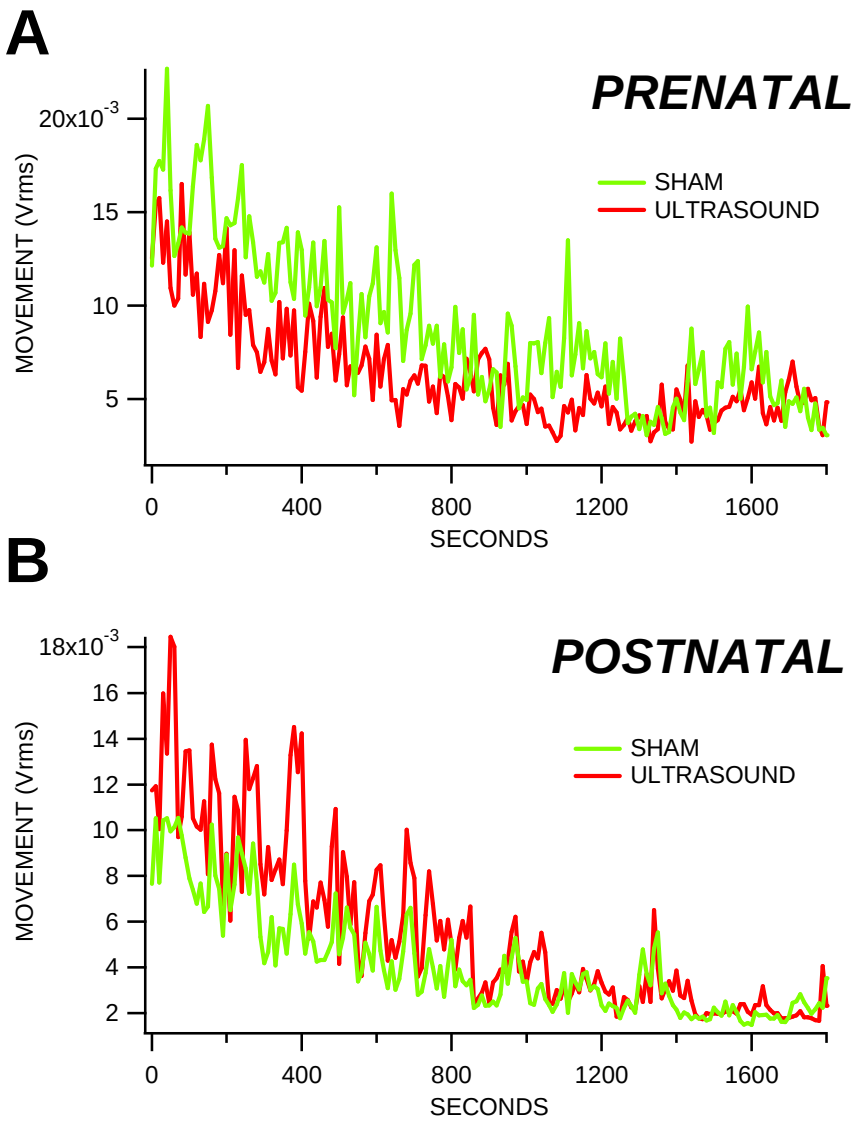


Figure 1. Perinatal ultrasound does not affect motor activity or habituation. Rats were placed in sound-attenuating chambers whose floor was suspended and

loaded with a piezoelectric transducer that measured their overall locomotor activity. (A) This figure shows the total rms voltage from the piezoelectric transducers (i.e., movement activity) during the first session for prenatal US versus sham-treated rats. The sham-treated rats are represented in green while the US-treated rats are represented in red. The traces represent the average of all rats. There was no difference in overall motor activity between sham and US-treated rats in this first session or between habituation in subsequent sessions (data not shown). (B) This figure shows the total rms voltage from the piezoelectric transducers during the first session for postnatal US versus sham-treated rats. The traces represent the average of all rats. There was no difference in overall motor activity between sham and US-treated rats in this first session or between habituation in subsequent sessions (data not shown)

Acoustic Startle Responses

Figure 2 shows ASRs obtained with random startle stimuli in postnatal US versus sham-treated controls. The startle stimuli were randomized to avoid habituation (see below). There was a significant difference in ASR amplitude between postnatal US and sham-treated rats (n=30 each; P=0.007 F(1,58)=7.62).

