Auditory Thresholds in Monkeys Asphyxiated at Birth

by

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INTRODUCTION

Nature of the Problem

There is both clinical and experimental evidence indicating that the auditory system may be particularly susceptible to damage as a result of perinatal asphyxia[1]. The sequelae most usually associated with neonatal asphyxia are cerebral palsy (Banker, 1967; Courville, 1961; Denhoff, Smirnoff, & Holden, 1951; Fraser, & Wilks, 1959; Fuldner, 1957; Lilienfeld, & Pasamanick, 1955; Norman, 1952) and mental retardation (Bailey, 1958; Ernhardt, Graham, & Thurston, 1960; Foale, & Paterson, 1955; Fraser, & Wilks, 1959; Perlstein, 1955; Towbin, 1969b); auditory impairment is a common finding among patients with these conditions (Fisch, 1956; Flottorp, Morley, & Skatvedt, 1957; Kodman, 1957; Lloyd,& Reid, 1967; Perlstein, 1966). The inferred association between perinatal anoxia and injury to the auditory system is, however, difficult to verify in human material. This is due not only to the usual difficulties inherent in clinical studies, such as attrition of cases and inadequacy of records, but also, in this instance, to inability to specify the variables in question. Oxygen lack can range along a continuum from brief episodes of hypoxia to prolonged and profound asphyxiation. Furthermore, there is no way to be sure that a period of anoxia of which the physician is aware has not been preceded by other episodes of which he has no knowledge. In addition, human studies cannot control for the interaction of anoxia with other variables affecting audition. Thus,

[1]See glossary, Appendix I

respiratory difficulties are more common in premature than in full term babies and it is not possible to dissociate the etiologic significance of such difficulties from the effects of the shortened intrauterine growth period.

The problems of adequately specifying the degree and duration of anoxia in infants are matched by the difficulties in determining any consequent damage. Hearing losses are often undetected by parents or physicians until the child is several years of age (Byers, 1955). Johnston (1967) found that hearing deficits had not been suspected in 50% of a group of 118 children until between 8 and 48 months of age, despite the fact that they were, in fact, deaf. Large-scale routine audiometric testing, which might be expected to reveal any association between respiratory difficulty at birth and subsequent auditory capacity, has not as yet been reported.

Pathological verification of lesions in the auditory system after neonatal anoxia is rarely possible. There is, however, some histopathological evidence that lends support to the view that neonatal asphyxia may be a specific causative factor in the production of damage to auditory structures (Hall, 1962, 1963, 1964). Furthermore, experimental evidence in monkeys indicates that auditory nuclei may be more susceptible to anoxia than other parts of the brain (Windle, 1960, 1963a, 1963b, 1966b, 1968a). If this is so, and if it may be assumed that the nervous system damage produced by asphyxia at birth extends on a continuum from the one extreme of death, to the other of a transient or minimal effect that is not evidenced by any neurological or behavioral aftermath

(Pasamanick, & Knobloch, 1960), then one of the earliest clinical indications of asphyxic brain damage should be altered auditory function.

The purpose of the present investigation was to determine whether neonatal asphyxia in the monkey does, in fact, result in a deficit in auditory function. This information would have obvious significance for the correlation of structure with function as well as for the clinical evaluation of asphyxiated human infants. In particular, if auditory deficits are present in asphyxiated animals that do not show other signs of nervous system damage, then this would have implications for the clinical management of all children who have undergone asphyxia at birth, whether or not they show obvious residua. The particular aspect of auditory function that was studied was pure tone intensity thresholds.

The introduction to this paper is organized into three principal sections. The first of these consists of review of the literature pertinent to the prevalence of neonatal asphyxia in the human, to indicate the magnitude and complexity of the problem. This is followed by a description of the neuropathology reported to follow neonatal asphyxia in the human and the monkey, upon which the expectation of an auditory deficit was based. In the third section, a review of previous studies of auditory thresholds in monkeys will be given. This will serve as background for the choice of methodology as well as for the normative results obtained.

Perinatal Asphyxia

Etiology

Hypoxia can occur at any stage before, during or after birth, be due to maternal or fetal factors, and be of long standing or sudden onset. Where hypoxia[1] is of long standing, there is some possibility that the fetus will adjust its metabolism to the available supply of oxygen. Saling (1966) found that maternal hypoxia of insidious onset induced circulatory adjustments in the fetus which resulted in a decreased oxygen consumption of its non-vital organs while maintaining the function of the vital organs. Acute conditions, however, would act upon the fetus without the intervention of any such self-protecting mechanism.

Prenatal factors. Factors that result in an abrupt cessation of oxygenation of the fetus include death or asphyxiation of the mother, as well as separation of the placenta, as in placenta praevia[1] or abruptio placenta[1] (Berendes, 1967; Faber, 1947; Perlstein, 1955). Conditions that result in chronic fetal hypoxia include maternal haemorrhage (Courville, 1952) or anemia (Denhoff, et al., 1951; Norman, 1952; Perlstein, 1955); placental or cord haemorrhage (Nesbitt, 1966); partial placental detachment, placental infarction or insufficiency (Courville, 1952; Denhoff, et al., 1951; Nesbitt, 1966; Perlstein, 1955); compression, strangulation or prolapse of the cord (Nesbitt, 1966; Norman, 1952); maternal infection, acidosis or metabolic disturbance (Nesbitt, 1966); cardiac or circulatory deficiencies in mother or fetus (Nesbitt, 1966); narcosis (Nesbitt, 1966); lowered blood pressure (Nesbitt, 1966); or exposure to high altitudes (Montagu, 1961).

[1]See glossary, Appendix I

The gestational age at which oxygen deprivation occurs is of considerable significance because it is generally held that the fetus is more resistant to hypoxia than the more mature organism (Dawes, 1968; Fazekas, Alexander, & Himwich, 1941; Jilék, Fischer, Krulich, & Trojan, 1964; Myers, 1967) and that this resistance decreases as a function of the structural maturity of the fetus (Glass, Snyder, & Webster, 1944). Fazekas, et al. (1941) reported that newborn rats and mice could survive immersion in pure nitrogen for periods of up to 50 minutes, although such a procedure was fatal to the adult animal within 1.5 minutes. Comparable results were reported for guinea pigs and dogs, with the relatively more mature guinea pig pup succumbing after the briefest period of time. Hall (1964a) observed that newborn kittens could survive a 96% nitrogen atmosphere for up to six hours but that at 1-2 weeks of age they would die after one hour under the same conditions. Myers (1970) found that barbiturate anesthetized monkey fetuses could withstand no more than 12-13 minutes total asphyxia at term without resultant brain lesions, whereas similarly anesthetized fetuses at 90-100 days of gestational age could tolerate up to 30 minutes of asphyxia without damage.

The capacity of young animals to withstand oxygen lack has been explained as due to the low level of energy turnover in the immature nervous system (Jilék, et al., 1964), as evidenced by the rate of degradation of adenosine triphosphate (Dawes, 1968), as well as to the existence of alternative pathways to glucose breakdown, and their dependence on anaerobic glycolysis (Himwich, 1951). In rats subjected to oligemia[1] of the brain as a result of ligation of the common carotids, survival rate is correlated inversely with postnatal age and positively

[1]See glossary, Appendix I

with decreases in the glycogen content of the brain and increased lactic acid production (Krulich, Jilék, & Trojan, 1962). Barbiturate anesthetized newborn monkeys subjected to asphyxia of 14-16 minutes duration show a 74% depletion of brain glycogen at the termination of the asphyxial period (Rivera, Brann, & Myers, 1970) with an abnormally high concentration of glycogen apparent twelve hours later in the glia of both grey and white matter (Klatzo, Mossakowski, Myers, Long, Rodriquez, & Walker, 1967).

Cross (1966) has pointed out that in the asphyxiated newborn of all species studied, cardiac activity continues for some time after respiratory efforts cease, whereas, in the adult, acute asphyxia results in abrupt circulatory failure before the cessation of respiratory activity. This difference is presumably a reflection of the relatively high carbohydrate reserves of the fetal and newborn heart (Mott, 1961). In this respect, Dawes, Mott, and Shelley (1959) showed that the time to last gasp during asphyxia in lambs is directly related to initial cardiac glycogen level.

Whatever the biochemical or physiological basis may be, it is clear that there is an inverse relationship between the age of the fetus or neonate and the degree of protection against the damaging effects of oxygen lack. Insofar as this protection extends to the maintenance of life, there should be a higher incidence of pathological sequelae of neonatal asphyxia among prematurely born than full term infants, due to the ability of the premature to survive periods of anoxia that would be fatal to the more mature infant. This does appear to be the case. The

role of maturation extends further, however, influencing the determination of the region of the brain that will be most sensitive to the effects of asphyxia. Phylogenetically older parts of the nervous system are reported to mature earlier than phylogenetically younger parts (Himwich, 1951). According to Jilek, et al. (1964), asphyxia damages phylogenetically older parts of the central nervous system in neonatal rats more than the newer parts, whereas, in older animals, the situation is reversed and phylogenetically newer parts of the brain are damaged the most severely. Himwich, Benaron, and Tucker (1956) compared data on six human fetuses and babies ranging in age from 20-40 weeks conceptional age, who had died during labor or shortly after birth. They found a transition, in the cortex particularly, from a relatively low metabolic rate at 20 weeks to a relatively high one at 40 weeks.

There is some evidence that the severity of the effects of anoxia may be a function of individual susceptibility (Fraser, & Wilks, 1959). Bailey (1958), in fact, suggested that the influence of hereditary factors on mental retardation might be via their contribution to individual susceptibility to natal or prenatal anoxia. Benaron, Tucker, Andrews, Bushes, Cohen, Fromm, and Yacorzynski (1960) observed a far greater variability on test scores among anoxic children than among normals and postulated that there was a factor of individual susceptibility governing the severity of reaction to anoxic stress. Along a similar line, Gesell and Amatruda (1941) suggested that children with an 'inferior' endowment might sustain more permanent ill effects from perinatal complications

than would children with 'superior' endowments. Corah (1966), however, found little support for this view. He investigated the effects of anoxia at birth on performance at 3 and 7 years of age, using four perceptual and cognitive measures at the earlier age and the WISC Full Scale IQ at 7 years. He found no greater recovery among those with initially higher ability than among those with lower starting scores. This does not, of course, mean that anoxia did not affect the intelligence of the children; only that the ability of the child to recover or adjust to his deficit was not a function of his initial level of performance.

There is considerable evidence for a sex-linked difference in individual sensitivity to oxygen lack. Female rats are more resistant to experimentally induced neonatal anoxia than are males (Britton, & Kline, 1945), and there is a tendency for more male than female infants to suffer asphyxia at birth (Benaron, et al., 1969), even when the predisposing factor of prematurity is excluded (Fraser, & Wilks, 1959). Thurston, Graham, Ernhart, and Craft (1960) drew attention to the fact that only 47% of the normal newborns in their survey were male while 60.6% of their anoxic group were boys. They suggested that more of the boys in the group with complicated births developed neurological signs than did the girls, a finding which is in accord with the fact that cerebral palsy is more common among boys than girls (Lilienfeld, & Parkhurst, 1951). If, in truth, males are more susceptible than females to the damaging effects of anoxia, then a study reporting a low correlation of abnormality with

anoxia, but composed mainly of girls, cannot be compared with a study reporting a high correlation, but composed mainly of boys.

Natal factors. The birth process itself involves the body in one of the most profound adjustments that it will ever be called upon to make. The fetus that has been the passive recipient of its metabolic requirements via its umbilical circulation is suddenly called upon to obtain its oxygen supply actively through a complex mechanism of neural, chemical and muscular elements. Anything that interferes with the establishment of rhythmic respiration will produce a state of relative or absolute oxygen need in the infant.

