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THE EFFECT OF NEONATAL INTENSIVE CARE UNIT
NOISE ON THE HABITUATION OF
NEONATAL CHICKS

Diane D. Ballweg, Capt, USAF, NC
1991
MSN Degree
Thesis 91 pages
The University of Texas Health Science Center at Houston
THE EFFECT OF NEONATAL INTENSIVE CARE UNIT
NOISE ON THE HABITUATION OF
NEONATAL CHICKS

By
Diane Doty Ballweg, Capt, USAF, NC

A Thesis
submitted in partial fulfillment
of the requirements for the degree of
Master of Science in Nursing
The University of Texas Health Science Center at Houston
School of Nursing
May, 1991
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THE EFFECT OF NEONATAL INTENSIVE CARE UNIT NOISE ON THE HABITUATION OF NEONATAL CHICKS

By

Diane Doty Ballweg, Capt, USAF, NC

APPROVED:

[Signatures]

vi
December 4, 1990

Mrs. Diane Ballweg, R.N.
School of Nursing
Student Affairs
HMB 5.526

Dear Diane,

It is my opinion that the essential elements of research you have proposed for your Master's Degree in Nursing, have already been reviewed and approved by the Health Science Center Animal Welfare Committee as part of my ongoing research (AWC-MS-86-207).

Rearing of the chickens in the small chambers has been approved. Our incubator room is an approved satellite animal care facility, and is inspected at regular intervals. Rearing of animals with exposure to sound and the testing procedures have been previously approved.

Although your research includes some theoretically important experimental manipulations, I do not believe that these minor changes make any difference to issues about the care and use of animals considered by the animal welfare committee. You propose to present noise recorded in the Neonatal Intensive Care Unit to chicks at a level that is equivalent to that experienced by human patients. (I have been previously approved to present pure tones at a similar level). You propose a slightly different stimulus (a single one-second burst of white noise rather than pulsing pure tones), and you will record the data in a slightly different way (measuring the time it takes for the chicks to return to a baseline rate of peeping, rather than just measuring the time to their second post-stimulus peep).

I think your project sets the stage for the extension of my research program beyond basic normal studies of hearing development in baby chickens. It is important to investigate the effects of the abnormal conditions experienced by increasingly large numbers of human neonates on measures of attentional and perceptual processing that are similar to those routinely made on humans.

Sincerely,

Lincoln Gray, Principle Investigator

LG/cp

xc: Animal Welfare Committee
ACKNOWLEDGEMENTS

I would like to acknowledge the many people who assisted in the completion of this thesis. I would like to thank my thesis committee for their continuous support and guidance: First of all, M. Kathleen Philbin, R.N., Ph.D. for her unyielding encouragement for excellence, her editorial expertise, and her genuine concern for the optimal development of the premature neonate. Secondly, Miguel da Cunha, Ph.D. for his editorial guidance and encouragement. Thirdly, Paul Femea, R.N., D.N.Sc. for his statistical knowledge and reassuring words. And last but definitely not least, Lincoln Gray, Ph.D. who not only provided laboratory facilities, equipment, and chicks, but also served as research consultant and Fourth Reader. His generosity and patience made this study possible.

I would also like to thank my family and friends (you know who you are) for their endless understanding and support throughout graduate school and my career as a nurse and an Air Force officer. (Yes, Evan, you can use the computer now).
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ABSTRACT

Prematurely born human neonates may experience weeks, even months, of auditory stimulation different from that known to exist in the uterus. Unlike full term neonates, many preterms do not demonstrate a consistent response decrement (i.e., habituation) to repeated stimuli. Habituation is regarded as an indicator of central nervous system competence.

The purpose of this experiment was to investigate the effect of Neonatal Intensive Care Unit (NICU) noise on the ability of neonatal chicks to habituate. Use of an animal model allowed for examination of this effect in isolation of confounding variables encountered in the population of sick preterm neonates. Neonatal human and chick auditory development is similar in both structural sequence and function at birth.

The effects of age, auditory experience, and test condition were tested in a two-by-two-by-two blocked design. Results indicated that 4-day-old chicks exposed to NICU noise failed to habituate to a white noise stimulus. These findings support recommendations for reducing auditory stimulation in the NICU. Although the results of animal research cannot be directly applied to the human situation, such research can indicate areas of potential risk meriting further investigation.
Chapter 1

The auditory environment of the neonatal intensive care unit (NICU) differs greatly from that in utero. Premature infants residing in hospital nurseries are exposed to mechanical and speech sounds varying in intensity, pitch, regularity, and complexity. In utero, the fetus is exposed to the rhythmic, low intensity, low frequency sounds of the maternal cardiovascular system and digestive processes (Bench, 1968; Walker, Grimwade, & Wood, 1971). Additionally, amniotic fluid, maternal tissues, and maternal physiologic sounds attenuate all other extraterine sounds. Thus, the intense, erratic auditory environment of the prematurly born infant is quite unlike the rhythmic, less intense, low pitched, diurnal uterine environment in which the term infant develops.

The status of audition at various stages of gestation is only partially understood. The fetus can hear and respond to auditory stimuli by 25-26 weeks of gestation (Birnholz & Benacerraf, 1983). Thereafter, a period of accelerated brain growth occurs between 26 and 40 weeks of gestation. The neural system which integrates auditory stimuli and neuromotor responsiveness develops during this period along with other neurosensory systems. Unlike the full term baby, the premature infant does not
complete this rapid growth phase prior to entering the extrauterine sound environment (Parmelee & Sigman, 1983).

It has been observed that preterm infants are more reactive to stimulation than are term infants. Healthy full term neonates, for example, consistently habituate to auditory stimuli (Brazelton, 1962). That is, they demonstrate a motor response decrement to a repeated auditory stimulus. Many preterm infants, in contrast, respond for longer periods and with less regular response decrement to the same stimulus (Als, Lester, & Brazelton, 1979).

The highly reactive preterm infant lives in an intense, irregular auditory environment. The effect of early exposure to some ontogenetically inappropriate conditions on the immature auditory system has been studied. Ambient NICU noise and incubator noise combined with other variables such as aminoglycoside use and hyperbilirubinemia have been shown to cause hearing loss (Abramovich, Gregory, Slemick, & Stewart, 1979). NICU noise has also been correlated negatively with sleep and oxygenation and positively with intracranial pressure (Gadeke, Doring, & Keller, 1969; Long, Lucey, & Philip, 1980). The study described here investigated the effect of early NICU noise exposure on habituation to sound. In order to examine this topic in isolation of confounding
variables encountered in the sick preterm, this study looked at the effect of NICU noise exposure on the habituation of neonatal chicks.

The development of audition in neonatal chickens and humans is similar (Rubel, 1978). For example, middle ear functional development follows an identical pattern of differentiation outward from the mid-basal region in both the chicken and the human (Rubel, 1978, 1984). Also, the ability to hear low frequency sounds develops initially in both species, followed by the ability to hear higher frequencies.

Both humans and chickens habituate to perceived auditory stimuli. Full term human infants demonstrate a motor response decrement to a repeated stimulus (Brazelton, 1962). For example, upon the initial presentation of an auditory stimulus such as a small, ringing bell, the sleeping, healthy term infant may move his arms and legs, make facial movements, or even startle. The newborn's motor response to repetitions of this stimulus decreases with each presentation and typically ceases altogether by the 10th stimulus (Brazelton, 1984). Neonatal chicks peep almost constantly but pause when a new auditory stimulus presents (Kerr, Ostapoff, & Rubel, 1979). After repeated stimulus presentation, the chicks no longer pause between peeps; they habituate to the
stimulus (Gray, 1987). This study took advantage of this response characteristic in order to investigate the effect of early atypical sound exposure on the ability of neonatal chicks to habituate at term.

Purpose

The purpose of this study was to test the following hypothesis: Neonatal chicks exposed to constant NICU noise beginning at post-set day 16 will have significantly altered habituation to white noise stimuli compared with chicks similarly reared but not exposed to NICU noise.

Theoretical Framework

The theoretical framework for this study was the model of neural plasticity presented by Wiesel and Hubel (1963, 1965). This model emphasizes the role of the environment in perceptual development. In their research with kittens, Wiesel and Hubel sutured one eyelid of the animals shut at birth. When the sutures were removed 16 weeks later, the kittens were blind in the eye which had been deprived of environmental stimuli; vision was normal in the nonsutured eye. The sutured eyes and the animals' brains were normal in all other respects. By contrast, the vision of adult cats was not affected by prolonged unilateral eye closure. Thus, Wiesel and Hubel established the existence of a critical period in early
development during which perception is affected by early experience.

Further investigations of neural plasticity have demonstrated neural changes following environmental overstimulation rather than deprivation. Spinelli and Jensen (1979) studied the effect of non-painful electric shock and simultaneous exposure to novel visual stimuli on the formation of brain cells and neuronal pathways in otherwise normally-reared kittens. Neuronal changes were seen in the animals receiving the additional, atypical stimulation.

It is believed that results from visual research are applicable to other physiologic systems including auditory and motor systems (Duffy, Mower, Jensen, & Als, 1984). Subsequent animal research has substantiated these findings in other animal species regarding auditory perception as well as visual perception. Rearing of animals in an abnormally quiet environment following monaural or binaural occlusion alters auditory neuronal structure and function (Gray, Smith, & Rubel, 1982; Rubel, 1984). The impact of the environment on development has also been applied to human neonatal developmental theory (Duffy & Als, 1983, 1988).
Hypothesis

Neonatal chicks exposed to constant NICU noise beginning at post-set day 16 will have significantly altered habituation to white noise stimuli compared with chicks similarly reared but not exposed to NICU noise.