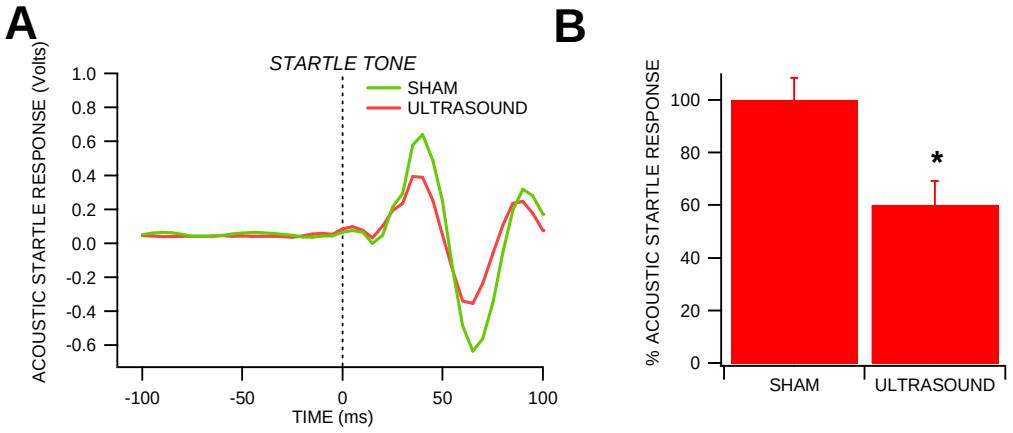


Figure 2. Ultrasound reduces acoustic startle in postnatally-exposed rats (PND 2 and 3 US-exposed). Rats were placed in sound-attenuating chambers whose

floors were suspended and loaded with piezoelectric transducers that measured their acoustic startle response (ASR) to 15 randomly-presented 120-dB 1000 Hz tone (20 ms) during a 15 min session. (A) These traces show the grand average ASR in sham and postnatal US-treated rats. The ASR of rats exposed to US on PND 2, 3 was smaller in amplitude than that of sham-treated rats. (B) There was a significant difference in ASR amplitude between postnatal US and sham-treated rats (n=30 each; P=0.007 F(1,58)=7.62).

Startle habituation

Learned habituation accrues to non-random startle stimuli. Typically, within 1 session of 10-15 non-random startle stimuli the ASRs will decrease in amplitude. Unlike the startle response above, by using non-random startle stimuli learned associations can be studied using the ASR. We studied the effects of non-random startle stimuli on postnatal US and sham-treated rats. **Figure 3** summarizes the effects of postnatal US exposure on startle habituation of the ASR. It shows a raster of the grand averaged ASRs for each non-random startle stimuli for sham and US-exposed rats. Habituation accrued to successive startle stimuli within 12 stimuli. There was no significant difference in startle habituation between postnatal US and sham-treated controls (n=24 each; P>0.05)