Breech delivery is more likely to result in asphyxia of the newborn than is the normal vertex presentation because exposure of the umbilical cord to the cool extrauterine atmosphere results in contraction of the umbilical artery before the head is delivered (Perlstein, 1955). Paradoxically, hyperventilation of the mother during delivery may also result in reduction of fetal PO_2 (Motoyama, Rivard, Acheson, & Cook, 1966). Haemorrhage, placenta praevia or strangulation of the umbilical cord (Courville, 1961; Denhoff, et al., 1951) as well as uterine contractions that are unusually strong or protracted (Faber, 1947) will deprive the fetus of placental blood. There is a tendency for more first born children to suffer from asphyxia at birth than children born later in the birth order (Fraser, & Wilks, 1959; Benaron, et al., 1960), presumably because of the higher incidence of prolonged or difficult labor with first births.

Any situation that prolongs the passage of the fetus through the birth canal will interfere with its oxygenation. Disproportion between the fetus and the birth canal due to an oversized fetus (Faber, 1947), or a contracted pelvis (Fraser, & Wilks, 1959) may result in a prolonged hypoxic state. A procedure which has been widely used, and criticized, is that of physically impeding birth in order to allow the attending physician to reach the scene in time (Carter, 1965).

Postnatal factors. After delivery, the establishment of respiration may be delayed, prevented or interrupted by a number of conditions. There may be obstruction of the airway by aspirated mucus or amniotic fluid (Nesbitt, 1966; Perlstein, 1955) as well as by suffocation or strangulation (Perlstein, 1955). There may be immaturity of the respiratory system (Denhoff, et al., 1951; Windle, 1968), disease states such as pneumonia, atelectasis, or hypoglycemia (Banker, 1967); or congenital defects of the pulmonary or circulatory system (Nesbitt, 1966). Respiratory distress, common among prematures (Vernon, 1967b), can be a consequence of neonatal asphyxia, rather than its cause, according to Windle (1968). Either way, there should be a higher incidence of hearing impairments among premature than full-term babies. That this is the case is borne out by the finding that the incidence of prematurity among deaf or profoundly hard of hearing children is several times higher than would be expected among normally hearing children (Vernon, 1967b).

Depression of the respiratory centers due to sedation, anesthesia, or other medication, has often been cited as a cause of neonatal

hypoxia (e.g. Eastman, 1940; Wedenberg, 1965). Schreiber and Gates (1938) in fact, thought that the majority of cases of early neural damage were due to asphyxia resulting from anesthetics and analgesics administered during labor and delivery and that such agents might also be responsible for subclinical levels of neurological damage. According to Perlstein (1955), dosages of morphine insufficient to cause narcosis in the mother may yet cause depression of fetal respiratory centers, while Taylor, Goven, & Scott (1951) found that a combination of scopolamine and demerol, commonly used in childbirth, produced a significant depression of blood oxygen in the infant, as measured by oxymetry within the first half hour of life. It is a common observation that far more babies breathe spontaneously that are born to non-drugged mothers than to mothers who have received anesthesia. McCormick (1941) reported that 98% of non-drugged and 65% of drugged babies in his series breathed spontaneously.

While it is true that anesthesia may postpone the initiation of respiration, there is considerable evidence that it may also serve as a defense against the ill effects of a prevailing anoxic condition and therefore be advantageous to both mother and child during delivery (Eastman, 1940; Secher, & Wilhjelm, 1968). The duration of primary apnea[1] and asphyxial gasping in fetal monkeys is prolonged and resuscitation is facilitated by sodium pentobarbitone (Dawes, 1968), while the incidence of brain damage is reduced (Cockburn, Daniel, Dawes, James, Myers, Niemann, & deCurer, 1969). Myers (1970) found that asphyxia of 12-13

[1]See glossary, Appendix I

minutes duration in barbiturized monkey fetuses resulted in brain damage identical in extent to that produced in unanesthetized fetuses by Windle (1963) after only seven minutes of asphyxia.

To add to the plethora of conditions resulting in hypoxia or anoxia in the fetus or infant, Fraser and Wilks (1959) renewed an old suggestion that "some infants may behave in an asphyxiated manner at birth because of intrinsic defect or because of brain damage sustained during fetal life - the asphyxia then being the result of neurological abnormality rather than the cause of it". This suggestion incorporates the possibility that the symptoms of asphyxia in a newborn are the secondary result of an episode of anoxia during fetal life.

In order to assess the relative importance of the various etiologic factors associated with perinatal asphyxia, Ernhart, Graham, and Thurston (1960) analysed a total of 116 cases of prenatal or neonatal anoxia. They reported that 21 of these were contributed to by maternal disease, 9 by intrauterine bleeding, 10 by abnormalities of labor and others by premature rupture of the membranes, abnormalities of the cord, polyhydramnios[1] and the like. Benaron, et al., (1960) used a more stringent criterion of asphyxiation, confining their interest to infants which had been anoxic for periods of more than 10 minutes, some more than an hour. Among 41 cases, they found that asphyxiation was the result of breech presentation with arrested head in six, gigantism with arrested shoulders in two, prolonged labor in seven, placental dysfunction in one, rapid, hard labor in four, premature separation of the placenta in two

[1]See glossary, Appendix I

and cord compression in one case. In two instances they attributed the anoxia to unspecified factors associated with an unassisted birth and in 16 cases felt that sedation caused or contributed to the condition.

Incidence

Estimates of the incidence of neonatal asphyxia vary, possibly as a result of differences in the criterion of asphyxia used. Mörstad (1953) reported that asphyxia occured in 2.8% of 44,533 live births, while Keith and Norval (1950) put the incidence at 4.8%, and Anderson (1952) at 13.8%. Asphyxia at the time of birth is considered to be responsible for more deaths in the perinatal period than any other cause (Clifford, 1941; Corner, & Anderson, 1958).

Physiology

Fetal asphyxiation is accompanied by a typical sequence of physiological and biochemical changes in every animal studied, although the time course differs according to species (Cross, 1966). The heart rate increases briefly as oxygen tension is reduced, but then slows to about half its usual rate; the systolic blood pressure shows a transient rise, then drops sharply (Dawes, Jacobson, Mott, Shelley, & Stafford, 1963); the pulse pressure falls, the pulmonary arterioles constrict, so as to offer a very high resistance to blood flow (Cooke, 1958). As the circulation deteriorates, chemical changes occur in the blood. Oxygen tension decreases, carbon dioxide content rises and pH levels drop markedly. This acidosis results from the rise in carbon dioxide and also from the anaerobic breakdown of glucose into lactic acid. A sharp rise

in lactates indicates the conversion of many cells to anaerobic glycolysis. Sodium, chlorine and potassium concentrations all rise, with potassium showing the greatest change. Phosphocreatine, adenosinetriphosphate and glycogen levels fall, signalling the exhaustion of the energy reserves. This stage of apnea with its resulting anoxia, carbon dioxide and lactic acid accumulations in the blood, lowered pH and reduced heart rate and blood pressure is termed asphyxia neonatorum (Windle, 1968).

At the cellular level, as the available oxygen decreases, the energy necessary for maintaining the difference in electrical potential and ionic concentration on the two sides of the cell membrane is diminished. As a consequence, plasma protein increases and enzymes are released from the soluble fraction of the cell cytoplasm into the blood. The first sign of physical damage within the cell itself, as seen by electron microscopy, is mitochondrial swelling and, according to Cross (1966), the first tissue to suffer irreparable damage appears to be the brain.

Pathology

Experimentally induced asphyxia. There are at least two procedures by which asphyxial brain damage may be produced in neonatal monkeys, and the resultant pathology differs in its distribution accordingly. Following total anoxia, lesions are found primarily in the brain stem

(Windle, 1968), whereas hypoxemia[1] or hypotension over a more prolonged period results in injury which is primarily cerebral in location (Myers, 1970).

Over the past 28 years, Windle and his associates have studied the neuropathological sequelae of asphyxia at birth in a number of species, but most extensively in the rhesus monkey. In the unanesthetized monkey, asphyxiation produced by either retaining the term fetus in the amniotic sac after clamping and cutting the umbilical cord, or preventing its respiration after caesarian delivery, resulted in a consistent pattern of brain pathology characterized by bilaterally symmetrical focal lesions of brain stem nuclei (Ranck,& Windle, 1959; Windle, 1961, 1963a, 1963b, 1966a, 1966b, 1968). If the asphyxial insult was sufficient to result in detectable histological change, then the inferior colliculi were consistently affected. Other nuclei of the auditory, somatosensory and vestibular systems were affected only after more prolonged anoxia. If the asphyxia was of considerably longer duration, or if the infant suffered episodes of postnatal respiratory distress (Windle, 1966b) then primary lesions were detectable in the cerebral cortex. Involvement of white matter appeared to be secondary to degeneration of associated neurons.

Animals sacrificed in the first days or months after asphyxia of six to seven minutes' duration showed neuronal loss confined to the central nucleus of the inferior colliculi in some, but not all, cases. With asphyxia lasting more than seven minutes, damage to the inferior colliculi was invariably present. The lesions were focal, bilaterally

[1]See glossary, Appendix I

symmetrical, non-haemorrhagic, and involved destruction of nerve cells and their replacement, in time, by glial scars. With increasing duration of asphyxia, other brain stem centers associated with hearing and general body sensation became involved. Windle (1970) emphasized that, without exception, lesions were found in second order afferent nuclei of the auditory, vestibular and somesthetic pathways, with the visual system untouched and the primary motor nuclei relatively undamaged.

With asphyxia lasting twelve or more minutes, the primary lesions in the brain stem and thalamus were larger and more severe and there were areas of cavitation, or of degeneration replaced by scar tissue. In addition, destructive changes were apparent in the basal ganglia, cerebellum and spinal cord. The cerebral cortex and primary motor nuclei remained relatively unaffected, although many of these severely asphyxiated monkeys presented symptoms resembling those of cerebral palsy. In some animals with particularly marked functional deficits, degeneration was apparent in the depths of cerebral sulci (Windle, Jacobson, de Arellano, & Combs, 1962).

When monkeys that had been severely asphyxiated at birth were allowed to survive for extended periods of time, up to ten years, the primary brain stem lesions were still apparent, as areas of scarring or cavitation. In addition, there was now more widespread neuronal loss, particularly in the cerebral cortex. This depletion of cortical cells, according to Faro and Windle (1969), must have resulted from transneuronal degeneration secondary to the earlier thalamic lesions.

The explanation most usually offered for the selective vulnerability

of certain brain structures to neonatal asphyxia is based on their high vascularity and/or high metabolic requirements. Circulation to a particular region of the brain presumably parellels and is determined by the metabolic needs of the tissue (Sokoloff, 1959). Differences in blood flow to a region of the brain, therefore, would be determined by the nature of the cell population (Courville, 1951; Scholz, 1963). Any reduction in systemic blood flow would be expected to result in damage to these regions of high need before affecting others with lower requirements. There is some support for this interpretation in the observation that neonatal monkeys asphyxiated in nitrogen do not develop asphyxial lesions such as are seen after asphyxiation by the prevention of respiration (Jacobson,& Windle, 1960). In the latter case, the pathology presumably results from the accumulation of metabolic breakdown products which, in the nitrogen environment, can be eliminated. It has been shown, in fact, that if steps are taken to combat the acidosis, the resultant pathology is reduced (Dawes, Hibbard, & Windle, 1964).