Definition of Terms

NICU Noise

**Conceptual Definition.** Naturally occurring or ambient sounds of any kind heard during representative periods in the neonatal intensive care unit including speech and mechanical sounds.

**Operational Definition.** Sounds recorded in a level III NICU in southeast Texas using a tape recorder with the microphone placed near the head of a newborn lying on a radiant warmer. Recordings were made over several visits and edited into a one hour tape representative of noise in that NICU. Recording level settings were verified at the onset of each recording session by a 94 dB national standard calibration tone (see methodology section).

Habituation

**Conceptual Definition.** The ability to perceive repeating stimuli as similar and, consequently, to decrease responses to subsequent repetitions.
Operational Definition. For the purposes of this study two operational definitions of habituation were used:

1. Rate Resumption (RR). Habituation was defined as the decrement in time to resume a baseline or pre-stimulus peep rate over 16 presentations of a white noise stimulus (rate resumption). Based on pilot studies, the baseline peep rate was set at 12 peeps per 10 seconds for 0-day old chicks and 16 peeps per 10 seconds for 4-day olds.

2. Peep Pause (PP). Habituation was also defined as the decrement in magnitude of the initial response to the onset of successive stimuli. This initial response was determined by measuring the delay of peeping beginning with the onset of the white noise stimulus and ending with the offset of a subject's second peep.

White Noise Stimulus

Conceptual Definition. Noise containing all frequencies of sound.

Operational Definition. A 1 second burst of noise containing all frequencies of sound repeated for 16 trials at 58 decibels (dB) to 0-day old chicks and at 54 dB to 4-day old chicks.

Neonatal Chick

Conceptual Definition. Newly hatched chickens.
Operational Definition. Domestic chickens (*Gallus gallus*, white leghorn strain) at 0 and 4 days post-hatch.

Significance of Problem

The ability of an infant to perceive repeated and novel stimuli and to habituate to that which is repeated has significance for both the immediate health status and future learning of the individual. The healthy neonate who is able to shut out repeated stimuli is likely to be able to sleep well and to use calories taken in from feedings for growth (Brazelton, 1962). Habituation enables the awake baby to focus attention on novel stimuli while disregarding the familiar or irrelevant. This ability is believed to enhance later learning (Miller et al., 1977).

Premature infants do not demonstrate consistent habituation (Als, Lester, & Brazelton, 1979). Rather, they are motorically and attentionally aroused by repeated stimuli. Effects of a limited ability to habituate include expending calories needed for growth on motoric activity, arousal from sleep states, and cardiorespiratory stress. Because many preterm infants present at later ages with attentional deficits affecting school performance, there is concern among clinicians that a causal relationship exists between early altered habituation and these later attentional difficulties.
(Holwerda-Kuipers, 1987; Miller et al., 1977). The nature of this relationship has not, however, been established empirically.

Early exposure to an altered environment has been shown to affect later perception (Wiesel & Hubel, 1963, 1965). As the hospitalized preterm infant lives in an ontogenetically inappropriate environment, the impact of these surroundings on perception warrants investigation.

Most sources of NICU noise are felt to be under the control of nursery personnel (Long et al., 1980). Conversation, laughter, trash disposal, closing doors and drawers, playing radios, ringing telephones, and moving equipment all generate noises which can be reduced by the hospital staff. If research demonstrates an effect of long-term NICU noise exposure on habituation, nursery personnel may be persuaded to control noise levels.

This study examines the effect of early inappropriate auditory stimuli on habituation in neonatal chicks. Demonstrated parallels exist between chicks and human infants, including the ability to habituate to auditory stimuli (Brazelton, 1984; Rubel, 1978). Although the results of animal studies cannot be directly applied to the human situation, animal research can indicate areas of potential risk for humans and provide direction for future human studies.
Assumptions

1. Because minimal variability exists among normally reared, individual chick subjects, a sample of 10 chicks was sufficient for a reliable group sample size.

2. One hour of recorded NICU noise represents a typical noise sample from a particular hospital in southeast Texas. A previous study of sound characteristics in this nursery indicated little variability in sound levels from hour to hour and between days (Gray, Deskins, Adcock, & Philbin, 1986).

Limitations

1. The exact correspondence between human and avian neonates' auditory development and acuity is not known. Therefore, the sound environment presented to the developing avian neonates may not have been equivalent in volume or most acutely perceived tone ranges to the sound environment experienced by human neonates.

2. Due to the constraints of programming a computer to document peeps, habituation may actually have occurred earlier during a given set of trials than the data indicated. The computer program tabulated the number of peeps within sequential 10 second intervals. The program did not tabulate peeps occurring across the boundaries of these 10 second intervals. It is possible, therefore, that the habituation criteria of 12 or 16 peeps
within 10 seconds may have been met by the chick but occurred across two successive intervals. Assuming that the chick maintained the criterion rate, it was picked up by the computer in a 10 second interval later than when it occurred.

3. The results of animal studies cannot be applied directly to understanding a parallel human condition.

4. Response to a white noise stimulus is not well studied. Unrecognized phenomena may be at work in the chick's response to this stimulus.
Chapter 2

REVIEW OF THE LITERATURE

Advances in medical technology have enhanced the survival of increasingly premature infants. Consequently, very early born neonates spend weeks, even months, in an auditory environment unlike that in utero. It is frequently observed that these preterm infants do not consistently demonstrate a response decrement to repeated auditory and other stimuli. The objective of this study was to investigate the effect of this early atypical sound exposure on the ability to habituate. An animal model was chosen to study the habituation of neonates exposed to continuous NICU noise with that of neonates not exposed to NICU noise. Use of an animal model allows for the examination of this topic in isolation of confounding variables encountered in the population of sick preterm neonates.

The literature reviewed for this investigation includes studies of sound levels in utero and in NICUs, the effect of NICU noise on the premature infant, neonatal habituation to auditory stimuli, and the possible relationship between altered habituation and later learning. The relevance of these topics to the human neonate will be addressed first. A discussion of information applicable to neonatal chicks will follow.
Sound Levels

Intrauterine

The intrauterine auditory environment differs from that of the NICU. In order to investigate the auditory environment of the fetus, Bench (1968) placed a microphone in the vagina near the cervix of a pregnant woman at 37 weeks of gestation. The mean internal sound level of 72 dB was attributed to uterine blood flow. A loudspeaker placed against the mother's abdomen generated sounds of 200 to 4000 Hz at 120 dB. All sounds external to the mother's uterus were attenuated by maternal tissues and amniotic fluid. The quantity of attenuation was positively correlated with sound frequency. Thus, the frequency of intrauterine sounds remained consistently low and at an intensity near 72 dB.

Walker, Grimwade, and Wood (1971) studied intrauterine sound in 16 pregnant women at term. A microphone was placed inside the uterus near the fetal head before and after rupture of the membranes. Neither sound intensity nor frequency were significantly affected by membrane rupture. The mean sound level of 85 dB SPL was attributed to the rhythmic, low frequency, turbulent blood flow in the vessels of the gravid uterus. This difference in intensity from that found by Bench (1968) was proposed to be due to the different positioning of the microphone.
The authors did not state whether the intensity level was measured on an A-weighted or linear scale.

To further investigate the quality of external sound reaching the fetus, Walker et al. (1971) played sounds of 100 to 3,000 Hz from a loudspeaker placed on the mother's umbilicus. Intrauterine sound intensities and frequencies were then measured via the microphone near the fetal head. As with the previous study (Bench, 1968), results showed that sounds external to the mother were attenuated by maternal tissues and amniotic fluid. The degree of attenuation increased as the sound frequency increased. The authors concluded that except for extreme situations (standing near aircraft or trains), all external sounds are masked by maternal sounds.

**Neonatal Intensive Care Unit**

Numerous studies have investigated sound intensity in neonatal intensive care units. Mean ambient sound levels range from 51 to 77 A-weighted decibels with peak values as high as 90 to 122.5 dB(A) (Anagnostakis & Messanitis, 1990; Anagnostakis, Petmezakis, Messanitis, & Matsaniotis, 1980; Gadeke et al., 1969; Gottfried, Wallace-Lande, Sherman-Brown, King, & Coen, 1981; Gray et al., 1986; Long et al., 1980). Sound level ranges of specific equipment, activities, and intensity levels inside incubators have been documented (Hilton,
These measurements of sound in the NICU show little variability from day to night allowing for no quiet time and no diurnal auditory pattern (Gottfried et al., 1981; Gray et al., 1986).

Most sources of NICU noise are under the control of nursery staff (Long et al., 1980; Thomas, 1989). Long et al. measured noise levels in an NICU noting those events causing increased intensity. These included conversation, laughter, trash disposal, closing doors and drawers, playing radios, ringing telephones, and moving equipment. After nursery personnel were asked to minimize or eliminate noisy activities, base line noise levels and sudden noise deflections decreased.

A descriptive study of activities in the NICU identified numerous sources of intense sound (Thomas, 1989). Actions such as bumping a metal waste basket and placing a plastic feeding bottle on top of an isolette produced sound levels of 85 to 117 dB(A) inside the incubator. The author states that noises such as these can be decreased, if not eliminated, by the staff.

