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Abstract

The purpose of this study was to determine the effects of an atypical pattern of early acoustic stimulation on auditory development. Previous human research suggests that the acoustic environment of pre-term human infants in the Neonatal Intensive Care Unit (NICU) negatively affects some aspects of auditory development. Animal research suggests that premature auditory stimulation interrupts auditory development. Because mice are born before their auditory systems are developed, they make an excellent model for research on fetal and postnatal plasticity of the auditory system. The premature auditory state of newborn mice is similar to that of the NICU pre-term infant, albeit, natural for mice

C57 mouse pups were exposed to an augmented acoustic environment (AAE) of a nightly 12-hour regiment of 70 dB SPL noise burst, beginning before age 12 days (onset of hearing) and lasting for one month. The prepulse inhibition (PPI) of mice exposed to the AAE was compared to that of non-exposed mice to observe short-term and long-term effects. Results showed that the prepulse inhibition of the AAE exposed mice did not differ significantly from that of the non-exposed mice. However, it is possible that the measurement used, PPI, may not have been appropriate or that the AAE may not have been an appropriate simulation of the NICU environment.

Introduction

Pre-term infants in the typical neonatal intensive care unit (NICU) are exposed to continuous light and a variety of necessary, but noisy equipment such as incubators, high frequency ventilators, and EKG monitors. The American Academy of Pediatrics (1997) raised concerns regarding the exposure of pre-term infants to high and continuous sound levels in the NICU. They reported sound levels inside incubators ranged from 50 dBA of background noise to 120 dBA of impact noise. These noise levels are in excess of the Environmental Protection Agency (EPA) recommendations for hospital levels of 45 dBA during the day and 35 dBA at night (Kahn, Cook, Carlisle, Nelson, Kramers, & Millman, 1998). This environment is very different, both visually and acoustically, from that experienced by a full-term infant during the same developmental period in the womb. Recently, the Study Group on Neonatal Intensive Care Unit Sound, convened by the Center for the Physical and Developmental Environment of the High Risk Infant, reviewed research on human and animal auditory development, including the effects of environmental light and noise exposure at different maturity levels in fetuses and neonates (Graven, 2000). Animal studies show that neurosensory development follows a certain pattern of development, a pattern that likely parallels that of human neurological development (Graven, 2000). This pattern begins with the sense of touch and is followed by vestibular, chemical, auditory, and, finally, visual development (Lickliter, 2000). Pre-term birth does not alter this sequence.

Animal models indicate that prenatal visual stimulation and augmented auditory stimulation interrupts auditory development (e.g., Lickliter, 1994; Lickliter, 1990; Lickliter and Stoumbos, 1992). For example, bobwhite quail chicks exposed prenatally to patterned light or to an unnatural pattern of auditory stimulation did not exhibit normal auditory responsiveness to the

maternal quail call (Lickliter, 1990; Lickliter and Stoumbos, 1992). It seems that, when a sensory system is typically, naturally stimulated at a particular period of development, that stimulation becomes essential for normal development. However, if the same stimulus is too intense, too early, or out of sequence, it can interfere with the normal pattern of neurosensory development. Therefore, the Study Group concluded that stimulation of the developing sensory system is dependant on the amount, type, and timing of stimulation (Graven, 2000). Pre-term infants are often exposed to patterned light and unfiltered sound at a stage in development when such stimulation is inappropriate. This suggests that visual and auditory exposure in the NICU may have long-term auditory effects.

Due to the ethical and practical issues surrounding studies of the effects of the early sensory environment on human infants, animal models are required for such studies. The use of animal models reduces between-subject variables and allows the control of environmental conditions. The use of inbred mice as a particular animal model for auditory research is advantageous because of the vast amount of information available on mouse genetics and on the mouse auditory system (Willott, 2001).

In the present study, a mouse model was used to address the issue of possible long-term effects from moderately intense (70 dB SPL) auditory stimulation during early development. The C57BL/6J (C57) inbred strain of mice was selected because it has been well studied. C57 mice hear normally during the first few months of life and into early adulthood. However, due to the Ahl gene, they exhibit gradual, progressive high frequency hearing loss parallel to that experienced by many humans as they age (Willott, 2001; Willott, Carson, & Chen, 1994). Thus, the present experiment may also shed light on the effects of early acoustic stimulation on the course of adult progressive hearing loss.