Kety (1963), using an autoradiographic technique, found that blood flow in the inferior colliculi of the adult cat was considerably higher than in other regions of the brain, deep or superficial. Kennedy (1967), working with the monkey, found the region of greatest blood flow to be the inferior colliculi in the adult; the olivary bodies, cerebellar nuclei and vestibular nuclei in the neonate. These results are consistent with the finding that these nuclei are high in the hierarchy of structures affected by neonatal asphyxia but not fully consistent with

the neuropathological picture. If the rate of blood flow to a structure were the only determinant of its susceptibility to anoxic damage, then neonatal asphyxia in the monkey should affect the lateral geniculate bodies, which have a high rate of blood flow (Kennedy, 1967). In fact, however, there seems to be sparing of the visual system (Windle, 1968). Furthermore, while the cochlear nuclei have a relatively high rate of blood flow in the cat (Kety, 1963), they are unaffected by neonatal asphyxia in that species (Hall, 1964). This is in contrast to the human, in whom neonatal asphyxia appears to be particularly damaging to the cochlear nuclei (Hall, 1962, 1963, 1964).

Neurological deficits were not seen with less than seven minutes of asphyxia but were always present after asphyxia lasting eight or more minutes. Depending on the severity of the insult, these deficits included loss of sucking and rooting, inability to swallow, high pitched or absent cry, loss of righting reflexes and difficulty in locomotion. In the most damaged animals there might be spasticity, seizures, flaccidity, pallor and respiratory distress. In general, these neurological findings were transitory; only rarely, and in the most severe cases, did they last more than a few days or weeks.

Myers (1967) confirmed the pattern of pathology produced by acute total asphyxiation of the monkey fetus at term, but felt that this distribution of brain stem damage, reflecting the rank order of vulnerability of brain structures to energy deprivation, occurred only after circulatory arrest (Myers, 1969a, 1969b, 1970). He suggested that prolonged partial asphyxiation of the monkey fetus resulted in a type of

brain pathology more comparable to that seen in the human infant asphyxiated in the perinatal period (Myers, 1969b). This pattern of pathology, produced by subjecting the mother to excessive oxytocic agents or to hypotension induced by halothane anesthesia, is characterized by brain swelling and sclerotic microgyria[1] (Myers, 1969, 1970). The atrophy is bilateral and affects principally the depths rather than the convexities of gyri. It is most marked in the paracentral region, but involves also the principal, arcuate, intraparietal and other sulci of the parasaggital region. There is bilaterally symmetrical neuronal destruction and astrocytic gliosis in the putamen, neads of the caudate nuclei, lateral and posterior ventral nuclei of the thalamus, with the process in both the cortex and basal nuclei having a perivenular distribution.

Clinical asphyxia. The task of interpreting the literature concerning the pathological changes occurring in the human brain as the result of neonatal asphyxia is an awesome one. The usual complexities of neuropathological diagnosis are here compounded by a number of factors, the significance of which, in each case, can only be determined by the pathologist involved.

Preparation of neonatal brain tissue is, at best, extremely difficult (Towbin, 1970). To add to this problem, it is rare to find a human infant with an authenticated history of birth asphyxia coming to autopsy within a sufficiently short time, and under circumstances which permit good fixation (Courville, 1961). Specification of anoxic etiology

[1]See glossary, Appendix I

must therefore often be equivocal. Furthermore, while the concern of the investigator may be with the effects of anoxia due to specific processes occuring in the perinatal period, asphyxia is an almost constant accompaniment of neonatal deaths, if only in the agonal period (Denhoff, et al., 1951; Hall, 1962). This is particularly true among prematures. The pathologist must be able to differentiate changes due to terminal asphyxia from those due to preceding, and more directly relevant, periods of anoxic insult. He must also be able to segregate the histopathological effects of asphyxia itself from processes antecedent to, and possibly causative of, the asphyxia (Lindenberg, 1967).

In 1940, Schreiber stated that, "In all deaths from cerebral anoxia, the microscopic lesions are identical, regardless of the cause of the oxygen deficiency in the cellular tissue, or age of the subject (p. 298)" and described these changes as microscopic degenerative areas in the cerebral cortex and ganglionic structures. Though an accurate distillation of the literature to that date, this statement appears ludicrous thirty odd years later. More recent reports of pathology in children who have succumbed to perinatal asphyxia speak of widespread and diverse destructive changes in the cerebral hemispheres, affecting both grey and white matter. The multitudinous lesions mentioned include: the presence of brain swelling (Lindenberg, 1967), or its absence (Brierley, 1966); gliosis (Courville, 1952), or its absence (Banker, 1966); general cerebral atrophy, cortical scars, nodular atrophy, cerebellar atrophy, cystic degeneration, cysts or atrophy of the lenticular nucleus, status

marmoratus[1] (Malamud, 1963); diffuse demyelination (Courville, 1952, 1961) or sclerosis (Malamud, 1963) of the cerebral white; porencephalic cysts[1] (Norman, 1952; Sharpe, & Hall, 1963); ulegyria[1] (Malamud, 1963; Norman, 1952); leukomalacia[1] and diffuse neuronal loss with ventricular enlargement (Banker, 1966); necrosis in the periventricular regions (Malamud, 1963); and degeneration in the basal ganglia, especially the globus pallidus (Norman, 1952; Perlstein, 1955). Many of the lesions found after anoxia in infants are thought to be secondary to a direct affect of anoxia on vascular endothelium, increasing its permeability and fragility and resulting in haemorrhage (Perlstein, 1955). According to Corner and Anderson (1958), 40% of intracranial haemorrhages in infants are due to anoxia. Windle (1968), on the other hand, believes that haemorrhage is not caused by neonatal asphyxia but, when it occurs, contributes to the severity of the neurological deficit.

Two of the most recent and comprehensive studies reported on the neuropathology of perinatal asphyxia are those of Banker (1967) and Towbin (1969a, 1969b, 1970). Banker (1967) described changes in both the grey and white matter. The changes in the grey matter were diffuse and affected particularly the cerebral cortex, thalamus, basal ganglia and hypothalamus. They were characterized by karyorrhexis, or dissolution of the nuclear chromatin, and absence of glial reaction. The late appearing effects of these early changes took the form of neuronal loss, evidenced by reduced size of the brain and ventricular enlargement. Lesions in the white matter consisted of zones of leukomalacia in

[1]See glossary, Appendix I

periventricular regions, occurring particularly in premature infants, and ischemic changes in subcortical white matter, occurring most often after two weeks of life.

Towbin (1969a, 1969b, 1970) emphasized the occurrence of cerebral infarcts, with secondary necrotic and haemorrhagic effects, as the principal pathological process with hypoxia, the lesions being localized in the deep periventricular strata in the premature fetus or premature newborn, and in the cortex in the mature fetus or mature newborn. With acutely developing anoxia, according to Towbin, there is sudden death and therefore no pathology.

It should be noted that remarkably few investigators mention examining the brain stem after asphyxia. In a case report of an infant who died after its birth had been forcibly held back for one-half hour, Schreiber (1940) mentioned two "devestation areas (p. 301)" in the region of the tenth nerve nucleus in the medulla, but dismissed these as of lesser interest than those in frontal and motor cortex. The only brain stem lesions that Towbin (1970) mentioned were those associated with the mechanical effects of traumatic birth. In a number of instances when the brain stem has been examined after asphyxia, however, lesions have been found. Gilles (1969) observed bilaterally symmetrical necrotic lesions in the brain stems of adult patients after cardiac arrest but felt that they were related to the acute circulatory failure rather than to hypoxia or hypoxemia <u>per se</u>. Brierley (1966) reported that, in 14 cases of circulatory arrest, there was cellular loss in the olives and inferior colliculi, in addition to diffuse alterations in the cerebral cortex,

necrosis of Ammons horn and degeneration of the anterior nuclear complex of the thalamus. Four children that were under the age of five years also showed lesions in the gracile and cuneate nuclei, spinal nuclei of the trigeminal nerve, vestibular nuclear complex, motor nuclei of the fifth nerve and oculomotor nuclei. Brain stem lesions involving the olives and inferior colliculi were found by Brierley (1966) in four cases of systemic hypotension in adults with "watershed"[1] lesions of the cerebral or cerebellar cortex and were extensive in one young child with a similar etiology. Slight neuronal loss in the inferior colliculi and olives were also seen in adults dying of hypoglycemic coma. In essence, then, Brierley's findings indicate a susceptibility of brain stem nuclei to hypoxia present at all ages, but most marked in young children (Brierley, 1967, 1970).

In considering the pathology in cases of circulatory arrest, it is important to recognize that cerebral anoxia may occur in any of several forms (Lampert, 1961; Schreiber, 1940) and it should be anticipated that its effects on nervous tissue would differ accordingly. In anoxic anoxia (lack of available oxygen), anemic anoxia (lack of oxygen carriers in the blood) or histotoxic anoxia (poisoning of the respiratory enzymes) there is deficiency in the amount of oxygen made available to the tissues but no deficit in the removal of the byproducts of metabolism. Stagnant anoxia, on the other hand, is produced by stasis of the blood and, in this case, there is no removal of catabolites.

[1]See glossary, Appendix I

Hall (1962, 1963, 1964) studied the auditory pathways (cochlea, cochlear nuclei, inferior colliculi, medial geniculates and auditory cortices) of 39 human infants who had died after perinatal asphyxiation. He found no changes from normal in the cochleas; the only unusual findings were attributable to autolytic changes or mechanical distortion during preparation. In every case, however, there were degenerative changes in the cochlear nuclei, particularly marked in the dorsal nuclei. These changes ranged from acute swelling of the neurons, with the cells enlarged and the cytoplasm poorly stained, the Nissl substance dissolved and the nucleus eccentrically displaced, to severe cell degeneration signalled by vacuolization of the cytoplasm with the cell borders blurred, the Nissl substance disintegrated and the nucleus dissolved. These changes were accompanied, in the more severe cases, by gliosis. Hall found a marked reduction in cell number in the cochlear nuclei of the asphyxiated children, with the magnitude of the loss roughly proportional to the duration of asphyxia. In the most severe cases, the cell population was reduced to one half the normal average. Degenerative changes were observed in the superior olives, inferior colliculi and the magnocellular division of the medial geniculate, but only in one case was there degeneration in the parvocellular, or auditory relay, division of the medial geniculate.

It is noteworthy that, in Hall's series, the clinical course in the asphyxiated infants consisted, most often, of episodic bouts of asphyxia punctuated by periods of more or less adequate oxygenation.

According to the model of Myers, these cases should have shown damage primarily in the cerebrum, rather than the brain stem. Hall, unfortunately, did not survey non-auditory cortex, but he did investigate the anterior transverse temporal gyrus and did not find any conclusive evidence of degenerative changes. Hall felt that the changes that he did detect could not be differentiated from artefactual changes due to fixation and sectioning.

It seems of interest to reexamine the literature on neonatal asphyxia with a view toward reconciling the results of either or both of the experimental methods of producing asphyxia in monkey fetuses (Myers, 1970; Windle, 1968) with the human data. Myers suggested that brain stem lesions seen in the monkey fetus or newborn after acute total asphyxiation are due to circulatory arrest, rather than to anoxia itself, and Gilles (1966) expressed a similar view with respect to brain stem lesions found in human adults after cardiac arrest. Banker and Larroche (1962), however, studied six cases of infantile cardiac arrest and reported periventricular leukomalacia as the predominant finding. Unfortunately, they did not mention examination of the brain stem in these children. As they referred to the work of Ranck and Windle (1959) in monkeys, however, it is tempting to think that they would have reported brain stem lesions in their cases if they knew them to be present.