Effect of Noise

The effects of noise are numerous and diverse. In 1859 Nightingale (1954) identified noise as a source of injury for the hospitalized patient:
Unnecessary noise, or noise that creates an expectation in the mind, is that which hurts a patient... But intermittent noise, or sudden and sharp noise, in these as in all other cases, affects far more than continuous noise - noise with jar far more than noise without... Unnecessary noise, then, is the most cruel absence of care which can be inflicted either on sick or well (p.149, 150, 152).

Nightingale described sources of unnecessary noise as including physician discussions, whispering personnel, and the rustling dresses of females.

Studies investigating the effects of noise exposure initially examined hearing loss. More recently, research has focused on central nervous system difficulties in processing auditory information.

**Hearing Loss**

**Safety standards.**

Sound level standards have been established to prevent hearing loss in adults but not infants. Occupational Safety and Health Administration (OSHA) regulations prohibit the exposure of working adults to levels greater than 90 dB for longer than 8 hours (Industrial Acoustics Company, 1982). Based upon regulations for adults and animal research, the American
Academy of Pediatrics and the American College of Obstetricians and Gynecologists (1988) recommend that nursery sound levels be evaluated periodically and be maintained below 75 dB.

Although average noise levels in NICUs do not exceed standards established to prevent hearing loss in adults, animal studies suggest that young animals are more vulnerable to auditory damage. Investigators have demonstrated the existence of a developmental period of increased sensitivity to acoustic trauma (Bock & Saunders, 1977; Douek, Dodson, Bannister, & Ashcroft, 1976; Falk, Cook, Haseman, & Sanders, 1974; Lenoir & Pujol, 1980; Price, 1976).

Falk et al. (1974) exposed guinea pigs to 30 continuous hours of 119-120 dBSPL white noise. Significantly greater cochlea damage was found in 2-day and 8-day-old subjects than in 8-month-old guinea pigs \( p < .01 \). Douek et al. (1976) subjected 1-week-old and adult guinea pigs to 7 days of continuous incubator noise up to 80 dBSPL. Young subjects receiving the noise had a significantly greater loss of cochlear outer hair cells than did control neonates \( p < .01 \). The percentage of hair cell loss in adults exposed to the noise was not significantly different from adult control subjects. This difference in auditory damage between adults and neonates
exposed to identical sources of noise has also been demonstrated in rats, hamsters, and cats (Bock & Saunders, 1977; Lenoir & Pujol, 1980; Price, 1976).

**Incidence.**

While the incidence of hearing loss is 0.1% in healthy term newborns (Simmons, 1980), 9% to 12.4% of all low birth weight infants develop a hearing deficit (Abramovich et al., 1979; Anagnostakis, Petmazakis, Papazissis, Messaritakis, & Matsaniotis, 1982; Stennert, Schulte, Vollrath, Brunner, & Frauenrath, 1978). In follow-up studies of infants failing auditory brain response (ABR) screenings upon NICU discharge, 2% to 3.6% demonstrated severe bilateral hearing loss (Cox, Hack, & Metz, 1981; Jacobson, Seitz, Mencher, & Parrott, 1981; Roberts et al., 1982).

**Etiology of hearing loss.**

In an attempt to determine the exact etiology of hearing loss in NICU infants, investigators have examined its association with NICU noise, perinatal complications, and ototoxic drug use. Anagnostakis et al. (1982) investigated the incidence of hearing loss in six year olds weighing less than 1800 g at birth. Of the 98 children, 9% had a hearing loss. Exudative otitis media was found in 14 of the subjects. Hearing loss due to ear infections disappeared after appropriate treatment. Using
chi-square analysis, mean birth weight, mean gestational age, mean length of incubator care, and use of ototoxic drugs were not significantly different for those with and without an auditory deficit. The duration of the infants' ototoxic medication regimens, however, was not addressed. Complications demonstrating a significant difference between the two groups were apneic spells with bradycardia and cyanosis, hyperbilirubinemia greater than 14 mg/dL, and hypothermia (rectal temperature less than 34.5°C) on admission to the NICU. Despite the lack of significance of the length of incubator care, the authors caution that the effect of noise can not be discounted. Loud equipment such as cardiac monitors and air compressors were not routinely used during the time these subjects were patients.

Abramovich et al. (1979) studied hearing loss in six year olds weighing less than 1500 g at birth. As with the previous study, 9% of the subjects had a hearing deficit. Also, mean birth weight, mean gestational age, mean length of incubator care, and use of ototoxic drugs were not significantly different between the hearing loss and non-hearing deficit groups. Using analysis of variance with stepwise regression, the incidence of apneic episodes was the only variable to significantly contribute to hearing loss (P < .05). Hyperbilirubinemia greater
than 10 mg/100ml made a significant addition. The authors conclude that hypoxia is the major etiologic factor in hearing loss with hyperbilirubinemia exerting a synergistic effect.

A combined effect of environmental noise with perinatal factors has been correlated with hearing damage in premature infants. Stennert et al. (1978) found hearing deficits in 12.4% of 193 infants between 28 and 36 weeks gestation. Hyperbilirubinemia, ototoxic drug use, gestational age, and noise exposure, when considered individually, were not correlated with auditory damage. The combination of perinatal risk factors including asphyxia and prematurity were correlated ($r=0.42$) with hearing loss. The authors conclude that long term noise exposure acts synergistically to affect hearing.

The overall conclusion suggested by these investigators is that NICU noise in combination with other perinatal factors affects neonatal auditory function. NICU noise alone does not appear to show such an effect. However, the atypical auditory environment of the NICU alters sleep states, other physiologic parameters, and possibly habituation (see below).

**Sleep States**

Intense noise interferes with sleep in the newborn. Gadeke et al. (1969) found that sounds of
100-7000 Hz played at levels of 70 to 75 dB for 3 minutes awakened 66% of sleeping fullterm infants. Those newborns not completely aroused shifted from deep sleep to light sleep. Identical sound frequencies played at 75 dB for 12 minutes awakened all 126 subjects.

Mann, Haddow, Stokes, Goodley, and Rutter (1986) examined the effect of a diurnal pattern of noise and light on the sleep and weight gain of 41 premature infants. Subjects were randomly assigned to one of two nurseries: (a) the control nursery where noise and light remained constant and (b) the "day/night" nursery where both noise and light were decreased from 7 p.m. to 7 a.m. The infants stayed at least 10 days and were discharged from the assigned nursery. In comparing the two groups, no significant differences were found with respect to gestation, birth weight, the amount of intensive care received, or the weights and ages on admission to the study. Using a t-test analysis, the infants in the "day/night" nursery slept longer, spent less time feeding, and gained more weight than those in the control nursery. However, these differences were not significant upon discharge. Time spent sleeping was significant post-discharge at the infants' expected delivery dates (p < .05), six weeks later than expected delivery (p < .01), and three months following expected delivery (p < .005).
The decrease in the number of hours required to take in the recommended amount of infant formula or breast milk per day became significant at three months after discharge \((p < .02)\). Weight gain was significant six weeks \((p < .05)\) and three months \((p < .02)\) after discharge. The authors conclude that premature infants should not be exposed to constant noise and light as the nursery environment appears to have long term effects.

**Pain Perception**

Although, historically, the infant was viewed as being incapable of feeling pain, current evidence indicates otherwise. The nervous system of the fetus and the newborn is known to be able to transmit and perceive pain (Anand, & Hickey, 1987). Average noise levels of 50 to 60 dB enhance pain perception in adults (Minckley, 1968). A study of 644 recovery room patients showed that the number of requests for pain medication increased as the noise level in the unit rose. A similar increase in pain perception following elevated noise levels may also occur in neonates.

**Other Physiologic Parameters**

Noise leads to increased heart and respiratory rates, hypoxemia, increased intracranial pressure, metabolic disturbances, and vasoconstriction (Long et al., 1980; Ciesielski, Kopka, & Kidawa, 1980). Long et al.
recorded the heart rate, respiratory rate, transcutaneous oxygen tension (TcPo2), and intracranial pressure (ICP) of two 7-day-old infants of 34-35 weeks gestation at birth. Following acute intense noises such as the closing of isolette portholes or a portable x-ray machine hitting the side of an incubator, the infants became agitated and began crying. A decrease in TcPo2 levels was followed by an elevation of ICP after 66% of the sudden noise episodes. At other times, ICP rose without any change in TcPo2. Increases in heart rate and respiratory rate were documented after all acute noises.

Furthermore, apnea, which is associated with hypoxemia, occurs more frequently in light sleep (Gabriel, Albani, & Schulte, 1976). As previously discussed, infants in noisy environments spend a greater portion of their sleep time in a light sleep state (Gadeke et al., 1969).

In a study by Ciesielski et al. (1980), continuous noise exposure over two weeks caused carbohydrate, fat and protein-enzymatic disturbances in guinea pigs. Blood sugar levels and proteins lowered while blood lipid levels increased.

Sounds greater than 70 dB linear result in a vasoconstrictive response throughout the cardiovascular system (Ciesielski et al., 1980). As the dB intensity
increases, vasoconstriction also increases in a linear relationship. The vasoconstriction does not decrease with repeated noise exposure.