It should be noted that the timetable of auditory system development of the C57 mouse differs from that of humans. During a 36-40 week gestational period, the human auditory system begins to develop by the end of the third week after conception (Peck, 1994). Between week 10 and week 21 of gestation, the organ of Corti begins to form, and hair cells begin to mature. By the seventh fetal month, all neural synapses are formed. At this time, the fetus is experiencing sound. Electrophysiological data indicate that, at birth, the full term infant has mature middle and inner ear structures, while the central auditory system continues to develop for the next 18 months (Ruben & Rapin, 1980; Hall, 1992; Hood, 1998).

The human visual system begins to develop by 22 days gestation. However, more than nine months of development are required for the visual system to reach completion. In fact, the eyelids, which fuse at the third gestational month, will not re-open until the fifth gestational month. The retinal vessels will not reach full maturity until after the ninth month. Some fetuses may demonstrate a response to light as early as the eighth gestational month indicating that at least some central nervous system pathways are established early (Sadler, 1985; Cibis, Anderson, Chew, Fishman, Kardon, Tripathi, Van Kujik, Weleber, & Balyeat, 1994).

In contrast, the gestational period for mice is 17 to 22 days and, at birth, the peripheral fibers within the auditory organ are still not oriented. It is not until the mouse is a few days old that the inner spiral bundle develops (Sobkowicz & Rose, 1983). By 12 days after birth, the eyes and external auditory canals open (Shnerson & Pujol, 1983). By 14 days, the cochlea is relatively mature, but the central auditory system continues to mature (Webster & Webster, 1980). Because mice are born before their peripheral auditory systems are fully developed, they make an excellent model for research on fetal and postnatal plasticity of the auditory system. The

premature auditory state of newborn mice is similar to that of the NICU pre-term infant, albeit, natural for mice.

In this study, the auditory behavior measured was prepulse inhibition (PPI) of the acoustic startle response (ASR). The ASR is elicited by an intense noise (e.g., 100 dB SPL) of abrupt onset. PPI occurs when a tone that does not have the potential to startle (e.g.. 70 dB SPL) is presented within 10 to 200 ms prior to the startle stimulus. If the prepulse tone is successful, a reduction, inhibition, of ASR amplitude will occur (Willott et al., 1994). PPI occurs readily in both humans and mice (Swerdlow & Geyer, 1999). PPI is the ratio of the startle with a prepulse to the baseline startle response, and can be used to assess the behavioral salience of sound. If the tone produces PPI, it can be heard by the animal, is assumed to be salient, and vice versa. In mice and in humans, the inhibition of the startle response when a prepulse is presented at a short interval prior to the startle stimulus can help determine the integrity of auditory pathways in the central nervous system (Blumenthal, 1999). Structures involved in the modification of the startle are the inferior colliculus along with other higher-order structures (Willott, Sundin & Jeskey, 2001). Thus, if normal PPI occurs, pathways in the auditory brainstem must be functional.

Past studies performed by Willott and colleagues have examined the effects of controlled auditory stimulation on the auditory function of mice. They found that auditory neural plasticity was induced by exposing adolescent/young adult C57 mice to an augmented acoustic environment (AAE), a broadband noise (rise/fall = 10 ms, duration = 200 ms, rate = 2/sec.) of 70 dB SPL presented nightly for 12 hours over a period of a few days to several weeks. Willott and colleagues have demonstrated that appropriate stimulation of the degenerating cochlea and the central auditory system by an appropriate AAE may have ameliorative effects, similar to the effects of "exercise" or increased neural activity in other neural systems (Willott et al., 2001;

Willott & Turner, 2000). PPI became stronger in mice when the AAE was maintained for a long period of time. This suggests that changes occur in the physiological and or anatomical properties of the central auditory system in response to chronic AAE treatment.

Thus far, studies of AAE have initiated treatment after weaning, at age 25 days. It is not known if very early AAE exposure might have weaker, stronger, or lasting effects on PPI and the ASR. Therefore, the goal of this project was to use an animal model to determine the effects of an atypical pattern of early acoustic stimulation on auditory development. The animal model used was the C57BL/6J (C57) inbred mouse. The testing procedures included the ASR and PPI.

Specifically, mouse pups were exposed to a nightly 12-hour regimen of 70 dB SPL noise burst beginning before age 12 days (onset of hearing) and lasting for one month. Then the PPI of mice exposed to the AAE was compared to that of non-exposed mice to observe short-term effects. Secondly, the mice were retested at several intervals after termination of noise exposure, with a maximum age of nine months to observe long-term effects of early exposure. It was expected that results of this study may have implications for early acoustic exposures found in the NICU.