The sequelae of acute total asphyxia in the monkey fetus and circulatory arrest in the human infant differ from each other in their effect on the visual system. Windle (1968) reported, and Myers (1968) confirmed, that the lesions affecting second order afferent neurons after

acute neonatal asphyxia in the monkey specifically spare the visual system. On the other hand, Brierley (1966) reported lesions of both the lateral geniculate bodies and calcarine cortex after circulatory arrest in children or adults, while Banker (1966) reported lesions interrupting the geniculocalcarine tract after infantile asphyxia or cardiac arrest.

Haemorrhage and brain swelling are concomitants of prolonged partial asphyxiation of the monkey fetus (Myers, 1969) but do not occur after acute asphyxia (Windle, 1968). Both have been reported in the human infant after perinatal asphyxia, although Brierley (1966) made specific note of the absence of brain swelling, and found haemorrhage in only a few of his cases.

When comparing experimentally induced lesions with neuropathology in the human, it has to be remembered that the damage in the human must be severe enough to result in death whereas, in the animal, the damage may be compatible with life. This distinction is particularly relevant with respect to the work of Windle. In his series, monkeys that showed brain stem damage without cortical involvement survived, whereas animals severely enough damaged by the experimental procedure or by subsequent respiratory distress to show cortical damage did not always survive. Windle's minimally damaged animals, those showing only afferent nuclei damage, must not be compared to human case material in which the damage is fatal.

It is important to recognize also, that just as under English law a defendant is presumed innocent until proven guilty, so in

pathology is a tissue considered normal by virtue of there being no evidence to the contrary. In other words, normality is defined by negative indices only, and subsequent refinements of technique may force a revision of diagnosis. For instance, Hicks, in 1953, reported that newborn rats immersed in nigrogen did not appear to have suffered nervous system damage. However, Hicks, Cavanaugh, & O'Brien (1962) pointed out nine years later "....that the infants' ability to survive periods of anoxia carries a price tag, and they virtually never escape injury....the injury (in the cerebral cortex) takes the form of inhibition of the proper development of the dendrites, yet no neurons are destroyed (p. 616)". Appropriate techniques revealed that asphyxiation of newborn rats results in inhibition of the maturation of nuclei of less mature neurons of the outer cortex, delayed formation of apical dendrites, inhibition of RNA production along apical dendrites of more mature neurons and stunting of dendritic growth.

Audiological sequelae

No prospective clinical audiological evaluation of children who had undergone asphyxia at the time of birth could be found, although studies of handicapped children draw attention to the fact that neonatal asphyxia may be a common denominator of the etiological factors under consideration. Thus, reports concerned with cerebral palsy and deafness will often mention some form of anoxia as an antecedent condition. Johnston (1967) correlated factors in the prenatal and neonatal histories of a group of 118 deaf children and 54 hearing children and found that

breech delivery and body blueness, among other factors, were significantly associated with deafness. Similarly, hearing deficits may be mentioned as an associated disorder with cerebral palsy or mental retardation resulting from perinatal anoxia (Billings, 1969).

Estimation of the incidence of hearing deficits in mentally retarded children vary according to the criteria of hearing loss used. Obviously, the lower the criterion, the greater will the incidence appear to be. Lloyd (1970) recently reviewed 31 audiometric surveys of mentally retarded populations. Despite differences between the studies in audiometric technique, acoustic environment, and calibration, as well as in definition of hearing loss, he found general agreement that a higher incidence of hearing impairment is found among the mentally retarded than would be expected from a non-retarded sample of the same age.

Johnston and Farrell (1953) pointed out that not only is the incidence of hearing difficulties disproportionately high among the mentally retarded, but the degree of the impairment, when it is present, is far greater than is usually found in impaired children with normal intelligence. This observation may have some relevance to the nature of the etiology of deafness in the two groups, or may merely reflect the poorer health habits of mentally retarded individuals. Johnston and Farrell noted that a larger percentage of mentally retarded boys than girls had hearing deficits. This is of some interest in view of the above mentioned greater susceptibility of males than females to anoxia.

Cerebral palsied children are typically multiply handicapped. Not only are there disorders of skeletal musculature, but there may be also disturbances of eye movement (Perlstein, 1955) as well as perceptual difficulties. Nevertheless, among the various deficits that these children suffer, auditory dysfunction seems to be particularly prominent. Partial or complete loss of hearing occurs in about 33% of such patients, being especially prevalent in athetoid[1] cerebral palsy, due to kernicterus[1] (Crabtree, & Gerrard, 1950; Markle, & Miller, 1963; Perlstein, 1955). Kernicterus is a degenerative condition of the brain in which groups of neurons in the brain become yellow stained and degenerate, apparently as a consequence of high levels of bilirubin in the blood. Children with kernicterus usually die shortly after birth, and in those that survive there may be extensive nervous system pathology, cerebral palsy or mental retardation. In addition, the majority of patients show some hearing loss (Byers, Paine, & Crothers, 1955; Perlstein, 1955). As many as 80% of surviving children with kernicterus have been reported to have complete or partial deafness (Cohen, 1956; Crabtree, & Gerrard, 1950). Windle (1968), however, found that experimental elevation of the bilirubin level in the blood of newborn monkeys resulted in jaundice, but not in kernicterus. It was only when asphyxia was superimposed on the hyperbilirubinemia, that kernicterus occurred. This association between hypoxia and hyperbilirubinemia with the subsequent development of cerebral

[1]See glossary, Appendix I

palsy, mental retardation and deafness has also been noted in the human (Nesbitt, 1966).

In general, the type of hearing deficit associated with a history of anoxia consists of a loss of acuity, particularly for high frequency tones (Fisch,& Osborne, 1954; Flottorp,et al., 1957; Perlstein, 1955; Zaner,& Miller, 1959) and primarily symmetrical in nature (Flottorp, et al., 1957; Goodhill, 1950, 1956). Most investigators believe that the deafness found in these cases is associated with lesions of the cochlear nuclei (Crabtree,& Gerrard, 1950; Fisch,& Norman, 1961; Goodhill, 1956). Fisch and Norman (1961) found no evidence of damage to the cochlea with hyperbilirubinemia, but did find staining of the cochlear nuclei. Crabtree and Gerrard (1950) examined the temporal bones and brain stem of one case of kernicterus and found no abnormality of the organs of Corti, the nerve fibers or ganglion cells. They did, however, find marked cellular losses in both dorsal and ventral cochlear nuclei.

Markle and Miller (1963) suggested another mechanism for the loss of auditory sensitivity in cerebral palsy. They hypothesized that the intratympanic muscles might be affected as part of the generalized neuromotor involvement, thus depriving the inner ear of the protection from intense acoustic stimuli that these muscles are believed to provide. They based this hypothesis not on direct evidence but on two cases of athetoid cerebral palsy in which the hearing loss developed several years subsequent to birth. The difficulty with this kind of reasoning is, of course, that by the time the patient is several years old, numerous other subject or environmental variables may have affected sensory function.

The same reservation applies to the conclusion of Flottorp, et al. (1957), that the lesion in athetosis was cochlear in nature, based on the observation that athetoid patients showed recruitment[1], intolerance for high intensity sounds and, in several cases, diplacusis[1]. As the testing was done at 11-28 years of age, it seems reasonable to assume that other secondary etiological factors might be involved.

[1]See glossary, Appendix I

Auditory Thresholds in Monkeys[1]

Most studies of auditory thresholds, including the present one, are restricted to the range of frequencies of clinical interest, 0.5 - 8 kHz. For this reason, only results concerned with these frequencies are reviewed. The relevant data are summarized in Table 1.

In nine studies, negatively reinforced behavior was used to obtain audiograms on several species of old and new world monkeys (Figures 1 & 2). In four of these (Behar, Gronholm, & Loeb, 1965; Clack, & Herman, 1963; Fujita, & Elliott, 1965 - bouble grill cage; Semenoff, & Young, 1964) an audiometric function similar in its essentials to the shape of the human audiogram (Davis, & Kranz, 1964) was reported, with maximum sensitivity between 1 and 2 kHz and a tapering off of sensitivity toward 0.5 and 8 kHz. Five studies showed a function decreasing from a minimum sensitivity at 0.5 kHz to a maximum acuity at 8 kHz, with, in three cases, a marked decrease in sensitivity (the so-called 'tonal dip') at 2 kHz (Fujita, & Elliott, 1965 - single-bar avoidance) or 4 kHz (Harris, 1943; Seiden, 1958).

Four studies have been reported that used a positively reinforced response to obtain audiometric data. Of these, one (Wendt, 1934) did not express the data in physical units and so cannot be compared to the other three. These showed considerable similarity from study to study (Figures 3 & 4). Regions of minimal sensitivity were at 0.5 kHz and between 2 and 4 kHz; maximal acuity was at 8 kHz, and in one instance also at 1 kHz.

[1] For an abbreviated classification of the species studied, see Appendix II.

TABLE 1

AUDITORY THRESHOLD MEASUREMENTS IN INFRAHUMAN PRIMATES: REVIEW OF LITERATURE

Author(s)	Criterion response	Species[1]	Number	Males	Females	Age[2]	Greatest acuity[3] kHz	Greatest acuity[3] dB[4]	Least acuity kHz	Least acuity dB[4]	Dip[5] kHz	Psycho-physical procedure[6]
Negatively reinforced												
Open-field Harris, 1943	Jump-cage avoidance	Mu,Ra	8	6	2	Adol. & juv.	8	-18.5	0.5	+10.5	4	d a d
Seiden, 1958	Shuttle-box avoidance	Marm	5	1	4	Adt. & yg. adt.	7	-8	0.5	+17	4-5	d a d a
Fujita & Elliott, ~~1965~~ Elder, 1934	Shuttle-box ~~avoidance~~	~~Squ~~ (monaural)	~~5~~	~~?~~	~~2~~	Yg. adt. ~~(monaural)~~ adol.	~~2~~	~~+2~~	~~0.5~~ ~~8~~	~~+11~~	-	~~d a~~
Stebbins, Green & Miller, 1966	Key-press	Ja,Nem	4	4	-	Yg. adt. & adt.	1	+2	4	+17		constant stimuli

1. Ba = baboon. Chimp = chimpanzee. Ja = M. java. Man = mangabey. Marm = marmoset Nem = M.nemestrina. Ra = M. radiata. Squ = squirrel monkey
2. Adt. = adult. Adol. = adolescent. Juv. = juvenile. Yg. = young
3. For averaged data.
4. re 0.0002 dyne/cm^2. Readings were extrapolated from figures when numerical values were not given in text.
5. Frequency band of decreased sensitivity interrupting audiometric function.
6. d = descending series. a = ascending series. Readings from left to right indicate successive series.
7. re 1 rms volt