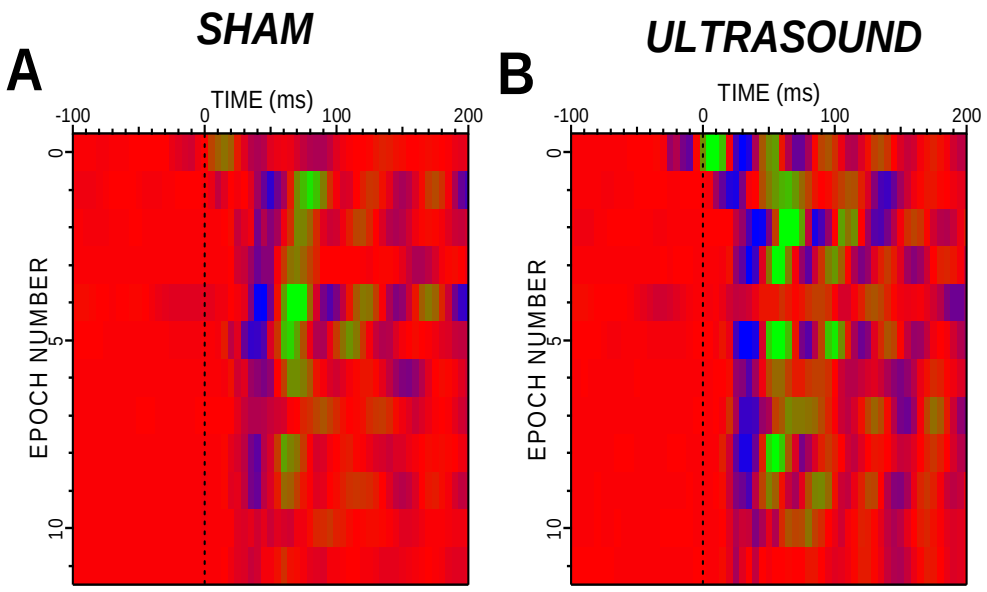


Figure 3. Postnatal ultrasound exposure has no effect on startle habituation. . Rats were placed in sound-attenuating chambers whose floors were suspended and loaded with piezoelectric transducers that measured their acoustic startle response (ASR) to 12 non-randomly presented 120-dB 1000 Hz tones (20 ms) during a 15 minute session. (A) This image plot shows the grand average sham ASR (blue indicates high motor activity, red indicates low motor activity) for each successive startle stimulus epoch of the 12 stimuli in the session. Note that the magnitude of the ASR decreases markedly after 10 successive startle stimuli. The zero line indicates the time of the presentation of the startle stimulus. (B) Startle habituation accrued in US rats in a manner similar to that of sham rats.

Prepulse inhibition

In normal rats and humans, the ASR previously observed can be inhibited by a prepulse occurring 100-500 msec before the ASR (Hagen and Jones, 2005). Prepulse inhibition of the ASR is thought to be a reliable measure of sensorimotor gating and is independent of learning. Prepulse inhibition tests were conducted on prenatal and postnatal US and sham rats by presenting random startle stimuli with randomized

epochs of a prepulse non-startle auditory stimulus. **Figure 4** summarizes the prepulse inhibition of the ASR experiments. There was no significant difference in the prepulse ASR amplitude between postnatal US and sham-treated rats (n=30 each; P=0.3 $F_{(1,58)}$=0.93).

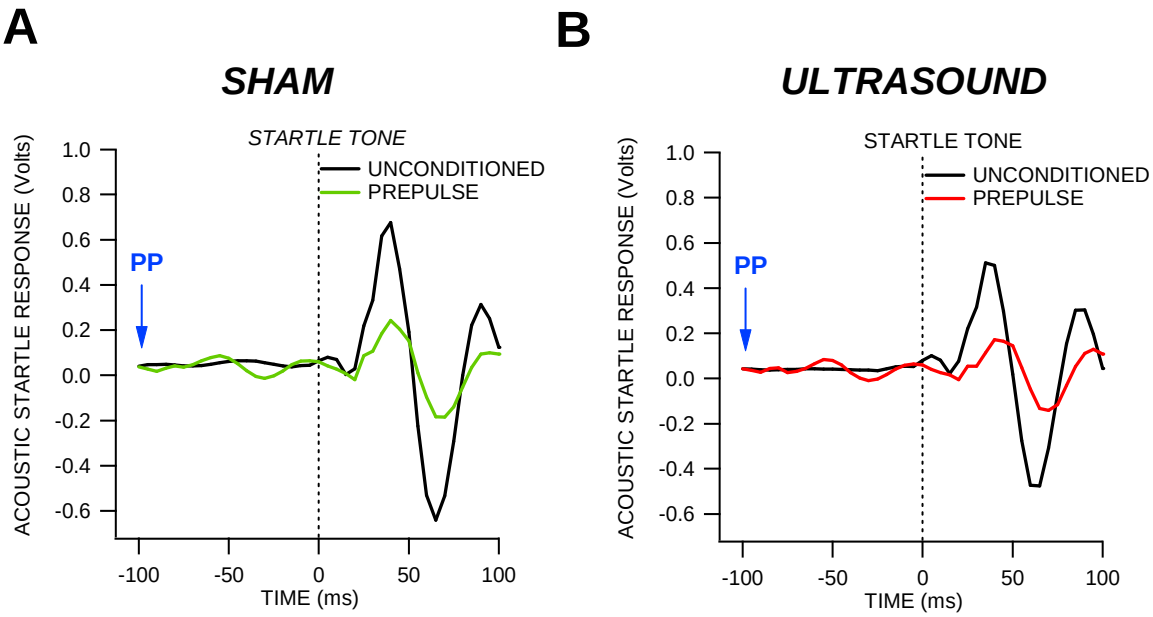


Figure 4. Ultrasound has no effect on prepulse inhibition of the acoustic startle response in rats exposed postnatally to ultrasound radiation. Rats were placed in sound-attenuating chambers whose floor was suspended and loaded with a piezoelectric transducer that measured their acoustic startle response (ASR) to a 120 dB 1000 Hz tone (20 msec) following a 60 dB 2000 Hz (20 msec) prepulse tone that occurred 100 msec before. (A) These traces show superimposed grand average ASRs and prepulse ASRs in sham-treated rats. The prepulse ASR in sham-treated rats was consistently smaller than the ASR alone. (B) These traces show superimposed grand averaged ASRs and prepulse ASRs in postnatal US-