Methods

Subjects

C57BL/6J mice of either sex were obtained from the University of South Florida rodent colony. Mice from this colony were the offspring of stock obtained from Jackson Laboratory (Bar Harbor, ME). They were randomly selected to form two groups: those that were exposed to an AAE (7 males and 10 females) and a non-exposed group (7 males and 6 females) housed in the vivarium.

Housing

Mice were kept with their mother until weaning. In the exposed group, the mother was also exposed to the AAE from the time the pups were 11 days old until weaning at 21-23 days of age. After weaning, the mice were separated into same sex cohort groups. Mice in exposed and non-exposed groups were kept in plastic shoebox cages with wire lids for unlimited access to tap water and rodent chow. Room temperature was maintained at 23 to 24 C°. A 12-hour night-day schedule was maintained. The exposed group received 12 hours of AAE treatment during their nocturnal active period, beginning at 11 days old. The non-exposed groups were kept in a relatively quiet vivarium.

AAE Treatment

The AAE was digitally synthesized and recorded on a compact disk (rise/fall= 10 ms, duration= 200 ms, rate= 2 noise bursts per sec.). The noise was amplified and sent to a Radio Shack Super tweeter, which was positioned over the top of each cage. The AAE was calibrated to 70 dB SPL using a sound level meter at various positions within the cage. The groups were exposed to a total of 30 days of the AAE environment, after which they were returned to the vivarium with the non-exposed groups.

Startle and PPI measurements

The stimulus used to elicit the startle was a 100 dB SPL broadband noise burst (4k – 25k range spectrum, 10 ms duration, 1 ms rise/fall time, delivered at a variable rate of 3 to 8 ms). The prepulse stimulus was a 70 dB SPL tone burst (4 kHz, 12 kHz or 20 kHz) with a 10 ms duration, and a 3 ms rise/fall time. The prepulse stimulus preceded the startle stimulus by 100 ms. A Med Associates startle measuring system was used to process and present the stimuli and record the startle responses. A speaker was mounted in the ceiling of a sound attenuated box. Beneath the speaker, the mouse was placed in a 32 ounce plastic cup placed on a movement-sensitive load cell to measure the mouse's movements.

Procedures

PPI testing began after one week of AAE exposure. For the first month, mice (exposed and non-exposed groups) were tested once per week. Thereafter, both groups were tested once per month for four months and once again when the mice were 9 months old. Each test included two runs. Each run consisted of 40 trials to test PPI. Trials consisting of startle stimulus only and trials consisting of the prepulse stimulus paired with the startle stimulus were presented in random order. For each session, the mean PPI for each frequency was calculated as a percent reduction of the startle amplitude with respect to that produced by the startle stimulus only.

Statistics

The PPI data were analyzed using separate 3-way mixed analyses of variance (ANOVA) for each prepulse frequency condition. For each ANOVA, age (1 month, 2 month and 9 month) was a repeated measure and environment condition (AAE, control) and gender (male, female) were between-groups measures. The alpha level was 0.05.

Results

The pattern of age-related change in PPI was different for each prepulse frequency. As shown in Figure 1, PPI improves with age for the 4 kHz prepulse, particularly for the female mice. This was true for both the AAE exposed group and the control group. These observations are supported by the statistical analysis. The main effect of age ($F(2,52) = 11.97$; $p < 0.0001$) and the interaction between gender and age ($F(2,52) = 3.76$; $p < 0.0297$) were statistically significant. Tukey post-hoc analyses revealed that PPI at 1 month was significantly poorer than PPI at 2 or 9 months ($p < 0.0001$) but PPI at 2 and 9 months did not differ significantly ($p > 0.05$). Also, the PPI of female mice was poorer than that of male mice at 2 months but the reverse was true at 9 months ($p < 0.05$).

For the 12 kHz prepulse (Figure 2), PPI appears similar across age and environment groups. None of the main effects or the interactions was statistically significant ($p > 0.05$).