**Developmental Effect of Noise Reduction**

Reducing sensory overload in the NICU enhances the medical and developmental outcome of premature infants (Als et al., 1986). A group of very low birthweight infants with bronchopulmonary dysplasia were shielded from light and noise and given individualized behavioral nursing care (e.g., protection from being roused from sleep states and handling tailored to individual infants' indicators of stress). These infants averaged 25 fewer days on ventilator therapy, needed less supplemental oxygen, established complete bottle or breastfeeding an average of 29 days earlier, and scored higher on behavioral and developmental exams than did matched control infants. Upon reevaluation at 3 years of age, those infants exposed to the less stressful environment continued to perform better on developmental and behavioral tests. These results must, however, be interpreted in the absence of information regarding advances in medical care which occurred during the study and which could have affected the subjects' outcomes.
Habituation

Harris (1943) defined habituation to be "response decrement as a result of repeated stimulation" (p. 385). This widely accepted definition excludes decreasing responses due to receptor, effector, or general organism fatigue (Harris, 1943; Thompson & Spencer, 1966). In these cases, response decrement occurs because of a progressive physiologic inability to respond. Habituation allows the organism to stop responding to irrelevant stimuli, enabling the organism to conserve energy (Lipsitt, 1986). The presence or absence of habituation reflects the functional status of the central nervous system (Brazelton, 1973; Prechtl & Bientema, 1964).

Although several models of habituation have been proposed, the two most frequently cited are the neurochemistry view of Thompson and Spencer (1966) and the learning theory approach of Sokolov (1963). Based on their research with spinal cats (i.e., decerebrate cats with spinal transections at the 12th vertebra), Thompson and Spencer (1966) suggested that habituation results from synaptic depression. Following repeated electrical shocks, habituation of the hindlimb flexion reflex of the spinal cat occurred between the afferent input terminals and the output motor neurons in the spinal cord. The authors conclude that the process of habituation occurs
during transmission through interneurons in the central nervous system. They further suggest that this process operates similarly in both the brain and the spinal cord. This view of habituation is widely held by animal neurophysiology researchers (Leaton & Tighe, 1976).

Developmental researchers, on the other hand, tend to favor hypotheses based upon Sokolov's model of habituation (Leaton & Tighe, 1976). Sokolov (1963) proposed that following a stimulus, the cerebral cortex constructs a "neuronal model", an organization of neural cells which processes and stores information regarding the stimulus pattern. Each subsequent stimulus is compared with the model previously formed. If the stimulus does not match the model or the model is not yet complete, excitation occurs eliciting a response and model reinforcement. As the model develops and the stimulus begins to match the pattern, negative feedback leads to the inhibition and ultimately the cessation of a response.

The view that habituation occurs at the level of the cortex is supported by studies in which neither hydrocephalic nor anencephalic neonates habituated well (Brackbill, 1971; Wolff, 1969). However, in a study of totally decorticated animals, habituation occurred but at a slower rate (Lebedinskaia & Rosenthal, 1935). Thompson and Welker (1963) found habituation to an auditory
stimulus to continue to occur in cats despite removal of the auditory cortex. Thus, disagreement continues regarding the exact mechanism of habituation.

**Neonatal Auditory Habituation**

Habituation occurs in all species from invertebrates to humans (Thompson & Glanzman, 1976; Thorpe, 1963). Both full term and premature human infants habituate to an auditory stimulus (Allen & Capute, 1986; Bartoshuk, 1962a, 1962b; Brazelton, 1962; Bridger, 1961; Leventhal & Lipsitt, 1964; Schulman, 1970). However, while healthy full term neonates demonstrate a consistent response decrement, preterms are either hypo- or hyperresponsive depending upon the stimulus used and the response being evaluated (Bench & Parker, 1971; Berkson, Wasserman, & Behrman, 1974; Field, Dempsey, Hatch, Ting, & Clifton, 1979; Garcia-Coll, Emmons, & Anderson, 1987; Howard, Parmelee, Kopp, & Littman, 1976).

This variability of response among premature neonates is also illustrated by conflicting reports concerning differences in habituation rates (Eisenberg, Coursin, & Rupp, 1966; Field et al., 1979; Schulman, 1970). Schulman (1970) compared heart rate response habituation in preterm infants with probable CNS impairment, preterms with unlikely CNS impairment, and healthy full term neonates. Over 30 trials, similar
decrement patterns were found in all groups following an 80 dBSPL auditory stimulus; however, more trials were required for both groups of premature infants to achieve cardiac response habituation. Also, latency of response (the time from stimulus onset to maximum heart rate change) following each stimulus was significantly longer (p < .01) for the preterms with greater risk for CNS impairment than for either of the other groups. The low risk premature infants also had a longer latency of response than the full term group but the value was statistically insignificant (p < .10).

Eisenberg et al. (1966) studied behavioral responses of neonates to an auditory stimulus. Full term infants achieved habituation to an 80 dBSPL tone (descending in frequency from 5000 to 200 cycles per second) after 20 to 37 trials. Even after the presentation of 100 trials, complete response decrement did not occur in 2 preterm subjects.

Field et al. (1979) investigated cardiac and behavioral responses following repeated presentation of an auditory stimulus. Term infants demonstrated both heart rate and motor habituation over 10 trials. Preterm neonates showed a only a response decrement of the number of limb movements to the stimulus; no cardiac decrement was found. The authors postulate, however, that heart
Rate habituation may have occurred in the premature subjects if more stimulus trials had been presented.

**Relation Between Later Attentional Ability and Habituation During Infancy**

As previously discussed, habituation is thought to reflect one aspect of the current functional status of the central nervous system (Brazelton, 1973; Prechtl & Bientema, 1964). A relationship is also proposed to exist between habituation in infancy and later cognitive functioning. Miller et al., (1977) found that infants at 2, 3, and 4 months of age who habituated faster to visual stimuli had higher cognitive functioning at 15 months than did those infants who habituated more slowly.

Many investigators have related prematurity with later behavioral and academic problems in school. It is proposed that such social and cognitive learning is related to attentional difficulties. Infants with birthweights ≤ 1500 g were found to have decreased IQ scores and increased behavioral difficulties (Drillien, 1961; Drillien, Thomson, & Burgoyne, 1980; Hunt, Cooper, & Tooley, 1988; Klein, Hack, & Breslau, 1989). Similar results were seen in neonates weighing ≤ 2500 g at birth (Bjerre & Hansen, 1976; Holwerda-Kuipers, 1987; Pasamanick, Rogers, & Lilienfeld, 1956).
However, Taub, Goldstein, & Caputo (1977) did not find such relationships. In a study of 64 preterm and term subjects tested at age seven to nine years, the group of those prematurely born had significantly lower performance IQ scores requiring visuomotor coordination ($p < .008$). However, there were no differences in verbal IQ scores, social behavior, or school performance (grade reports) between those born prematurely and those born term. The mean gestational age of the preterm infants was 34.4 weeks with a mean birth weight of 2152 g. The authors propose that the visuomotor deficit results from extremely "subtle brain dysfunction or a limitation of brain cell growth to which the visual system may be most susceptible" (p. 803).

The differences in cognitive and behavioral outcomes shown by the above studies may be due to environmental factors occurring during infancy and early childhood. Achenbach, Phares, Howell, Rauh, and Nurcombe (1990) demonstrated the effect of maternal caregiving behaviors on the long-term outcome of premature neonates. Following interventions designed to increase maternal knowledge of infants' unique behavioral and temperamental characteristics, previously low-birthweight infants scored higher on cognitive tests at age seven than did infants of mothers not receiving this teaching.
Use of the Neonatal Chick Model

Many confounding variables are encountered in investigations of sick preterm infants. Use of an animal model allows for greater control of these variables. For example, the use of healthy animals prevents illness from influencing study results.

Parallels exist between chicks and human infants which support research use of the chick model (Rubel, 1978, 1984). The auditory functional status of term chicks at hatch approximates that of term human infants at birth. Also, auditory development prior to hatch and birth proceeds in a similar sequence. For example, middle ear functional development follows an identical pattern of differentiation outward from the mid-basal region in both species. In chicks and humans the ability to hear low frequency sounds develops initially, followed by the ability to hear higher frequencies.

Like human infants, chicks habituate to repeated stimuli. Neonatal chicks consistently demonstrate habituation by decreasing their peep delay response following repeated auditory stimuli (Kerr et al., 1979). Chicks peep almost constantly but pause when a new stimulus presents. After repeated stimuli, the chicks no longer pause between peeps, having habituated to the stimulus (Gray, 1987a, 1987b).
Habituation in chicks is such an extremely robust phenomenon that means of circumventing it must be included in the design of studies of auditory perception using chick subjects (Gray, 1987a, 1987b; Kerr et al., 1979; Schneider & Gray, 1991). The robust nature of this phenomenon makes a demonstrated effect of NICU noise on habituation even more impressive.

Summary

NICU noise is shown to adversely affect the prematurely born human neonate. The ability to demonstrate a response decrement to a repeated stimulus indicates an intact central nervous system. Habituation is also proposed to relate to later learning in humans. Preterm infants, however, do not habituate consistently; they may be either hypo- or hyperresponsive to stimuli. Because many preterm neonates present at later ages with attentional deficits, there is concern among clinicians that a causal relationship exists between early altered habituation and these later difficulties.