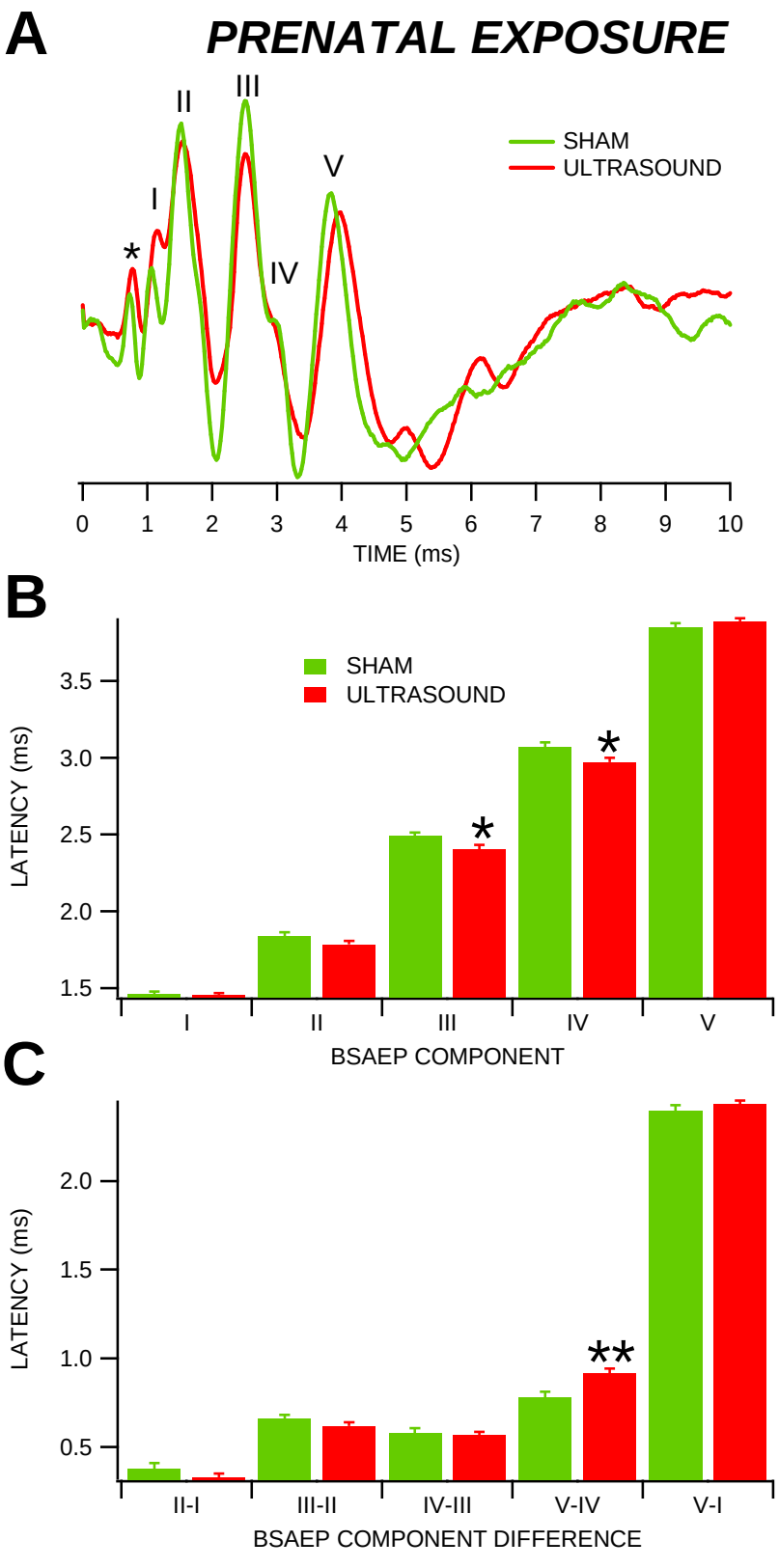
treated rats. The prepulse ASR in US-treated rats was consistently smaller than the ASR alone. There was no significant difference in the prepulse ASR amplitude between postnatal US and sham-treated rats (n=30 each; P=0.3 F(1,58)=0.93).