For the 20 kHz prepulse (Figure 3), PPI appears to worsen with age for mice of both genders, however the PPI of the female mice is poorer than that of the male mice. There are no apparent differences in PPI between the AAE and control groups for either gender. The main effects of age ($F(2,52) = 5.26$; $p < 0.0083$) and gender ($F(1,26) = 4.79$; $p< 0.0377$) were statistically significant. Tukey post-hoc analyses revealed that the PPI of the female mice was poorer than that of the male mice ($p = 0.037$) and PPI was poorer at 9 months than at 2 months ($p = 0.005$). The PPI at 1 month and 2 months did not differ, nor did PPI at 1 month and 9 months ($p > 0.05$)

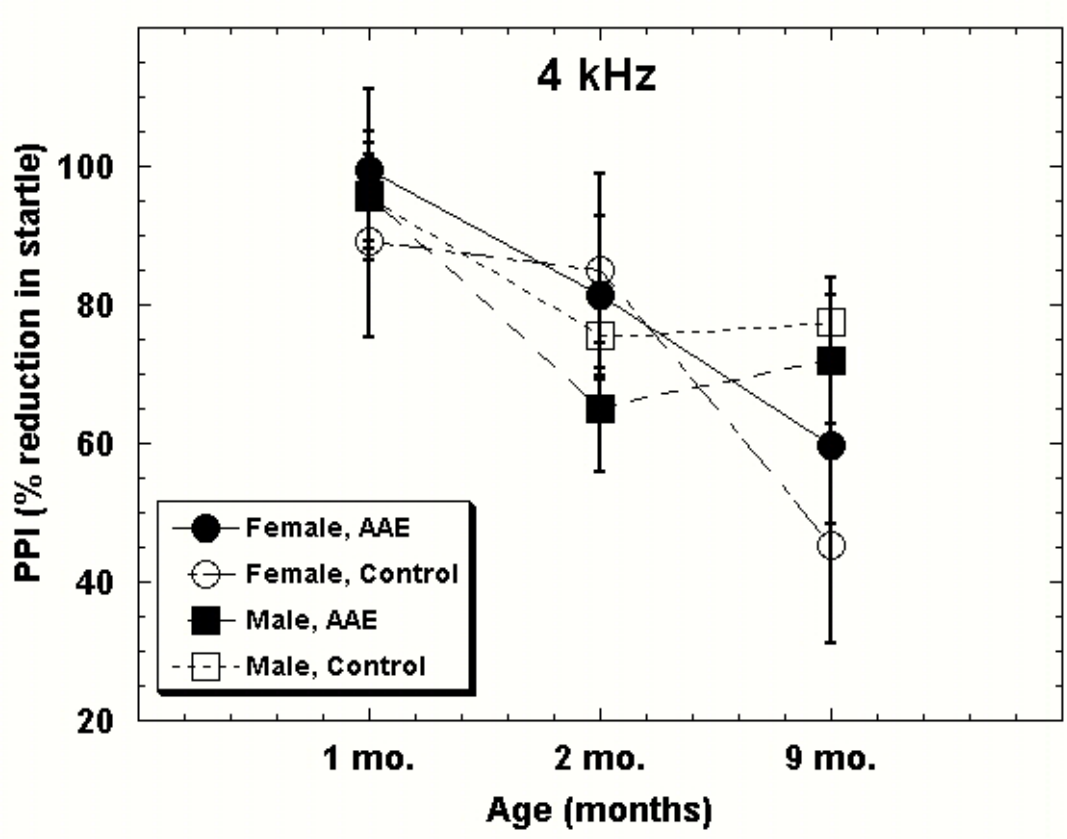

Figure 1. PPI amplitude for the 4 kHz prepulse stimuli across age. Filled circles represent the female C57 mice exposed to the AAE. Open circles represent the female C57 mice not exposed to the AAE. Filled squares represent the male C57 mice exposed to the AAE. Open squares represent the male C57 mice not exposed to the AAE. Standard error bars are shown for each group.