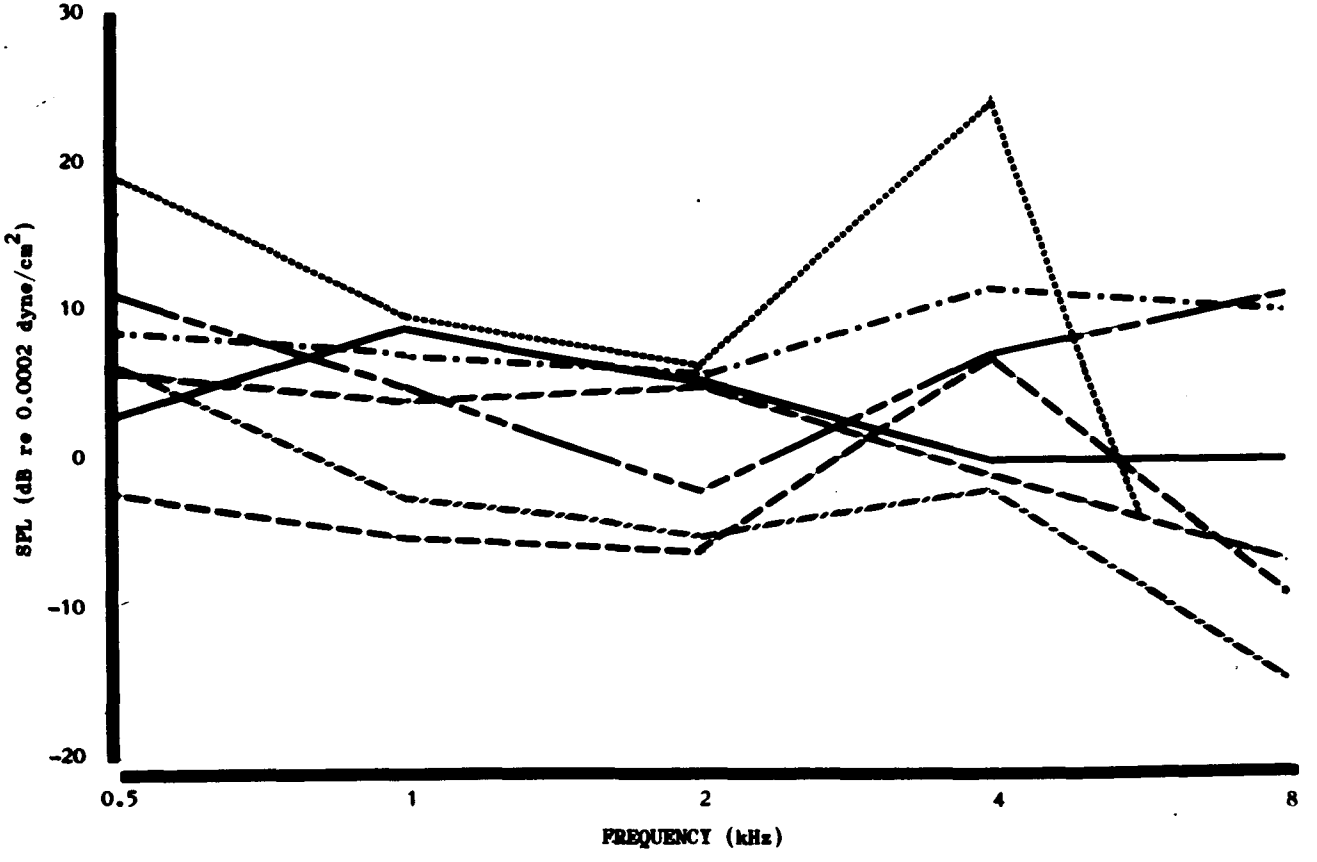


FIG. 1. Audiograms using infrahuman primates, free-field stimulus presentation and negative reinforcement. Allen, Dalton, Henton & Taylor, 1968 ——— Behar, Cronholm & Loeb, 1965 .—.— Clack & Herman, 1963 — — — Fujita & Elliott, 1965 (double-grill cage) — — — Fujita & Elliott, 1965 (single-bar avoidance) — — —. Harris, 1943 ——— Seiden, 1958 ———

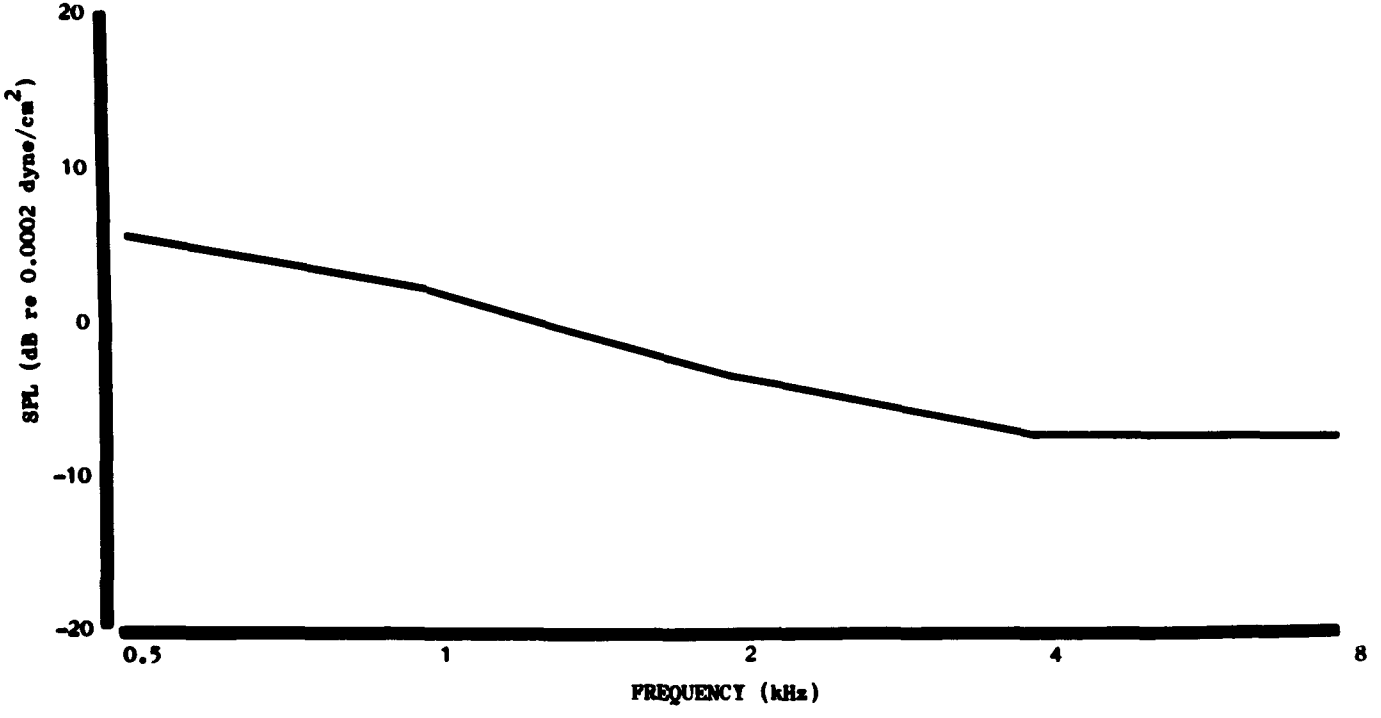


FIG. 2. Audiogram using infrahuman primates, closed-field stimulus presentation and negative reinforcement. Dalton, Taylor, Henton & Allen, 1969

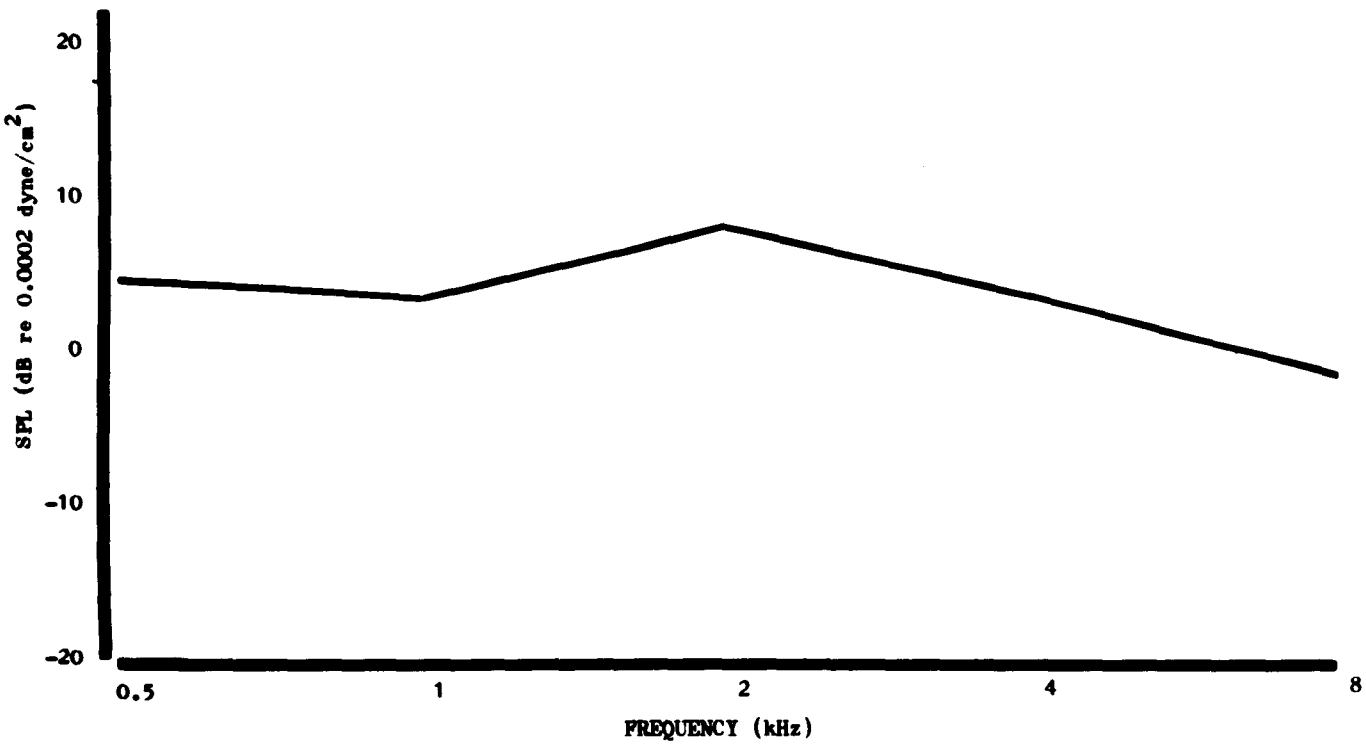


FIG. 3. Audiogram using infrahuman primates, free-field stimulus presentation and positive reinforcement. Fujita & Elliott, 1965.