Like human infants, neonatal chicks possess the ability to habituate to auditory stimuli. The development of auditory structures as well as the functional status of audition at birth is similar in both species. The purpose of this study was to compare the habituation of neonates exposed to continuous NICU noise with that of neonates not
exposed to NICU noise. The chick model allowed for the investigation of this topic without the confounding variables encountered in the sick human preterm.
Chapter 3

METHODOLOGY

The purpose of this experiment was to compare habituation to white noise stimuli in neonatal chicks exposed to constant NICU noise beginning at post-set day 16 with neonatal chicks not exposed to NICU noise. All subjects were tested at 0 and 4 days post-hatch to either a sound stimulus or a control, silent, condition.

Sample

Subjects were 58 domestic chickens (Gallus gallus, white leghorn strain) incubated and hatched in the Otolaryngology laboratory of The University of Texas Medical School at Houston per lab protocol (Schneider & Gray, 1991). All birds were obtained from Texas A & M, Department of Poultry Science as eggs and placed in a commercial incubator (Petersime Model 1) soon after receipt. Subjects were randomly assigned to either the normally-reared (quiet-reared) or the atypically-reared (noise-exposed) group. All subjects were tested between 4 and 24 hours of age (0-day olds) and retested between 96 and 120 hours of age (4-day olds).

Several criteria were required for subjects' data to be included in the study. The chicks had to remain healthy throughout the study. The various conditions of the experiment (i.e., levels of quiet and noise, stimulus
level, and operation of the computing equipment) had to remain the same for all subjects according to the experiment requirements of their respective groups.

In order to determine sample size, a pilot study of 10 chicks was used to obtain estimates of response variability used for power analysis. Using an Alpha level of .05 and a power of .90, the sample size required to detect a difference in habituation was 10.6 subjects per experimental cell (Zar, 1984). For this calculation, habituation was defined as the subject's recovery time to the baseline peeping rate in trial 1 minus recovery time in trial 16. The two groups compared were subjects tested following the presentation of a white noise stimulus at 10 dB above threshold and subjects evaluated without the presentation of an auditory stimulus.

Design

This study was an experimental study in which the auditory environment was the independent variable. Habituation to a white noise stimulus was the dependent variable. Subjects were randomly assigned to be reared normally (quiet-reared group) or atypically (noise-reared group).

All subjects (quiet- and noise-reared) were randomly assigned to be tested for responsiveness to one of two conditions: (a) a control, mock-test condition with
no auditory stimulus (silence), or (b) white noise stimulus at 10 dB above the estimated absolute threshold level (sound). The first testing condition yielded baseline data regarding chicks' peeping without exposure to an auditory stimulus. To evaluate the effect of development, all subjects were tested at 0 days post-hatch and retested under their respective testing parameters at 4 days post-hatch.

All eggs used in this experimental study were moved on post-set day 16 to 20 x 20 cm sound-attenuating chambers (Brower Electronics, Raleigh, NC). These triple-walled boxes have heat, air, and sound controls which allow hatching of birds in isolation from outside sounds and provide exposure to specific auditory stimuli (Schneider & Gray, in press). Two to four birds were hatched and maintained in each box.

The quiet-reared group inhabited the sound-attenuating boxes until 2 days post-hatch. At this time the birds were moved to regular 94 x 33 x 30 cm "battery" brooders in the Animal Care Center of The University of Texas Medical School at Houston. This change in location was required by animal welfare protocols to allow for proper feeding and watering of the chicks. These subjects received no exposure to NICU noise.
The noise-exposed group was reared in continuous NICU noise beginning on post-set day 16 when the subjects were transferred as eggs to the sound-attenuating boxes. Because the auditory status of chicks at post-set day 16 corresponds approximately to that of humans at 30 weeks gestation, noise exposure began on that day and continued through post-hatch day 4. At 2 days post-hatch, the chicks were moved to a 74 x 44 x 29 cm brooder (Marsh Hobby model HB76) located in the same room as the sound-attenuating chambers to allow for feeding and watering of the birds. Exposure to NICU noise continued via a speaker (Fisher Coaxial) placed on top of the brooder until the subjects were retested at 4 days post-hatch.

Atypically-reared Condition

Noise-reared animals were exposed to a continuously repeating 60-minute recording of NICU noise. These sounds were recorded in a southeast Texas level III NICU using a portable reel-to-reel tape recorder (Uher Report Monitor model 4400). A microphone (Bruel & Kjaer type 2235) was placed near the head of a newborn lying on a radiant warmer. Recordings were made over several visits. Segments of recorded noise were then copied to a 60-minute cassette. Recording level and volume settings were verified at the onset of each recording session by a
94 dB national standard calibration tone (Brüel & Kjaer Sound Level Calibrator type 4230). Because the sound level in this NICU varies little from hour to hour, a one-hour tape of noise is representative of sounds in this NICU (Gray et al., 1986). The mean noise intensity of the recording was 76.61 dB.

Due to the higher auditory threshold of neonatal chickens as compared to neonatal humans, the noise recording was presented to subjects at 21 dB above the actual recorded level. This level was derived by taking the average of the differences between the threshold of chicks and the threshold of adult humans to sounds at 125, 250, 500, 1000, and 2000 Hz (ANSI, 1969; Gray & Rubel, 1987). This averaged difference was 26 dB. Next, 5 dB was subtracted from the 26 dB difference because human neonates are thought to be approximately 5 dB less sensitive than human adults over this range of frequencies (J. Jacobson, personal communication, November, 1990). This yielded an expected effective difference between neonatal chicks and humans; accordingly, the recorded sounds were presented to the chick 21 dB above recorded level to render an equivalent auditory environment to the chicks as to human neonates.

The NICU noise was transmitted to the speakers in the sound-attenuating boxes via an amplifier (TOA model
A-906A) attached to an auto-reverse cassette player (Panasonic model RX-FS450). The intensity of noise within the boxes was calibrated with a small calibrated microphone (Knowles BT1759) placed in the center of one of the boxes at the level of a chick's head and connected to a signal analyzer (Hewlett-Packard model 3561A). The volume settings of the cassette player and the amplifier were adjusted until a recording of a 94 dB national standard calibration tone (Bruel & Kjaer Sound Level Calibrator type 4230) was verified to be 94 dB by the signal analyzer. This calibration tone recording had been taped at the same recording level settings on the tape recorder as the NICU noise was taped. Thus, a 94 dB tone found in the NICU noise corresponded with the 94 dB calibration tone. The volume control settings of the tape player and the amplifier were then increased until the calibration tone was 115 dB, an increase of 21 dB.

To further verify that the NICU noise was elevated by 21 dB, the signal analyzer determined the average dB level of a two minute segment of the tape. When this particular 82 dB segment became 103 dB, the volume control settings were sufficiently adjusted to produce a 21 dB increase. Identical volume settings of the cassette player and the amplifier were obtained following both methods of achieving a 21 dB increase.
At the onset of the study, the variability of the noise intensity between the sound-attenuating boxes was measured with the volume controls of the tape player and the amplifier at these settings. The Knowles microphone was placed in each of the sound-attenuating boxes and the noise level measured by the signal analyzer. The dB levels ranged from 101.57 dB to 104.02 dB with a mean of 102.8 dB. All noise intensity measurements were calculated with the doors of the boxes closed.

At 2 days post-hatch, noise-exposed subjects were transferred to a brooder located in the room with the sound-attenuating boxes. Using a speaker resting on top of the brooder, the same tape player and amplifier which delivered noise to the boxes transmitted NICU noise to the brooder. The intensity of the noise was verified prior to presentation to the subjects. A sound level meter (Bruel & Kjaer type 2235) placed in the center of the brooder at the height of a chick's head relayed the dB measurements to the signal analyzer. Employing the same calibration procedure used with the sound-attenuating boxes, the volume control settings of the tape player and the amplifier were adjusted to deliver noise at 21 dB above the recorded level.

Due to time constraints in collecting data, some quiet-reared subjects were maintained in the sound-
attenuating boxes while noise-reared subjects were being exposed to noise in the brooder. To check for the leakage of NICU noise into the boxes, the dB level inside the chambers was measured. Without the tape playing, dB levels in the sound-attenuating boxes at 125 to 2000 Hz ranged from 41.85 dB to 50.46 dB with a mean of 47.02 dB. When the noise was played to the brooder, dB levels in the boxes at 125 to 2000 Hz ranged from 40.09 dB to 52.90 dB with a mean of 47.94 dB. Thus, a negligible amount of noise leaked into the boxes when the tape was played to the brooder. To control for the possible effect of external NICU noise on the subjects' responses, however, data collected from quiet-reared chicks occupying the boxes while sound was played to the brooder were not included in the statistical analysis.

Instrumentation

The instrument used for this study was a computerized system in the laboratory of Lincoln Gray, Department of Otolaryngology - Head and Neck Surgery, The University of Texas Medical School at Houston. A microphone registered the chicks' peeps and sent this information to a calibrated vocalization detector which signaled a computer. The validity of the detector was determined by comparing its detection of over 4000 peeps and 500 inter-peep intervals with measurements made by an
experienced human observer. The results of the detector were in 99 percent agreement with those of the observer (Severns, Gray, & Rubel, 1985).

The computer program designed by L. Gray measured and recorded the time delay to the 2nd, 4th, 8th, 12th, and 16th peeps to the nearest 1/60 second starting at the onset of the white noise stimulus and ending with the offset of the chick's peep. The delay to the second peep, termed peep pause, yielded information regarding subjects' initial responses to the auditory stimulus. Previous studies have shown the pause to the second peep to be a highly sensitive measure of response in the chick (Gray, 1987b, 1990).