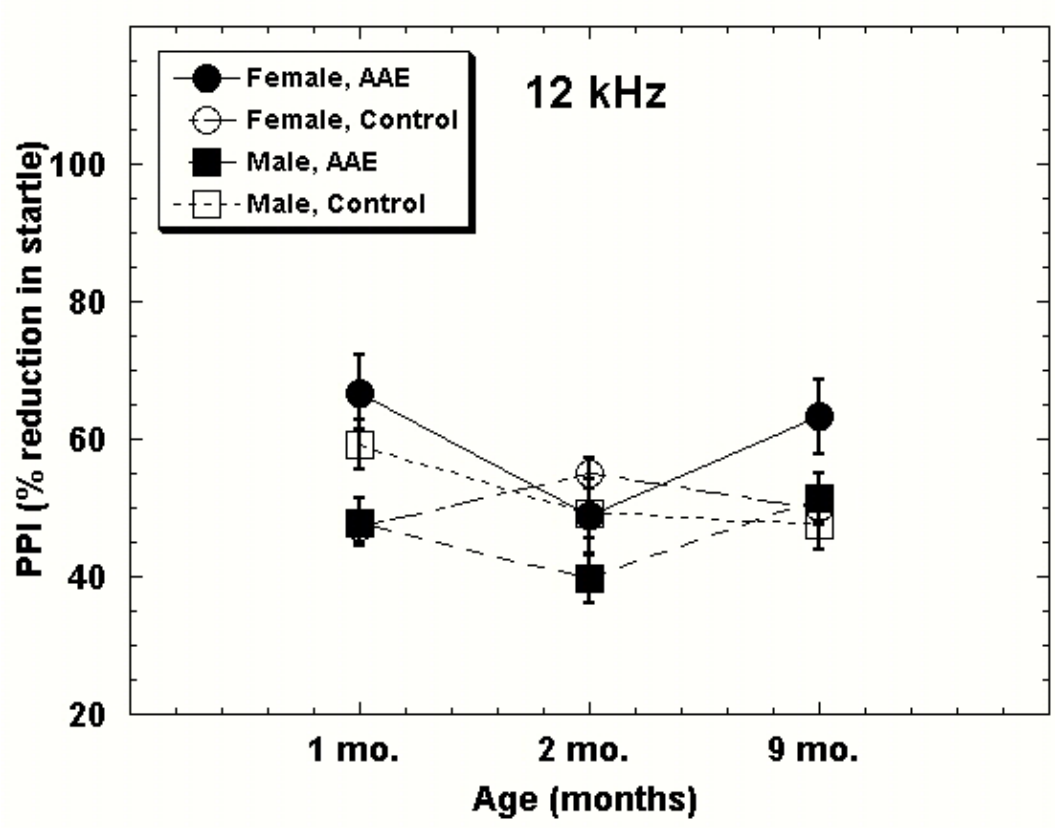

Figure 2. PPI amplitude for the 12 kHz prepulse stimuli across age. Symbols and legend as shown in Figure 1.

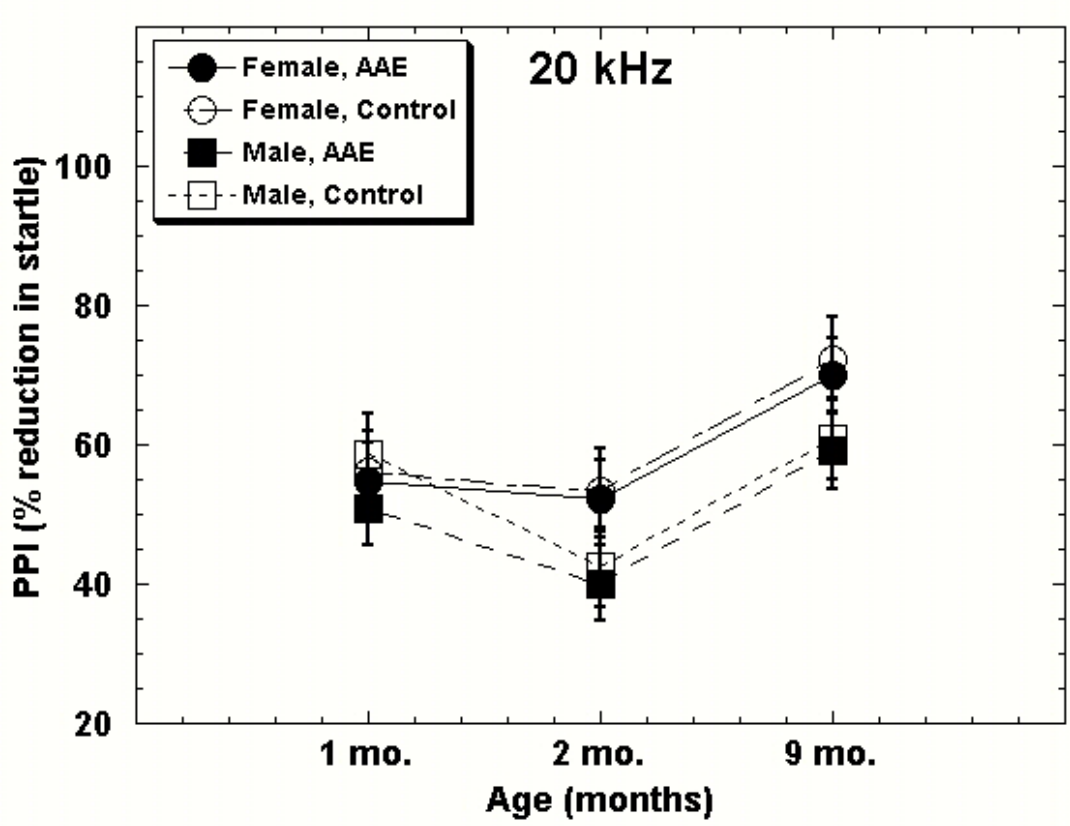

Figure 3. PPI amplitude for the 20 kHz prepulse stimuli across age. Symbols and legend as shown in Figure 1.