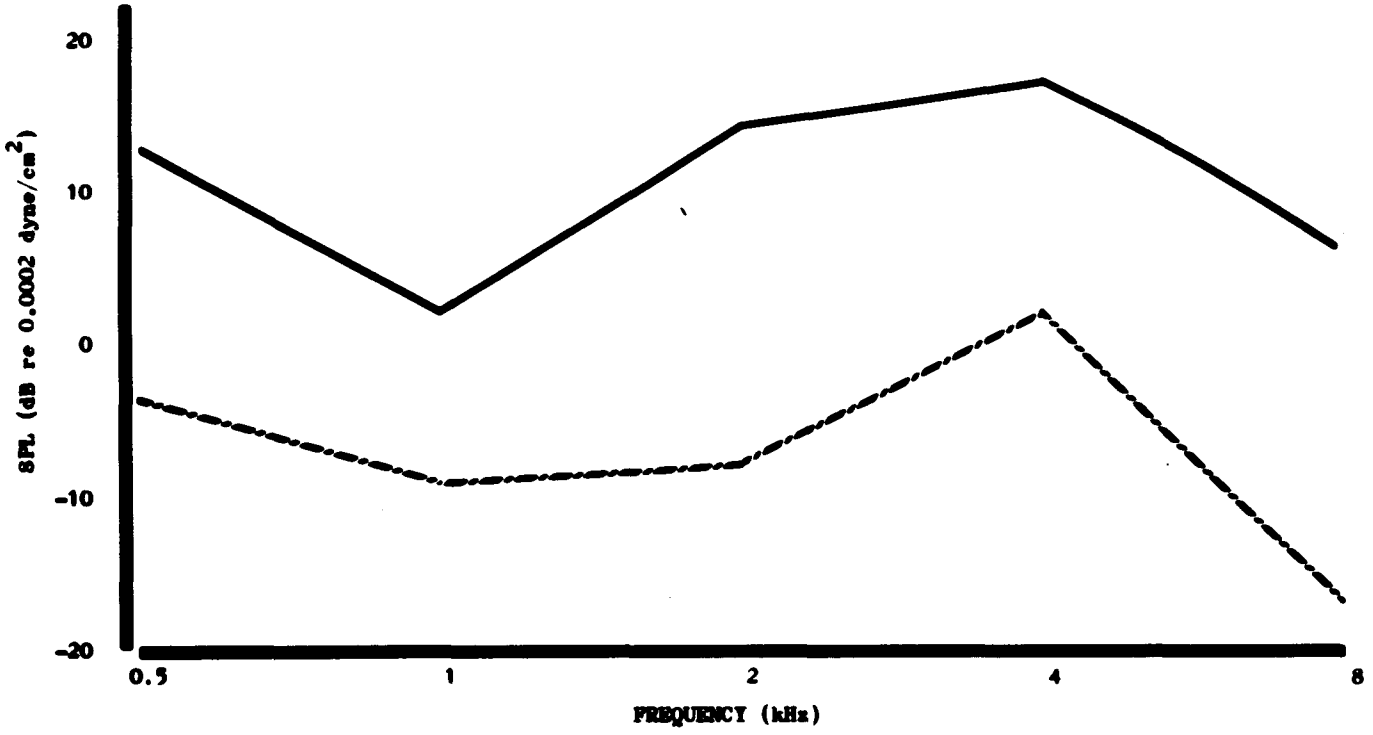


FIG. 4. Audiograms using infrahuman primates, closed-field stimulus presentation and positive reinforcement. Elder, 1934 ~~~~ Stebbins, Green & Miller, 1966 ——

It is possible to ascribe at least some of the variability in the auditory threshold data reported to procedural differences between studies. Threshold levels must be considered to be a function of factors such as the psychophysical method, the type of response required and the nature of the reinforcement contingencies. Any of these could be responsible for an elevation or depression of the audiogram in one study relative to the audiogram in another. They could not, however, account for differences in shape between audiograms. Whatever effect they exercise should operate more or less equally for all frequencies tested, provided only that the animal has generalized his training to the different test frequencies.

A number of studies (e.g. Fujita, & Elliott, 1965; Harris, 1943) used tones of various frequencies during training, thereby precluding this difficulty. Some reports (Clack, & Herman, 1963; Dalton, Taylor, Henton, & Allen, 1969), unfortunately, do not mention the nature of the training stimuli. One study (Semenoff, & Young, 1963) involved training the subjects with a 1 kHz tone only, and, once threshold values at that frequency had been established, testing other frequencies on other testing days. As lowest readings were obtained at the initial 1 kHz tone, the question arises as to whether values to frequencies above and below this might not have been lowered with further training at these frequencies.

The two obvious factors that might be responsible for differences between studies in the shape of the audiometric function are the method

of stimulus presentation and the species of monkey studied. In a free-field, standing waves may result in an artificially high or low threshold at a particular frequency, dependent on the dimensions of the sound field. Inadequate shielding from extraneous sources of sound may allow interference from those parts of the ambient coincident in frequency with the test tones, particularly at near-threshold intensities. With earphones, there are problems of fit, and of standing waves within the ear canal at the higher frequencies.

The question as to whether there may be species differences in hearing cannot be answered with certainty. Fujita and Elliott (1965) used three species and found similarity between them. The number of animals tested, however, was small, and the authors did not feel justified in drawing any firm conclusions.

One possible basis for a difference in frequency response between species would be in the shape of the external ear. Differences in behavioral response between species might, in addition, be responsible for shifts in the level of the audiometric function. Dalton (1968), for instance, found that habituation to the UCS in a GSR procedure was a problem with M. mulatta but not with C. capuchinus. On the other hand, he was able to use conditioned suppression with M. mulatta but not with C. capuchinus, which did not develop sufficiently stable base-line responding.

CHAPTER II

METHODS

In choosing the behavioral method used in this study, we were guided by our own experience with infant monkeys, as well as by the experience of other investigators in obtaining auditory threshold data in monkeys. It was apparent that any paradigm that depended on a response made for positive reinforcement would be impractical. Previous studies of this nature have had the disadvantage of false positives, particularly with near threshold stimuli (Elder, 1934; Wendt, 1934). In addition, Wendt (1934) found that not all animals could be trained to perform the necessary discrimination and, in fact, four out of eleven animals had to be eliminated from the experiment because they developed "persistent interfering habits (p. 29)". Further, the training of his remaining animals proved to be excessively long, 1203-3895 trials or 80-166 sessions. The use of food reinforcement is particularly inappropriate with infant monkeys because of the necessary dietary restriction. Previous attempts to use food pellets to reinforce a bar-pressing response in pre-adolescent monkeys (Berman, Waizer, & Dalton, in press) had to be abandoned because the food deprivation interfered with the animals' normal physical development.

The choice of an avoidance conditioning paradigm was dictated not only by indications that positively reinforced procedures were unsuitable, but also by the fact that avoidance methods, in one form

or another, had been used successfully in several studies of auditory function in monkeys (e.g. Clack,& Herman, 1963; Fujita,& Elliott, 1965; Harris, 1943; Seiden, 1958). Furthermore Kaye, Povar and Schrier (1966) found that shock used in classical conditioning of infant monkeys did not affect weight gain or constitute a health hazard. Avoidance procedures have the double advantage of not requiring either food deprivation or the training of complex response patterns. It was considered necessary to use as simple a response measure as possible so that differences in learning or performance between brain damaged and normal animals would not influence the results. The double-grill cage was selected principally because of the favorable experience of Fujita and Elliott (1965), who found it to be quicker and more efficient than bar-pressing procedures, although they conceded that this finding might have been a reflection of their greater familiarity with this technique.

The presentation of pure-tone stimuli, either through earphones or in a free-field, always presents problems in terms of possible stimulus artefacts. In this study, the difficulties involved in fitting earphones to infants with widely differing head sizes, plus the decision to use a shuttling response, resulted in the choice of free-field presentation.

Subjects

Fourteen monkeys, eleven M. mulatta and three M. fascicularis, were used in this study. The six experimental animals, all M. mulatta, were of known gestational age, 153-164 days (gestation for M. mulatta averages 164 days in this colony). Surgery was performed under aseptic conditions. No premedication was used, induction being effected by placing the animal in a box into which halothane (5%) was introduced through a tube passed through the wall. After induction, anesthesia was maintained with a mixture of halothane ($\frac{1}{4}$ - $\frac{3}{4}$%), nitrous oxide (60%) and oxygen (40%). A midline longitudinal incision was made through the abdominal wall. The location of the placenta was then determined by transilluminating the exposed uterus. The uterus was opened at a location free of large vessels or placental attachments and the fetal head was brought through the incision. Before respiratory exchange could take place the fetal head was covered with a rubber sac (Figure 5) and the umbilical cord clamped and cut. The infant was then transferred to a resuscitation table and maintained at a body temperature between 84 and 88°F. Seven to 10 minutes after the onset of secondary apnea[1], an endotracheal tube was introduced and resuscitation initiated.

Two of the eight control animals were of known gestational age (159 and 162 days), while the other six were judged by x-ray evidence to be at or near term. One control, 40, was delivered by

[1]See glossary, Appendix I

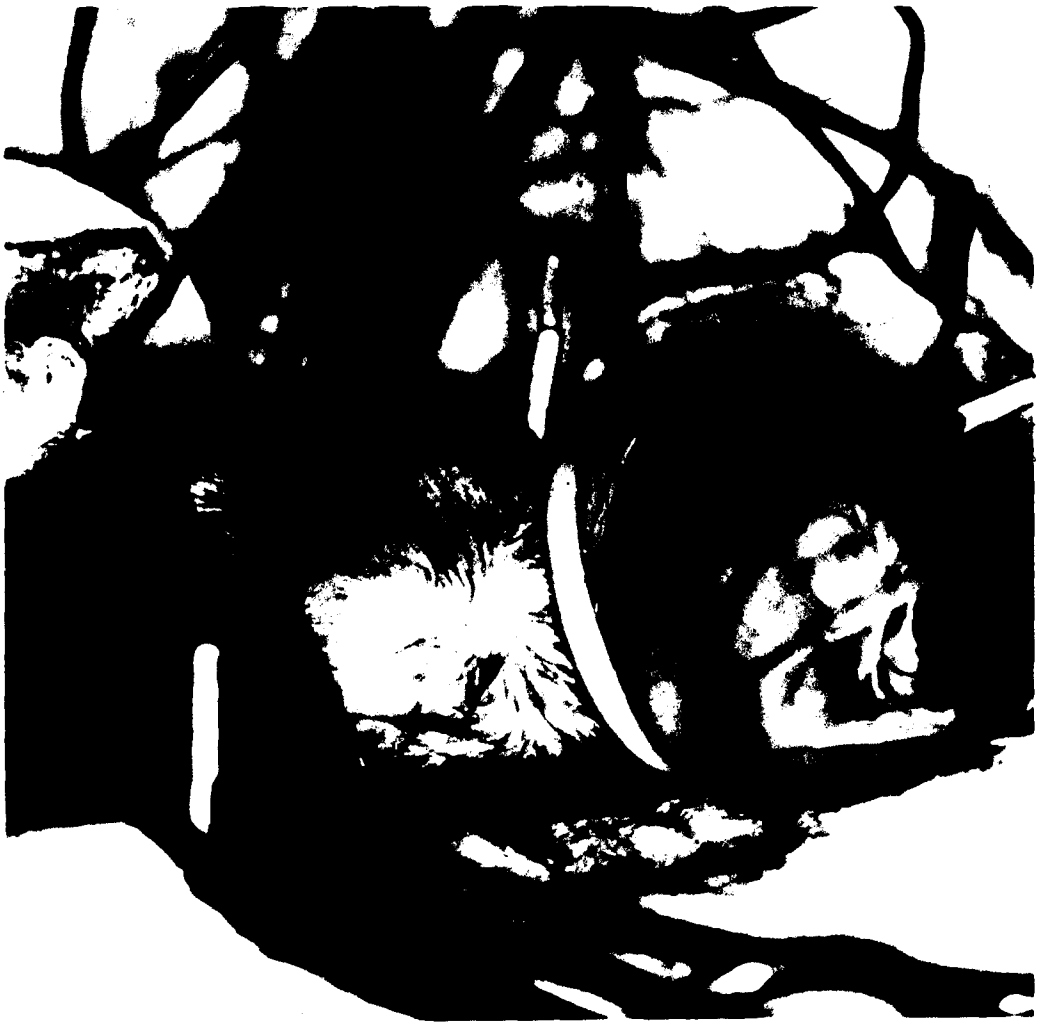


FIG. 5. Fetus, asphyxiated at birth. The fetus is still attached to the placenta via the umbilical cord. The head is covered with a rubber sac and EEG and EKG electrodes have been applied.

caesarean section, using nembutal anesthesia, while the remaining seven were born spontaneously and removed from their mothers within a few hours of birth. Age and birth data are summarized in Table 2.