Following each of 16 presentations of the white noise stimulus, the computer also recorded the time in seconds required for the subject to return to a baseline pre-stimulus peeping rate. This baseline rate was defined as 12 peeps per 10 seconds for 0-day olds and 16 peeps per 10 seconds for 4-day old chicks. These values were derived from pilot data from a sample of 10 chicks and from previous published data (Gray, 1987a, 1987b).

The validity of the computer program to record chicks' peeps was evaluated at the onset of the pilot study. Avian peeps per 10 second intervals were counted by a human observer and by the vocalization detector and
the results agreed with the computer's tabulations. Timing was verified with a digital oscilloscope. Because the computer and the vocalization detector are mechanical devices they are unbiased; thus, reliability was maintained.

Once a subject met the baseline peeping criteria, the computer triggered the presentation of the white noise stimulus to those subjects assigned to the auditory stimulus testing groups. Because previous studies of chickens used stimuli of 33 to 60 dB above threshold levels, the stimulus in this study was initially to be presented at 30 dB above threshold (Gray, 1987a, 1987b; Schneider & Gray, 1991). However, in pilot studies the subjects did not return to the baseline peeping rate following presentation of a white noise stimulus of this intensity. Thus, in this study, subjects assigned to receive an auditory stimulus were presented with a white noise burst at 10 dB above threshold. The chick's white noise threshold is 48 dB at 0-days post-hatch and 44 dB at 4-days (Gray, 1989). Thus, the stimulus was delivered at 58 dB to 0-day old subjects and at 54 dB to 4-day old chicks. As long as the subject continued to return to the baseline peeping rate within 5 minutes post-stimulus, the 1 second white noise burst was repeated for a maximum of 16 trials.
To determine the variability of stimulus intensity, the decibel sound pressure level (dBSPL) of the stimulus was measured at 15 locations within the testing chamber. A microphone (Bruel & Kjaer type 2235) was suspended at the level of a chick's head if the bird were standing, lying down, or standing with its neck extended at the center, northern, southern, eastern, and western areas of the chamber. The dBSPL at these locations were then measured by a signal analyzer (Hewlett-Packard model 3561A). The difference between the lowest and the highest dBSPL within the testing chamber was 1.2 A-weighted dB.

To further ensure that the desired stimulus was consistently presented, the computer measured the voltage of the stimulus delivered to the speaker (dBV) before and after each group of subjects was tested. The computer program was written to allow the dBV to vary only 0.5 dBV either negatively or positively from the voltage required to present the desired dBSPL.

Mock-tested birds were incubated in either noise or quiet and tested without sound stimuli. During the test procedure, the computer recorded peeps as if a stimulus had been given, just as with the chicks which did receive test stimuli.
Data Collection

Following the laboratory protocol of Lincoln Gray (Schneider & Gray, 1991), subjects were placed inside a small (1 liter) cylindrical Plexiglas chamber in a well-lit, room-temperature, double-walled, sound-attenuating room. Presentation of the stimulus and the collection of the data were automated, but a video image, audio output from the microphone, and the digital signal from the peep detector were monitored at all times.

After an acclimation period of about one minute, subjects began to peep at a steady rate. Testing trials began after the chicks met the baseline peeping criterion of 12 peeps for 0-day-old and 16 peeps for 4-day-old birds. Because somnolent subjects are unresponsive, testing was ended whenever a chick failed to resume at least the baseline peeping criterion within five minutes (Gray, 1990).

Analysis of Data

Descriptive statistics were used to give information about the testing groups. Analysis of covariance was used to evaluate the effects of age and experience on habituation of rate resumption and peep pause. Regressions were done and slopes compared to evaluate the rapidity of habituation in the quiet-reared and noise-exposed groups at 0 and 4 days of age.
Protection of Subjects

Approval for this study was granted by the University of Texas Health Science Center at Houston Animal Welfare Committee. Approval to record NICU noise was given by the University of Texas Health Science Center at Houston Committee for the Protection of Human Subjects. The Level III NICU Nurse Manager reviewed and approved the edited, one-hour tape of NICU noise for the protection of patient and staff confidentiality. All recordings not used for the study were erased.
Chapter 4
ANALYSIS OF DATA
Data Preparation

Subjects Who Failed to Meet Inclusion Criteria

Approximately 24% of subjects experienced some uncontrolled abnormality during the course of the experiment. These abnormalities included death between 0 and 4 days and minor changes in their rearing conditions due to temporary equipment failures. All of the reported statistical analyses were performed only on the 58 birds that completed the experiment without any problems. The exact number of subjects changed across trials within particular subject groups as individual birds occasionally ceased peeping due to somnolence (see Table 1).

In order to determine whether the results would be affected by excluding the atypical animals, statistical analysis were also done on the groups of all animals. These results showed no significant effect of these minor abnormalities on group indicators of central tendency.

Change in Peep-rate Criteria for 4-day-old Birds

Preliminary analysis of the data suggested that the baseline peep rate of 16 peeps per 10 seconds for the 4-day-old subjects was too severe. Using this criteria, most of the quiet-reared, 4-day subjects failed to return to baseline in the first 10 seconds even after the 16th
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<th>Experience</th>
<th>Stimulus</th>
<th>Age</th>
<th>Trial 1</th>
<th>Trial 16</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quiet</td>
<td>None</td>
<td>0-day</td>
<td>12</td>
<td>11</td>
</tr>
<tr>
<td>Quiet</td>
<td>Sound</td>
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<td>13</td>
<td>9</td>
</tr>
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<td>None</td>
<td>0-day</td>
<td>15</td>
<td>14</td>
</tr>
<tr>
<td>Noise</td>
<td>Sound</td>
<td>0-day</td>
<td>16</td>
<td>15</td>
</tr>
<tr>
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<td>None</td>
<td>4-day</td>
<td>12</td>
<td>8</td>
</tr>
<tr>
<td>Quiet</td>
<td>Sound</td>
<td>4-day</td>
<td>16</td>
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</tr>
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<td>None</td>
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</tr>
<tr>
<td>Noise</td>
<td>Sound</td>
<td>4-day</td>
<td>16</td>
<td>9</td>
</tr>
</tbody>
</table>
stimulus (i.e., last trial). These normally-reared birds, therefore, could not be shown to habituate to the test stimulus. This result was not logical, however, as prior work in the laboratory routinely showed a capacity for habituation among these normal birds. Their failure to meet the habituation criteria, therefore, was likely due to the criteria itself, rather than to the subjects.

The data showed that habituation could be demonstrated with a slightly relaxed criteria of 12 peeps per 10 seconds. Measures of rate resumption (RR) were therefore adjusted to estimate what would have been the recovery had a criteria of 12 peeps per 10 seconds been used for both ages. This could be done accurately because the computer recorded the time to the 12th peep if it occurred in the first 10 seconds after the stimulus and also recorded the number of peeps in each subsequent 10-second segment of a given trial. To calculate the time to the 12th peep in a given trial, it was assumed that the peeps occurred regularly throughout the 10-second period. With this assumption, and making X equal the number of peeps in a given 10-second period, rate was assumed to have recovered at the 120/X second in the earliest occurring 10-second period where the number of peeps was greater than 12.
Data Analysis

In Figure 1, the mean values of the two outcome measures, length in seconds of the peep pause (PP) and time in seconds to resumption of the pre-stimulus peep rate (RR), are plotted as a function of trial for each of eight groups in a two-by-two-by-two blocked design. The blocks comprise the three experimental variables: (a) perinatal exposure to quiet or noise, (b) tested in silence or sound, and (c) tested at 0 and again at 4 days of age.

All of the trends discussed below were evident by inspection of the mean values (Figure 1). First, responses in the silent or mock-test condition (circles) varied slightly over both age and trials. Although these differences were small, they were statistically significant as shown below. Previous studies have shown similar age-related differences in baseline peep rates (Gray, 1987a, 1987b, 1990). Because of the changing baseline response rates, outcome measures in sound must be interpreted in relation to responses in the silent or mock-test condition at the same age and trial. This was accomplished by a process of normalizing the outcome data against the mock-test data as described below.

A second trend evident in Figure 1 is that responses to the sound stimulus (squares) generally
Figure 1

Means of Outcome Measures by Trial

Experience Stimulus
- - - Quiet Sound
- - - Noise Sound
- - - Quiet Silence
- - - Noise Silence

0-Day

Peep Pause (sec.)

4-Day

Role Resumption (sec.)

Trials
decreased rapidly across the early trials. These changes in response indicate rapid habituation to the stimulus. The 4-day-old chicks reared in noise did not, however, show this rapid decrease, particularly in the initial response to the stimuli or PP. In fact, the most important trend evident in Figure 1 is that 4-day-olds reared in noise were more responsive to the test stimulus at the end of the series of trials than animals reared in quiet. This trend suggests that neonatal chicks exposed to noise for a relatively long period habituate to sound less well than neonatal chicks exposed to quiet rearing conditions.