Brain stem auditory evoked potentials (BSAEPs)

In order to evaluate the auditory system effects of perinatal US radiation, we performed BSAEPs. BSAEPs have been used by many labs to assess the normal physiology of the neuroaxis from peripheral auditory nervous system structures to cortical auditory areas (Kadner, 2006). They have also been used to assess myelination along each of the central pathways. Because of the heavy myelination of auditory pathways and the susceptibility of the cochlea to ultrasound we determined the threshold for elicitation of BSAEPs as well as BSAEP waveforms to evaluate the functionality of the cochlea and its projection pathways in the CNS. Although there was no significant difference in BSAEP threshold between prenatal US and sham rats (mean sham threshold = 29.4 ± 1.42 dB (n=31) versus mean US threshold = 32.2 ± 1.46 dB (n=29); P = 0.13, $F_{(1,68)} = 2.3$), there were significant differences between some BSAEP waveform components. The BSAEP waveform components are typically five positive peaks (I-V) that are recorded when an electrode over the vertex is referenced to mastoidal electrodes. Specifically, the auditory nerve and the cochlear nucleus are the generators of peaks I and II, the superior olivary complex generates peak III, the lateral lemniscus generates peak IV, and the inferior colliculus generates peak V (**Figure 5A**). Together, the series of waveforms encompass these nuclei and the relays between them. The BSAEPs are used to demonstrate the integrity of the neuronal pathway from the

cochlea, via the auditory nerve to the brain stem, allowing localization of dysfunction within this pathway. These are very short latency responses with very tight interpeak latencies that are not easily perturbed by experimental manipulations. The interpeak latencies between BSAEP components are the most independent of subject, stimulus, and recording parameters compared with other measures derived from the BSAEP.

Figures 5 B and C summarize the results of the BSAEP studies in prenatally-exposed rats. There were small, but significant, differences in BSAEP peaks III and IV latencies between prenatal US rats versus sham controls (**Figure 5B; III:** $P = 0.023$, $F_{(1, 66)} = 5.42$; IV: $P = 0.054$, $F_{(1, 66)} = 5.31$). There was also a significant difference in inter-peak latencies between BSAEP peaks IV-V in prenatal US rats versus sham controls (Figure 5C; IV-V: $P = 0.002$, $F_{(1, 65)} = 10.76$).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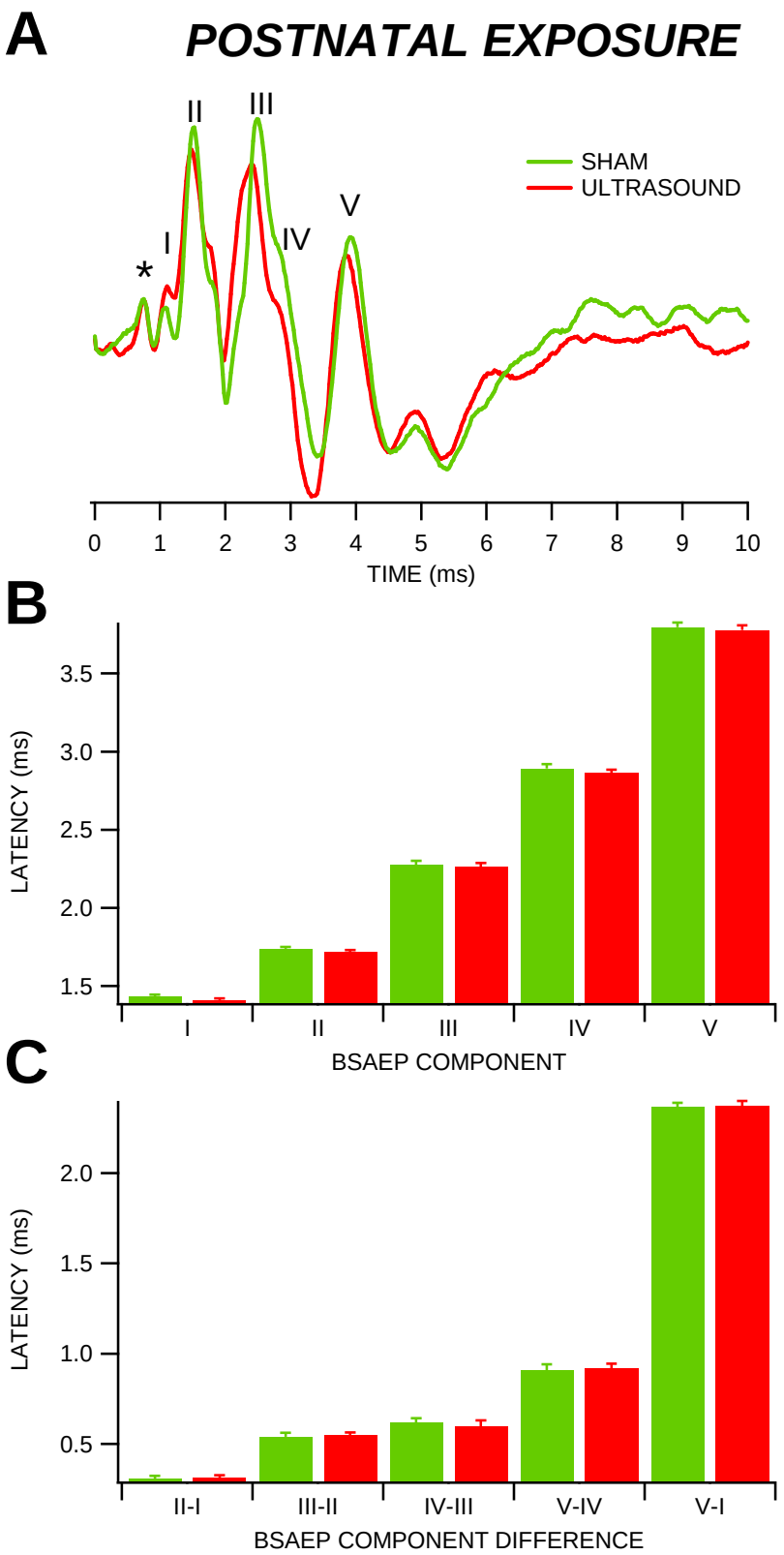
BSAEP COMPONENT DIFFERENCE