TABLE 2

SUBJECT DATA

Subject	Species[1]	Sex	Mode of birth[2]	Gestational age (days)	Birth weight (gm)	Duration Secondary apnea (min)	Age at threshold testing (mos)
Asphyxiates							
83	R	F	C	161	355	$6\frac{1}{2}$	7
84	R	F	C	162	523	7	9
20	R	M	C	155	483	10	17
16	R	M	C	158	383	9	19
18	R	M	C	157	359	10	19
13	R	M	C	158	284	7	21
Controls							
76	R	M	S	159	468	-	7
42	R	F	S	162	483	-	10
71	F	F	S	feral	298	-	11
40	F	F	C	feral	284	-	16
31	R	M	S	feral	454	-	18
9	R	F	S	feral	520	-	19
10	R	F	S	feral	340	-	19
28	F	F	S	feral	348	-	19

1. R = M. mulatta
   F = M. fascicularis
2. C = caesarean
   S = spontaneous

All infants were kept in incubators for the first weeks of life, the exact time depending on the animal's condition. If there was respiratory difficulty, oxygen was administered. This was done through an endotracheal tube, or via a tube placed near the animal's nose and mouth, or was pumped into the atmosphere of the incubator; the choice of method depending on the severity of the animal's state. The infants were transferred to individual cages when they could move about freely, at 2–4 weeks of age. They were provided with terrycloth towels or lap-pads at all times.

Animals were hand-fed from small nursing bottles (Nip Pet Nurser, Poly Nurser, Brooklyn, N. Y.) for the first days of life, or until they were able to reach and suck from a bottle suspended from the side of their cage. The first feeding, of glucose 10% in water, was given at approximately 12 hours of age. For the next 24 hours the infant was given Similac formula diluted 1:1 with glucose 10% in water, after which it was switched to full strength Similac. Six feedings a day were given for the first 14 days, after which the number of daily feedings were reduced to four. At two months of age, the infants were given oranges and Purina crackers in addition to the milk and, by four to six months, were weaned to solid food and water. All animals were given multivitamins daily.

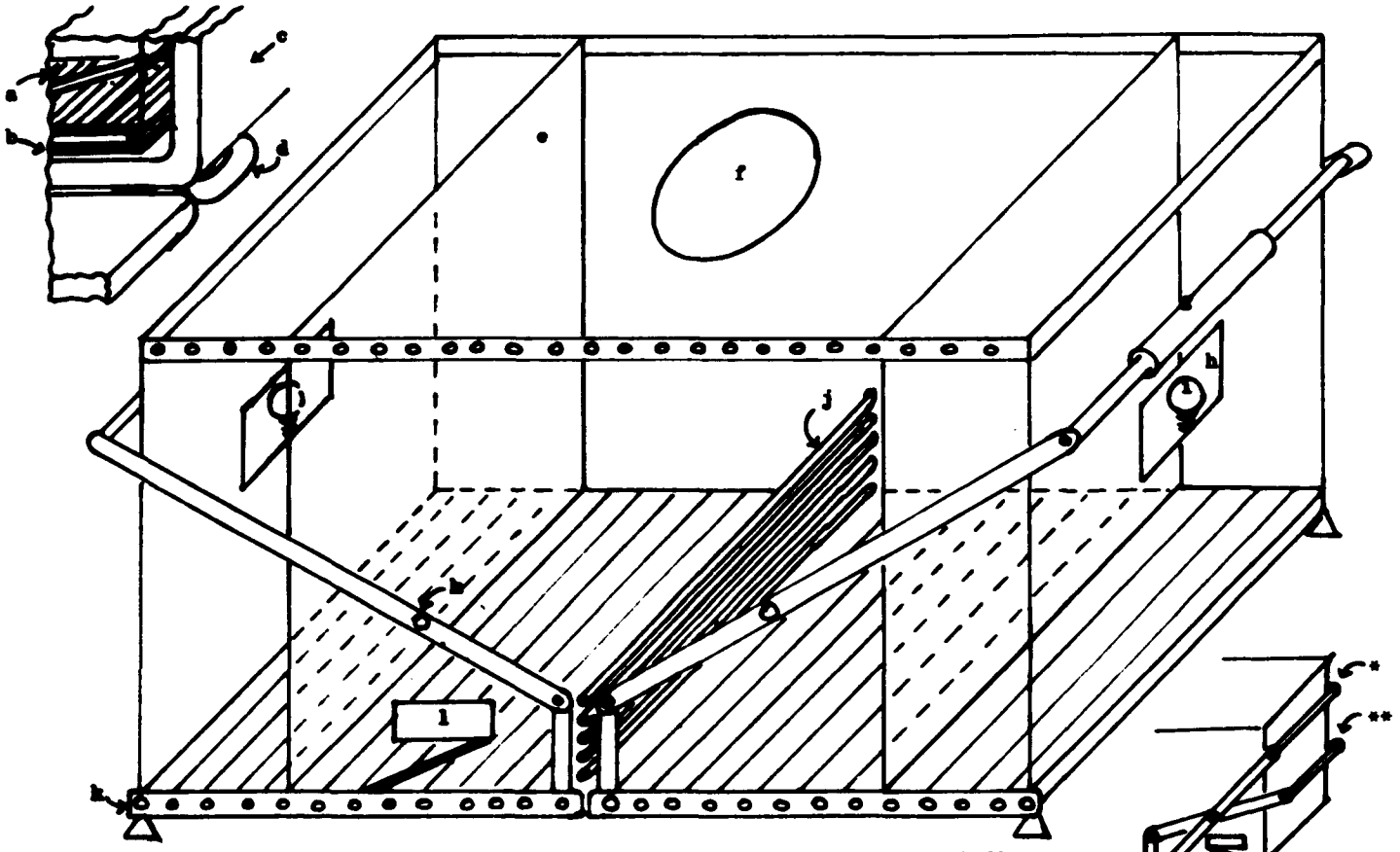
Double grill box training was started when the subjects were between five and 18 months of age and threshold data were obtained when they were seven to 21 months old.

Apparatus

Double-grill cage

A diagramatic representation of the double-grill cage is shown in Figure 6. The basic structure consisted of four walls of plexiglass mounted on a grid of stainless steel rods, the outer dimensions measuring 90 x 45 cm. The inner surfaces of the walls were lined with foam rubber, covered with smooth surfaced tape, except for two 6 x 9 cm cut-outs, one at each end of the box, made to accomodate a light source.

Within this framework, the box was divided into two 45 x 45 cm compartments separated by a hurdle. A copper mesh screen placed 15 cm from each end of the box reduced the effective size of the two compartments to 30 x 45 cm. The floor and hurdle were composed of steel rods, 6 mm in diameter and spaced 13 mm apart. The hurdle was adjustable up to a height of 6 cm, by adding or removing rods which were attached to the cage by means of threaded ends inserted through holes in the plexiglass walls. The lid of the cage was constructed of steel rods, with copper mesh suspended beneath. A speaker was mounted centrally on the lid of the box, with the speaker faced upward. This location and direction of the speaker were determined empirically to minimize standing waves in the testing area. A small light bulb was situated behind an opaque plexiglass window at each end of the box. Each side of the cage was made to pivot on its transverse axis



a. Double-grill cage
b. Pull-out shelf
c. Sound-retardant chamber
d. Rubber tire
e. Copper screen
f. Speaker
g. Counterweight
h. Semi-opaque window
i. Light bulb
j. Hurdle
k. Pivot
l. Microswitch, normally closed: * not activated
** activated

FIG. 6. Double-grill cage

so that the presence of a monkey in either compartment would open a normally-closed microswitch. The weight necessary to trigger the switch was 565 gm close to the hurdle and 770 gm at the furthest point from the hurdle.

Shock was provided by a Lehigh Valley 113-04 constant current shocker connected in parallel to the two sides of the cage through independent scrambler units. The necessary scrambler circuit was activated by the logic programming to provide shock to the appropriate side of the cage. Shock was pulsed for 50 msec/sec in order to reduce the possibility of habituation.

The double-grill cage was contained within a sound attenuating chamber and was mounted on tracks to permit its partial extraction and replacement when the access door to the chamber was opened. The attenuating chamber was mounted on inflated rubber inner tubes to help reduce the transmission of extraneous low frequency noise to its interior. The entire unit was situated in a carpeted and heavily draped room, with the programming equipment in an adjacent room.

Auditory stimuli

A block diagram of the audio equipment is shown in Figure 7. Pure tones of 0.5, 1, 2, 4 and 8 kHz were generated by a General Radio (GR) 1311-Au audiometric oscillator, with a rise-fall time of 100 msec controlled by a Grason Stadler 1387 exponential-envelope switch. They were attenuated via a 600 ohm Hewlett Packard 350D attenuator and led to a McIntosh 60 amplifier, the output of which (60 watts rms)

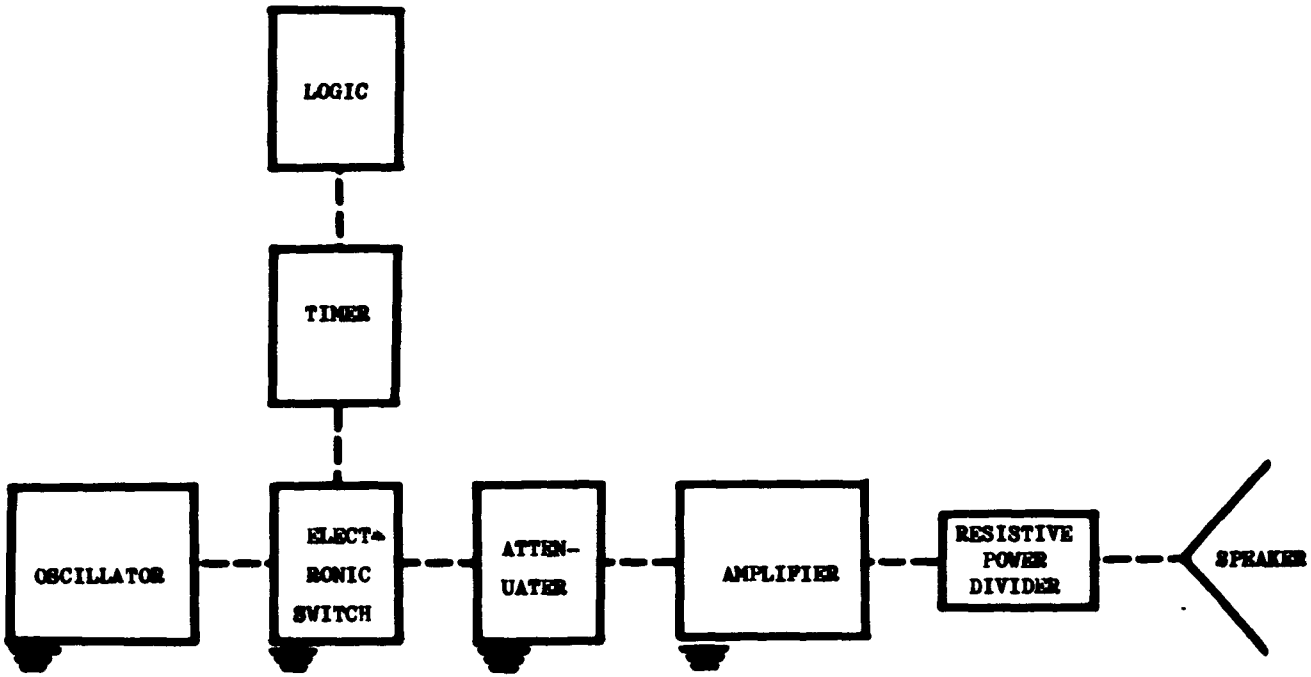


FIG. 7. Block diagram of audio equipment.

was reduced via a resistive power divider network to deliver 48-60 watts to an Acoustic Research 4 ohm, 1½ in, hemispherical direct radiator speaker, mounted on the lid of the double-grill cage.