**Transforming the Data**

The means in Figure 1 for both outcome measures (PP and RR) were not normally distributed ($p = .0000$ for each) with skewness of 1.2251 for PP and of 3.8655 for RR. This type of positively skewed distribution is expected in latency data such as length of peep pause and time to rate resumption because these reactions are more often short than long. Statistics planned for analysis, however, were more appropriate for use with normal distributions. A logarithmic transformation of the data was used, therefore, to create more nearly normal distributions. Following the transformations, the data were still not normally distributed ($p = .0000$ for each), but skewness of
PP had been reduced by a factor of almost 7 to 0.1824 and skewness of RR by a factor of slightly more than 3 to 1.2312. The distributions also appeared more normal on inspection.

Normalizing the Data

Removal of Extraneous Habituation Effects

The two groups of birds were incubated in either noise or quiet and mock-tested in the sound attenuated room without sound stimuli. During this test procedure, the computer preserved maximally attenuated stimulus sounds well below the subjects' hearing threshold and recorded peeps as if a stimulus had been given, just as with the chicks which did receive test stimuli.

In this silent or mock-test situation one would expect a steady rate of peeping across the mock trials. The recorded peeps, however, revealed a pattern of changing peep rates which resembled a habituation pattern. An analysis of variance of log-transformed data from the mock-test conditions showed effects of age and trial but not of experience. The effect of age was expected as was the lack of effect of experience. As

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1 A marginally significant age-by-experience interaction in recoveries (F1, 742 = 4.1, p = .044 is ignored to make the analysis tractable.
there was no functional difference between one trial and the next in terms of perceived sound, the effect of trial was difficult to understand. A possible explanation was that the birds were habituating to the test chamber itself.

The next step in preparing the data for analysis, therefore, was to remove the effects of this extraneous habituation to the test environment. Assuming that both the tested and mock-tested birds were reacting similarly to the chamber, the log transformed mean score of each mock-test trial was subtracted from each tested subject's score for the parallel trial. For example, the log transformed mean from trial 3 of all 0-day, noise-exposed, mock-tested subjects was subtracted from the trial 3 score of each 0-day, noise-exposed, sound-tested bird. These normalized scores are similar to z-scores and are used in all the following statistical analyses.

Positive z-scores were expected, indicating greater response to the sound stimulus than to silence. A z-score of 1, for example, would mean that PP or RR was 1 standard deviation above that which was expected in silence. Similarly, a z-score of 0 would mean that PP or RR was the same in response to a sound stimulus as to silence. The evaluation of whether responses to the sound
stimulus are significant then involved a test of whether the z-scores were greater than 0.

**Different Rates of Habituation**

Two, 2-way analyses of covariance were used to evaluate the effects of age and experience on habituation as measured by PP and RR. Trials were the covariate (Norusis, 1990). These analyses showed significant age-by-experience interactions.

For both outcome measures the effect of experience was different at each of the two ages. This interaction was more significant for PP than for RR ($F_{1, 710} = 13.3$ vs $7.5$, $p << .001$ vs $.006$). More specifically, the age-by-experience-by-trials interaction for PP expressed statistically the aberrantly flat slope of responses in the 4-day, noise-reared group. This interaction was highly significant ($F_{1, 716} = 6.4$, $p = .01$). This interaction can be seen in Figures 1 and 2. Parallel curves are observed at 0-days whereas crossing curves are observed at 4-days. These curves suggest that habituation occurred in all groups except the 4-day-olds reared in noise.

The more significant interaction for the PP measure could be due to the more precise nature of the measurement of this variable as compared with the measurement of RR. Alternately, the more significant
Figure 2

Mean Normalized Delays by Quartile of Test Trials

Experience

- Quiet
- Noise

0-Day

4-Day

Quartile of Test
values for PP could indicate that the initial response to the stimulus (i.e., the length of the first pause) is the more dramatic of the two measures.

Regressions were used to evaluate the rapidity of habituation in each group. At 0 days of age both outcome measures showed negative slopes in both rearing conditions and also had highly significant intercepts. These results indicate a reliable response that habituates rapidly.

At 4 days of age there was rapid habituation in quiet-reared subjects: The length of the peep pause shortened by a standard deviation over 16 trials ($p = .0001$). There was, however, no change in PP over trials in the 4-day animals that had been exposed to noise ($p = .88$). The flat slope, however, does not indicate the absence of the response because there is a highly significant intercept of that regression ($p < .003$). Subjects exposed to noise showed a highly reliable response that failed to habituate over trials. Confidence intervals in the slopes of these regressions from 4-day-olds reared in quiet and noise do not overlap, showing that a significant difference in rate of habituation is clearly associated with atypical early experience.

Approximately the same trend was present in habituation as measured by rate resumption, but the
difference between noise-reared and quiet-reared groups was not statistically significant.

Summary of Results

Figure 2 provides a summary of these results. Mean normalized delays are shown averaged over blocks of 4 trials with 1-tailed, 95% confidence intervals. Responses to sound are, therefore, statistically reliable if confidence intervals do not cross 0. The age-by-experience interaction is clear. At 4 but not at 0 days the animals exposed to noise show significantly different rates of habituation than animals reared in quiet. This pattern indicates an absence of habituation in one group. Comparing the last trials of the noise-reared and quiet-reared animals, the noise-reared animals do not show significantly greater responsiveness. In other words, there is a significant difference in rates of response decrement between the noise-reared and quiet-reared 4-day birds although not between the response rates of the two groups when particular trials are compared.
Chapter 5

DISCUSSION, CONCLUSIONS, IMPLICATIONS, AND RECOMMENDATIONS

The purpose of this study was to investigate the effect of early, atypical, sound experience on neonatal habituation to auditory stimuli. Due to the numerous confounding variables encountered in a study involving a population of sick preterm infants, an animal model was used to examine this topic.

This chapter discusses the study results, their support of the proposed hypothesis, and their relationship to the theoretical framework. Conclusions, implications, and recommendations based upon the results will follow.

Discussion

The model of neural plasticity presented by Wiesel & Hubel (1963, 1965) emphasizes the role of early experience in perceptual development. Structural and functional neuronal changes affecting perception are known to occur in animals reared in atypical environments (Duffy et al., 1984; Gray et al., 1982; Rubel, 1984; Spinelli & Jensen, 1979; Wiesel & Hubel, 1963, 1965). Students of human behavior also postulate the role of neural plasticity in the human species (e.g., Duffy & Als, 1983, 1988).

Wiesel and Hubel identified critical periods in the development of perception in animals. That is,
segments of time in early development during which an organism must receive particular sensory experiences if the brain is to develop normally. It is unknown whether development of the human brain involves this type of critical period.

The auditory environment of the NICU exposes human neonates to sound frequencies and intensities not heard by the fetus in utero (Bench, 1968; Walker et al., 1971). NICU speech and mechanical sounds are intense, erratic, and contain many frequencies. In the uterus, the fetus hears rhythmic, low intensity, low frequency sounds. Applying the model of neural plasticity, this altered environment may affect perceptual development.

As NICU sounds also present an ontogenetically inappropriate auditory environment to the developing and post-hatch chick, the proposed hypothesis was: Neonatal chicks exposed to constant NICU noise beginning at post-set day 16 will have significantly altered habituation to white noise stimuli compared with chicks similarly reared but not exposed to NICU noise. The results of this study indicated that experiencing atypical NICU sounds does alter habituation in neonatal chicks.

Habituation curves of both outcome measures (i.e., peep pause and rate resumption) showed that 0-day-old, noise-exposed birds appeared to habituate faster to the 58
dB white noise stimulus than did similarly tested quiet-reared subjects. This difference in habituation rate was not, however, statistically significant. The lack of significance was possibly due to wide variation among subjects in both experience groups. Large variability is expected in 0-day neonates, and could be addressed experimentally by increasing the sample size. With a large enough sample, small differences in group means could be statistically significant despite large variability among subjects.

It is unclear why noise-reared subjects responded less to the presented stimulus at 0 days. Constant noise exposure may have familiarized the chicks to the frequencies and intensity of the white noise stimulus. The chicks, then, may have regarded the stimulus as familiar and demonstrated a relatively smaller response to the stimulus than did the quiet-reared birds.

A previous study of the effect of early auditory experience with a rhythmically alternating narrow band of pure tones demonstrated an apparent increase in familiarity to similar test tones post-hatch (Schneider & Gray, 1991). Even though responses to all test tone changes were less than anticipated in the exposed subjects, the apparent increased familiarity suggests support for the diminished response to a white noise
stimulus found in 0-day-old, noise-exposed chicks. However, Schneider and Gray did not specifically address the effect of early experience on habituation. In addition, the early auditory experience used in the previous study consisted of pure tones delivered at regular intervals at 70 to 90 dB as opposed to the cacophony of sounds found in NICU noise.

At 4 days the effect of age was demonstrated by the increased speed of habituation of the quiet-reared subjects when compared with the habituation of the same birds at 0 days of age. This change reflects the maturation of the subjects and was expected (Gray, 1987, 1990). Noise-exposed chicks, however, did not demonstrate more rapid habituation at 4 days of age. They, in fact, failed to habituate to the white noise stimulus.

There are two possible explanations for this failure of noise-reared chicks to habituate at 4 days. First, an accumulation effect may have occurred. That is, the cumulative amount of noise exposure experienced from post-set day 16 through hatch day may not have been enough to interfere with habituation in this test situation. However, the continued exposure to NICU noise through post-hatch day 4 may have been sufficient to interfere with habituation.
A second explanation is that a critical period may exist between 0 and 4 days of age during which those aspects of the neurosensory system involved in habituation are particularly sensitive to auditory experience. Subjects at 0-days may not have passed through this critical period and so would continue to habituate to the white noise stimulus. These same chicks at 4 days, having experienced an atypical auditory environment during a critical period, might then fail to demonstrate habituation to a repeated stimulus. Thus, it could be hypothesized that the effect of the NICU noise was particularly strengthened by exposure between testing on day 0 and day 4.