Figure 5. Ultrasound effects in prenatal US-exposed rats. (A) These are superimposed grand-averaged BSAEP waveforms from prenatal US and sham control rats. Note the 5 peaks of the BSAEP. The early peak denoted by the asterisk is not biologically relevant, but represents microphonics. The waveform components (I-V) of the BSAEP were measured at 80-dB. (B) There was a mild difference in BSAEP peak latencies of peaks III and IV between prenatal US and sham-treated rats (n=30 each). (C) There was a moderate difference in BSAEP interpeak latencies IV-V between postnatal US and sham-treated rats (n=30 each).

We also evaluated BSAEPs in postnatal US rats versus sham controls. There was no significant difference in BSAEP threshold between postnatal US and sham rats (mean sham threshold = 33.4 ± 1.397 dB (n=31) versus mean US threshold = 32.7 ± 1.6 dB (n=29); P = 0.73, $F_{(1, 59)} = 0.12$). **Figure 6** summarize the results of the BSAEP studies in postnatal US rats versus sham controls. There were no significant differences in BSAEP peak latencies between groups (n=30 each).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BSAEP COMPONENT DIFFERENCE

Figure 6. Ultrasound effects in postnatal US-exposed rats. (A) These are superimposed grand-averaged BSAEP waveforms from postnatal US and sham control rats. Note the five peaks of the BSAEP. The early peak denoted by the asterisk is not biologically relevant, but represents microphonics. The waveform components (I-V) of the BSAEP were measured at 80dB. (B) There was no difference in BSAEP peak latencies of peaks III and IV between postnatal US and sham-treated rats (n=30 each). (C) There was no difference in BSAEP interpeak latencies between postnatal US and sham-treated rats (n=30 each).

Summary of results

1) There were no significant differences in various indices of developmental landmarks, including weight gain, in prenatal or postnatal US rats compared to sham controls

2) Postnatal exposure to US radiation significantly decreases ASR amplitudes, but did not significantly alter prepulse inhibition of ASR responses.

3) There was no significant difference in motor activity or locomotor habituation in prenatal or postnatal US rats compared to sham controls.

4) There were no significant differences in hearing thresholds in prenatal or postnatal US rats compared to sham controls. There was, however a statistically significant increase in transmission in some components of the BSAEP in prenatal US rats compared to sham controls.