To summarize, PPI changed across age, but the AAE had no significant effect on PPI. Gender differences were present, but these interacted with prepulse frequency. Females (both AAE and control) showed greater improvement of PPI with age for the 4 kHz prepulse as compared to males. By contrast, females (AAE and control) exhibited weaker PPI for the 20 kHz prepulse than males. PPI with the 12 kHz prepulse was unaffected by age, gender, or AAE.

Discussion

Previous studies have shown that PPI with a 4 kHz prepulse improves with age in C57 mice, as found in the present study (Willott et al., 1994; Willott & Turner, 2000). Gender differences were not analyzed, however. The improvement probably reflects central auditory plasticity previously demonstrated in this strain. As high frequency sensitivity is lost, the auditory salience of low frequencies has been shown to improve (Jeskey & Willott, 2000; Willott & Turner, 2000; Willott & Carlson, 1995; Willott, 1984). Worsening of PPI using a high-frequency prepulse, shown here with 20 kHz, was also observed in the earlier studies (Willott et al., 1994; Willott & Turner, 2000), and presumably reflects the loss of sensitivity to high frequencies. The improvement seen in the salience of the 4 kHz prepulse for female mice, coupled with the poorer PPI for the higher (20 kHz) prepulse frequency suggests that the female mice suffered more high frequency hearing loss (and resultant plasticity for 4 kHz) than did the male mice.

Whereas age and gender effects were found in the present study, the results do not support the hypothesis that early acoustic stimulation affects auditory development. Even immediately following exposure (1 month of age), AAE-exposed and control mice had similar PPI. Of course, it is possible that an effect might be demonstrable using exposure parameters different from those employed here. It is possible that the testing method used (i.e., PPI) was not the appropriate testing method; therefore, another testing method, such as the auditory brainstem response may have a higher sensitivity.

The question still remains: does the NICU environment affect the auditory development of pre-term infants? The traditional American NICU environment consists of patterned light and continuous sound. The sounds are airborne, intense, continuous, and cover a wide range of

frequencies. These sounds are generally lacking in pattern and rhythm, unlike the sounds experienced in utero (for review see Philbin, 2000). Typical NICU sound levels vary from 50 to 75 dBA with peaks of 105 dBA and frequent prolonged sounds in the 70-80 dBA range. Because conversational speech falls between 60 and 75 dBA, maternal speech is often masked and rendered less intelligible by NICU sounds (for review see Morris, Philbin & Bose, 2000). Thus, it is possible that the pre-term infant may experience a disrupted pattern of auditory learning due to the auditory deprivation caused by the masking (League, Parker, Robertson, Valentine & Powell, 1972; Peltzman, Kitterman, Ostwald, Manchester, & Heath, 1970).

In contrast, the fetus in the womb is exposed to low frequency and temporal aspects of speech (e.g., intonation, stress, rhythm), but not light. Prenatal animal research has shown that early visual experience can alter postnatal perception in the visual modality as well as earlier developing sensory systems (e.g., olfactory and auditory). Studies of precocial birds showed that embryos do not exhibit prenatal auditory learning when the maternal call is presented simultaneously with visual experience. This suggests that early exposure or deprivation of normal or typical sensory stimulation can have negative effects on auditory development (Lickliter, 2000).

In an attempt to determine the effects of early acoustic stimulation on auditory development using C57 mice exposed to an AAE, the analyzed data indicated that early acoustic stimulation of the auditory systems of C57 mice does not affect the auditory processing of tonal stimuli in three frequency regions (4k Hz, 12k Hz and 20k Hz). It is possible that it is the early visual stimulation that disrupts the auditory development of premature human infants in the NICU and not the early exposure to instrument noise. These results are supportive of such a hypothesis. Of course, the results illustrate the need for more research within the area of auditory

development of premature infants in the NICU, such as, the synergism effects of noise, drugs, oxygen, low-birth weight and early visual stimulation.

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