Study Strengths

The following were considered strengths of this study:

1. The two-by-two-by-two blocked design permitted investigation of the separate effects of age and testing condition as well as auditory experience. Testing at two ages increased the possibility of detecting changes in habituation over time due to a developmental effect. Subjects at 4 days of age were tested under the same condition as they were tested at 0 days. For example, 0-day-old birds tested to a sound stimulus were tested to the same stimulus at 4 days of age. This permitted
evaluation of a developmental effect. This design feature did, in fact, reveal a developmental trend not unlike those seen in earlier studies using this test condition. Testing with a mock-test condition (i.e., silence) provided control data regarding the test stimulus. This design feature did reveal an unexpected habituation response to some aspect of the test situation other than the sound stimulus.

2. Because data collection procedures were automated, the possibility of extraneous human error was diminished.

3. This study contributed to a continuing program of developmental auditory studies in the laboratory of Lincoln Gray, Department of Otolaryngology - Head and Neck Surgery, The University of Texas Medical School at Houston. The laboratory has a history of studies of normal development. This study of atypical rearing conditions furthered an emerging mission of investigating atypical development.

4. A new way to study the development of a higher order central nervous system function in an animal model was identified. The study of habituation itself is scant in avian literature. Habituation has been viewed as an investigational problem which must be controlled. This
study demonstrated the value of investigating the phenomenon of habituation in chicks.

Study Weaknesses

The following were identified as weaknesses of the study:

1. Developing chicks were exposed to recorded NICU sounds while still in the egg. It is unknown to what extent the egg shell and fluids attenuate external sounds (L. Gray, personal communication, 1990). For this reason, some researchers open the shell in the area of the head, uncover the external ear, and clear the ear canal of its fluids before exposing the embryo to sound (Cousillas & Rebillard, 1985). Other researchers do not open the shell prior to sound exposure (Schneider & Gray, 1991). If the surrounding shell and fluids alter the intensity or quality of external sounds, then NICU sounds reaching the chick prior to hatch may be at a lower volume and lacking in some higher frequencies than the researcher was able to measure in the air. The experimental rearing condition, therefore, may have been less similar to the NICU noise condition than the investigator intended.

2. The activity level or behavioral state of the subjects may have affected the animals' responses during testing. State changes from an alert state to a sleep state were controlled for by the automatic cessation of
testing when somnolent chicks failed to resume at least the baseline peeping criterion within five minutes. However, changes from an alert state to a drowsy state during testing would result in a decreased peep rate possibly confounding the lack of habituation with somnolence. Because behavioral state changes may affect responsiveness and the measurement of habituation, further studies should provide more control of this phenomenon (Gray, 1990; Leaton & Tighe, 1976).

3. The computer program recorded the number of peeps within sequential 10-second intervals. However, the program did not tabulate peeps occurring across the boundaries of those 10-second intervals. Because of this method of recording peeps, habituation may actually have occurred earlier during a given set of trials than the data indicated.

Conclusions

The findings of this study support the following conclusion:

1. Four-day-old chicks exposed to continuous NICU noise from post-set day 16 to post-hatch day 4 do not habituate to a white noise stimulus whereas 4-day-old chicks not exposed to NICU noise do habituate to the same stimulus. The conclusion from this is that early atypical
auditory experience does decrease habituation in avian neonates.

Implications

As stated earlier, the neonatal chick is an appropriate animal model for some types of research which cannot be done with preterm humans. Care must be taken when attempting to apply results from animal studies to the nursing care of human infants. However, such research does indicate areas of potential risk and provides direction for patient care.

The results of this study demonstrate a clear effect of continuous early NICU noise exposure on the perceptual development of neonates. The major implication for practice is that noise in the NICU needs to be altered as it may be affecting the habituation and related perceptual development of the premature neonates residing there.

Many sources of NICU noise can be controlled by the personnel (Long et al., 1980; Thomas,1989). Sounds generated by human voices, machinery, and care-related activities can be reduced by hospital staff. Patient care rounds and conversations can be held outside the range of hearing of the neonates. If it is necessary to talk at the bedside, personnel can speak softly. Radios can be eliminated from patient care areas.
Centrifuges, addressograph machines, telephones, and other ancillary equipment can be moved to an adjoining area. If telephones cannot be moved, the bells can be muffled. Staff can monitor their own behavior to avoid personal calls at the bedside. Desktop phones can be quieted by placing them on a folded towel.

The volume of the continuous heartbeat on the cardiac monitor can be reduced and ringing alarms can be quieted quickly. The staff can turn off alarms altogether while they are at the bedside if unit policy allows for this. Eliminating unnecessary monitoring eliminates the numerous false alarms which occur with active babies. Personnel involved in equipment purchases can make the decibel level produced by equipment a criterion for selection. The demand for quieter operating machinery will influence future manufacturers’ designs.

When caring for infants in isolettes, personnel should avoid using the isolette top for a desk, placing items on the top, or tapping the isolette. Care should be taken to close porthole and cabinet doors quietly. Trash disposal can be quieted by replacing metal receptacles with plastic containers. Staff can make a conscious effort to avoid banging nursery doors, drawers, or equipment.
Everyone entering the NICU needs to be aware of the need for noise reduction. Nurses can inform others of the importance of maintaining a less intense environment through informal conversations and structured inservice education.

Recommendations

The following are recommendations for future study:

1. Repeat this study using the criterion of 12 peeps per 10 seconds for 4-day-old chicks. Also, record peeps continuously rather than in 10 second segments.

2. To control for the attenuation of external sounds by the egg shell and fluids, repeat this study with the shells opened and the external ear canals exposed.

3. Repeat this study with greater control of behavioral states.

4. If there is a critical period, it may occur between 0 days and 4 days. This study should be repeated with each of several subject groups receiving a different period of noise exposure in the post-hatch period while keeping the amount of total noise exposure constant.

5. Repeat the study but test the subjects at an age greater than 4 days to see whether the absence of habituation continues or whether a recovery period in quiet allows habituation to return.
6. Repeat with a larger sample size due to the variability expected of neonates at 0-days. Variability can also be explained by more precise knowledge of post-hatch age by hour at time of testing.
NOTICE OF ANNUAL REVIEW APPROVAL

HSC-MS-85-059 - "Evaluation of Ambient Noise Levels in the Newborn ICU"
P.I.: Lincoln Gray, Ph.D.

APPROVED: At a Convened Meeting

APPROVAL DATE: 4/20/90
EXPIRATION DATE: April 30, 1991

CHAIRPERSON: William H. Radentz, D.D.S

Upon review, the CPHS finds that this research is being conducted in accord with its guidelines and with the methods agreed upon by the P.I. and approved by the Committee. This approval, subject to any listed provisions and contingent upon compliance with the following stipulations, will expire as noted above:

CHANGES - The P.I. must receive approval from the CPHS before initiating any changes, including those required by the sponsor, which would affect human subjects, e.g. changes in methods or procedures, numbers or kinds of human subjects, or revisions to the informed consent document or procedures. The addition of co-investigators must also receive approval from the CPHS.

INFORMED CONSENT - Informed consent must be obtained by the P.I. or designee using the format and procedures approved by the CPHS. The P.I. must instruct the designee in the methods approved by the CPHS for the consent process. The individual obtaining informed consent must also sign the consent document.

UNANTICIPATED RISK OR HARM, OR ADVERSE DRUG REACTIONS - The P.I. will immediately inform the CPHS of any unanticipated problems involving risks to subjects or others, of any serious harm to subjects, and of any adverse drug reactions.

RECORDS - The P.I. will maintain adequate records, including signed consent documents if required, in a manner which ensures confidentiality.

COPY: Contracts & Grants
Hermann Hospital
APPROVAL - ANNUAL REVIEW OF ANIMAL PROTOCOL

March 2, 1990

* AWC-MS-86-207 - "Neonatal Development of Auditory Responsiveness"

P.I.: Lincoln Gray, Ph.D.

SPECIES: chicken

NUMBER OF ANIMALS APPROVED: 1000

FUNDING AGENCY: NIH

PROVISIONS:

APPROVED: At a Convened Meeting

APPROVAL DATE: 2/28/90 EXPIRATION DATE: 2/28/91

CHAIRPERSON: Kathleen Rose, Ph.D.

Upon review, approval is given to continue this protocol for the use of animals, contingent upon compliance with any noted provisions.

REVISIONS REQUIRED BY THE AWC - If the AWC requires that revisions be made to a protocol which has already been submitted for funding, the P.I. must submit the revisions to the funding agency as soon as the revised protocol/materials have been approved by the AWC.

* ANIMAL PROTOCOL REVIEW NUMBER - Animals purchased with the number listed above may be used only for the protocol(s) approved under this number.

PLEASE NOTE - It is your responsibility to ensure that all personnel working with animals are adequately trained. If individuals on your projects require training, contact Dr. Chris Smith x5127 to discuss training options.

COPY: Animal Care Center

Contracts & Grants
References


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