DISCUSSION

In this study, prenatal and postnatal US in rats did not produce any significant differences in developmental indices compared to sham-treated controls. In addition, there were no significant differences in overall motor activity or motor habituation in US rats versus sham controls, indicating that this gross measure of CNS development was not affected.

We performed all three components of the ASR test: non-random ASR habituation, random ASR, and prepulse inhibition of the ASR. There was a significant difference in the amplitude of the startle response between postnatal US rats and their sham controls. This might indicate a deficit in hearing, a deficit in sensorimotor gating or a deficit in motor output. As it was fairly evident from the locomotor activity experiments that motor output was not affected, we looked at prepulse inhibition of the ASR. There was no difference in prepulse inhibition of the ASR in postnatal US rats versus sham controls, indicating that sensorimotor gating was intact. Therefore, an US-induced deficit in hearing might have occurred. To further evaluate the amplitude differences of the startle response, the rats were studied with hearing tests.

While there was no difference in BSAEP threshold in prenatal or postnatal US rats, indicating the ability to hear isn't affected, there was significant speeding up of some of the component peaks of the BSAEP in prenatal US rats compared to their sham controls. The faster waveforms correspond to the olivary complex and lateral lemniscus, respectively. These findings suggest that there might be labile pathways in the brainstem that are sensitive to US exposure and that hearing might be disrupted somewhat by US exposure. It is also possible that US exposure on days G15 and 20

disrupted development of the superior olivary nucleus and lateral lemniscus pathway, the two components that showed a decreased latency. Interestingly, these two structures develop during the same time period that we exposed the rats to US. The superior olivary nucleus begins to appear on day G16 and the lateral lemniscus pathway appears at approximately G18 (Altman *et al.,* 1995). The decrease in BSAEP peak latencies suggests that neural processing of auditory stimuli by these structures has been altered in US exposed rats.

Previous studies have correlated decreased BSAEP peak latencies with abnormal auditory circuitry. Hall (1992) reviewed the findings of several studies that explored the BSAEP findings in Down syndrome, noting that human subjects with Down syndrome have a reduction in the wave I-II and III-IV latency intervals. Hall suggested that the conduction time was reduced because of a high frequency hearing impairment. However, the shortened interwave latency time still occurred in subjects with normal hearing. Other studies demonstrated a decrease in latency for BSAEP waves with increased stimulus intensity in high-frequency cochlear impairment (Folsom *et al.,* 1983; Squires *et al*., 1980, 1982; Hall, 1992). There is also a possibility that the decreased latency could be due to decreased inhibitory synaptic connections in the auditory pathway or absences in points of transmission or neurons in the auditory pathway.

Strengths

This study offers new methods of evaluating effects of US exposure. We carefully identified rat litters and US exposed rats in order to produce clearly defined results. We were also able to systematically evaluate the outcome of preliminary

measures and apply them to further tests to track the associated deficits within the rat's physiology. Further, we applied the use of BSAEP and acoustic startle response to evaluate possible deficits, a novel combination to evaluate US effects.

Limitations

With the complexity of monitoring US exposure, this study posed some limitations worth considering. One limitation of this study is that we did not measure the amount of US radiation actually delivered to each animal. Because of this, we are unable to explicitly say how the radiation levels compare to other studies or uses of US. It is possible that the rats exposed prenatally to US were given a different amount of US than those who were exposed postnatally. The unknown amount of US each rat received makes it impossible to use the US exposure as a variable and to increase or decrease levels to monitor effects. The most considerable limitation of this study was the inability to use US exposure on human subjects and monitor those effects. While the US effects on rats are important, the most beneficial information would be how US exposure affects human development and causes possible defects *in utero*.

There is also the concern that we weren't able to complete the ASR studies in the prenatal US exposed rats. Approximately half-way into the study period the equipment had to be moved from one room to the next (Rm1220 to Rm1296 SWKT) due to departmental expediencies. As a result, we could not obtain the same calibration values for auditory stimuli in the new room as previously obtained in the former room. This was most disappointing and precluded us from comparing prenatal US exposure to their sham controls. The only reliable data was obtained from postnatal US exposure experiments as indicated in the results.

Implications

Our findings indicate a decrease in acoustic startle response in postnatally exposed US rats and a speeding up of the BSAEP in rats who were prenatally exposed. If these results can be replicated, further research needs to be done in animal models that would better the understanding of possible US teratogenic effects. Eventually, conclusions could be linked to human conditions such as speech problems and other deficits discussed earlier that may be associated with US exposure. Our study may implicate changes in human physiology when an individual is exposed to US. Research has already been done in the past linking our findings in animals to human pathology. One study (Kouni *et al.,* 2006) discovered that subjects with dyslexia showed delayed peak and interpeak latencies verses normal subjects when given verbal stimuli in the BSAEP test. Other studies have also demonstrated variations in the brainstem related to dyslexia (McAnally *et al*., 1996). Eventually, tests of the auditory pathway could show a link to learning disorders such as dyslexia and lead to treatment.

Speech and other learning problems may be related to problems in the auditory pathway (Song *et al.,* 2006). It can be difficult for a person to correctly form words and speech if they do not hear the words correctly. It is possible that damage to the auditory pathway due to US exposure could alter speech development in some people. Previous studies (Akshoomoff *et al.,* 1989) have studied learning disorders and the brainstem with varying results. Further research on the subject could lead to a better understanding of these disorders and hopefully better treatment. In any case, our results indicate a strong need for more US research and correlated effects.

Future Direction

We were unable to collect data on the acoustic startle response of prenatally exposed rats. Because of this, data should be collected and compared to sham exposed rats to see if prenatally exposed rats were affected by the US exposure. Also, the prenatally exposed rats showed a speeding up of the neural firing in the auditory pathway. Further research is needed to determine the cause of this decrease in latency. It would be beneficial to use a myelin stain in a control and an ultrasound exposed rat to determine variations in the auditory pathway between the two rats or differences in myelin distribution. It is possible that one pathway has more connections or branching of neurons than the other pathway.

Further study could be done by causing a partial lesion of the superior olivary nucleus and the lateral lemniscus pathway, the portions of the BSAEP where variations appear to have occurred. The partial lesions could be followed with a BSAEP test to determine if damage to these areas alters the results of the BSAEP. It is possible that US exposure causes variations in the superior olivary nucleus or in the lateral lemniscus pathway which in turn is causing the decreased latency of the BSAEP. Each of these portions of the pathway could also be removed post-mortem and compared to determine variations in size, neuron density, shape, and structure.

It is possible that the US exposure could have an effect on the number of inhibitory connections being made in the superior olivary nucleus or in the lateral lemniscus pathway. It would be possible to test for this by immunostaining tissue sections with an antibody against glutamic acid decarboxylase, the key enzyme in the biosynthesis of GABA, which is the main inhibitory neurotransmitter in the brain. With analysis of these results, it would be possible to determine if the number and density of

inhibitory neurons varied between US exposed rats and sham exposed rats.

To rule out any variables other than US exposure, better controls are needed in the future to ensure the validity of results. This could be accomplished by replicating the study using BSAEP equipment that automatically calculates all values. The equipment used in this study left some room for human error because the threshold was determined visually by the administrator of the test. Better controls could also be ensured by using better methods to restrain the prenatally exposed rats. It is possible that rats were not placed exactly over the transducer when they were restrained allowing for the possibility that a rat may have received more exposure on its stomach while another on its head. This could have caused variation in the US exposure and its effects.

Conclusion

The main finding emerging from this study is that rats exposed to US on PND 2 and 3 show a decreased acoustic startle response, a finding that suggests a decreased ability of the auditory system to process auditory stimuli. In addition, US exposure on G15 and G20 disrupt the auditory pathway as demonstrated in the results of BSAEP testing. In contrast, developmental indicies, motor function, and memory appear unaffected by prenatal and postnatal US exposure. Although the implications for humans prenatally exposed to US are unclear, the results discussed in the thesis show a need for further studies analyzing affects of US on the auditory system in humans.

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Worked with researchers at ONPRC on collecting dominance data as well as activity monitoring on hundreds of rhesus macaque monkeys. Also acted as the human intruder during several hours of hidden intruder testing and worked with veterinarians during round-up health checks.

2005-present **Master's thesis**
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Studied the effects of perinatal ultrasound exposure on Wistar rats. Conducted ultrasound exposures, hearing and acoustic startle testing as well as motor habituation tasks and tests of hippocampal memory. Supervised other students in these tasks as well.

2004-2005 **Undergraduate Research**
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Conducted electroencephalogram recordings of human subjects during visual recognition tasks. Helped to analyze data and write up conclusions.

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Participated in efforts to educate people about health effects associated with tobacco including a health parade, street booths, and teaching school children.

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