Control and recording

Tone and shock schedules were controlled by Foringer BRS equipment, with the experimenter able to override the program. Frequencies were switched manually. Counters recorded the number of trials, escapes and avoidances within each session.

Calibration

All sound pressure level (SPL) measurements were made with a GR 1560-P5 ceramic microphone and GR 1560-P40 preamplifier attached by extension cable to a GR 1551-C sound level meter. The sound level meter was calibrated at intervals via a GR 1562 sound level calibrator. The microphone was placed in the desired location within the double-grill cage and readings were made from the adjacent room. All readings were made on the 20KC scale, which gives a flat response from 0.2 - 20 kHz. At the start of the study, the frequency composition of the ambient noise was analysed by means of a GR 1558-AP octave-band analyser. At intervals thereafter, usually in response to known changes in laboratory conditions but sometimes because of a subjectively noticed change in the nature of the ambient, the frequency distribution was checked by displaying the output of the sound level meter on an oscilloscope face.

Ambient SPL was measured at the start of each session and was

found to vary from 48-64 dB, depending on the time of day and the nature of the other activities going on in the laboratory. Threshold testing was carried out before 8:00 a.m., when the ambient was always below 58 dB. Initial analysis showed the frequency composition of the ambient to be predominantly below 250 kHz (Figure 8) and subsequent oscilloscope checks confirmed the stability of this distribution.

The possible existence of standing waves was checked periodically by measuring the unattentuated intensity of each frequency tone at a total of 36 positions within the box, including 18 locations 3 in above grid level and 18 locations 12 in above grid level. The difference in SPL between measurements taken 3 in and 12 in above the floor was usually 1-2 dB (re 0.0002 dyne/cm^2) and did not exceed 4 dB at any time. Differences in intensity for any given frequency between different horizontal positions were usually of the order of 3 dB, never more than 6 dB. These readings compare favorably with readings regarded as 'homogeneous' by other workers (Lidén, 1970).

At the start of each threshold session, the SPL of the unattenuated output of the oscillator was recorded for each frequency, with the microphone placed 3 in above the grill and adjacent to the hurdle, midway across the cage. This position was found to provide readings that did not differ significantly from the average of 36 different positions within the cage (Table 3). In order to calculate threshold intensities, the dB of applied attenuation was subtracted from the initial reading.

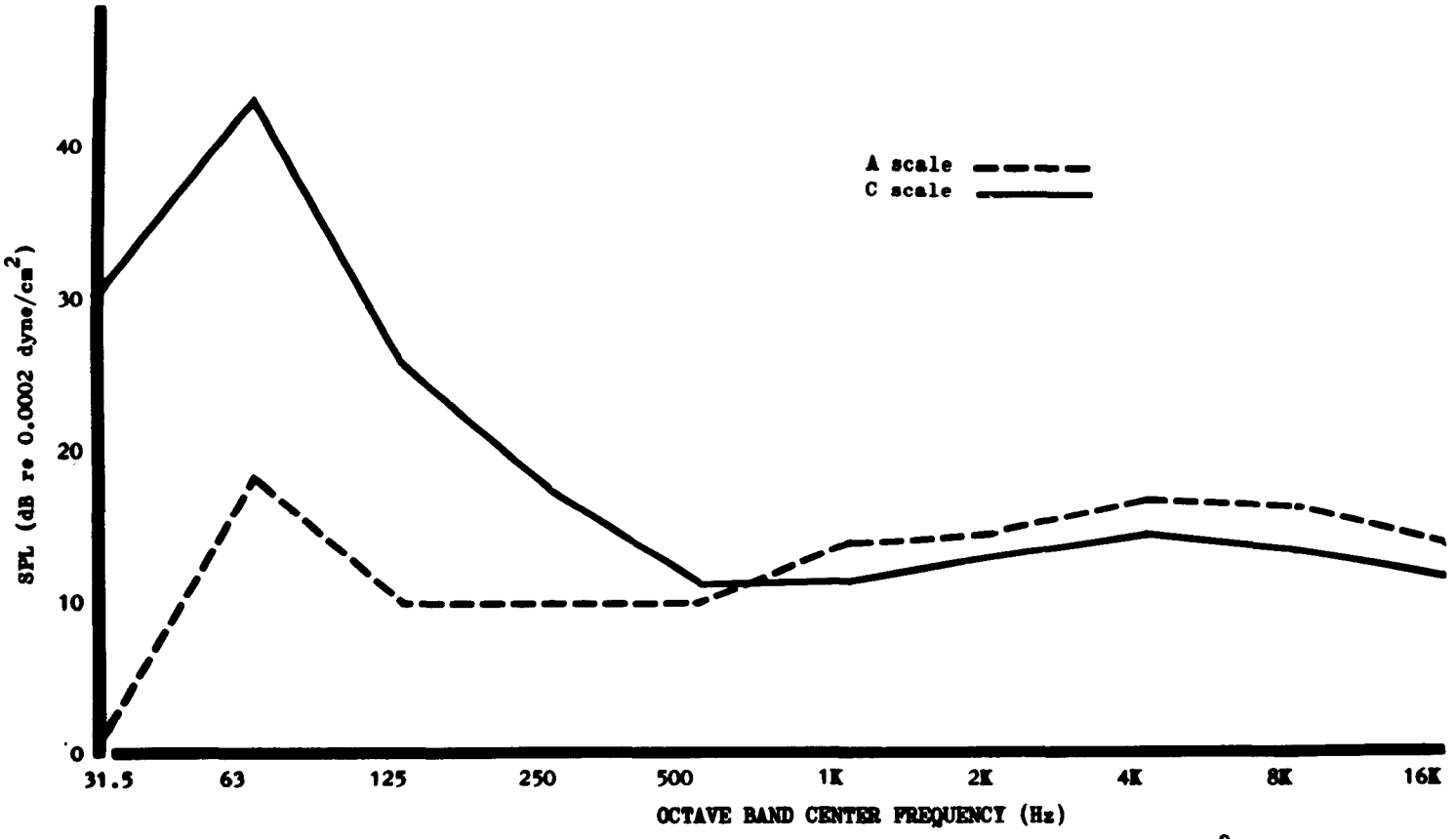


FIG. 8. Octave band analysis of ambient noise, 10.00 a.m., October 14, 1969. Temperature 78°F. Relative humidity 50%.

TABLE 3

COMPARISON OF CALIBRATION READINGS OBTAINED FROM CENTRAL POSITION WITH READINGS AVERAGED FROM ENTIRE BOX

(in dB re 0.0002 dyne/cm^2)

Frequency (kHz)	Central position		Entire box		Difference between means (t test)
	$\bar{X}$	SD	$\bar{X}$	SD	
0.5	85.5	1.44	83.8	1.14	not sig.
1	81.1	1.15	80.0	0.69	not sig.
2	88.9	1.53	88.8	1.75	not sig.
4	75.3	2.01	75.1	0.97	not sig.
8	73.3	1.41	72.6	1.45	not sig.

As a further calibration procedure, audiograms were obtained on six human subjects (for rationale, see Discussion). The microphone was placed in a central location within the double-grill cage and connected via an amplifier to a sound level meter, the output of which was led to earphones. Tones were presented in the same manner as for the monkeys, in groups of three at one intensity and starting below anticipated threshold. The intensity was increased in 10 dB steps until the subject responded positively on at least two out of three presentations, and was then reduced by a 5 dB step for three further presentations.

Procedures

In training the subjects for this study, emphasis was placed on having them reach a criterion of performance suitable for threshold testing as rapidly as possible. Pilot work had indicated that certain infants, unless shaped, would fail to escape and would simply curl up, cry and take shock. As it was imperative that all subjects acquire the desired response, and it was not known whether excessive durations of moderate intensity shock would be harmful to young animals, the monkeys were shaped by manually assisting them over the hurdle on those trials during which they failed to escape within the first 30 sec of shock.

Daily training sessions consisted of 50 trials randomly spaced 15-120 sec apart. For each trial, the conditional stimulus (CS) was presented for 10 sec during which time no current was passed through either side of the box. If the animal avoided by crossing to the opposite compartment during this period, the CS was terminated and the trial was over. If he failed to avoid, the CS remained on and his compartment floor was electrified until the subject escaped by crossing, thus terminating both shock and the CS. The intensity of shock was adjusted for each animal at each session to the lowest level at which responding could be maintained. Intertrial crossing was discouraged by energizing the side of the box opposite to the monkey except when the CS was presented.

Initial trials were carried out with light as the CS, in order to preclude the possibility that a hearing deficit might affect training.

When the subject achieved 75% avoidance for four sessions, supra-threshold tone (80-90 dB) was paired with the light, with frequencies manually switched to appear in quasi-random order. The light was then gradually dimmed over successive trials until the animal was responding to tone alone. If a monkey did not show acquisition with light as the CS, he was switched to light-plus-tone, to safeguard against the possibility that he might be suffering from a visual deficit.

When subjects reached a criterion of 80% avoidance to tone alone on at least three sessions, threshold determination was begun. Experimental and control animals were tested in a quasi-random order. Shock was turned off and, at each frequency tested, three trials at a given intensity were presented. Two avoidances out of three trials were taken as a 'positive' response; two failures to avoid out of three trials were taken as a 'negative' response. Tones were first presented at a level below expected threshold, after which the intensity was raised by 10 dB steps until a positive response was obtained. False positives (intertrial responses) did not occur in trained animals. The possibility of false negatives was considered and if, during ascending trials, a positive response was not obtained for six consecutive tone presentations, tone intensity was returned to training level. Shock was then turned on for six trials before threshold determination was resumed. Threshold for each day of testing was taken as the SPL halfway between that resulting in a 'positive' response and that resulting in a 'negative' response. All five frequencies were tested during each session, using a balanced order of presentation, and

testing was continued until the audiograms obtained on five out of six consecutive sessions did not differ from each other at any frequency by more than 5 dB. When this criterion was met, threshold at each frequency was taken to be the average of the five values obtained.

As a check on the psychophysical procedure used, four control and five asphyxiated monkeys were further tested, using a method of constant stimuli. A range of intensities was chosen for each frequency to be tested, such that the previously determined threshold value was centered among a group of 6-8 intensities separated by either 5 dB or 1 dB steps. At each frequency, the order of presentation of the different intensities was determined by a random sequence, with the constraint that each intensity appeared 10 times in all. In the case of two of the controls, two additional frequencies, 1.5 and 3 kHz, were included, in order to clarify some of the experimental findings.

Data Analysis

Threshold data on the two groups of animals were subjected to analysis of variance, using a two-factor design with repeated measures on one factor (Winer, 1962). For the purpose of this analysis, it was necessary to equalize the number of animals in the two groups. This was done in two ways, and the analysis carried out for each case. In the first, the two control animals that had the highest overall audiograms (76 and 31) were eliminated, thus accentuating the differences between groups. In the second case, the two control animals with the lowest overall audiograms (9 and 28) were discarded from the analysis, thus weighting the results against the finding of a difference between the two treatment groups. Threshold data were also compared between male and female control animals, male and female asphyxiated animals, and M. mulatta and M. fascicularis females, using, in each case, the Mann-Whitney U test.

Data obtained using the method of constant stimuli were graphed. For each frequency, the number of correct responses out of 10 presentations of the stimulus was plotted against intensity, and a reading made of the intensity at which the curve crossed the 50% ordinate.

Acquisition data, in terms of the number of sessions required to reach a criterion of 80% avoidance to tone, were compared for the two groups, using the Mann-Whitney U test. The association between rate of acquisition and age at the time of training was measured by the Spearman rank correlation coefficient.

Pathology

While it is recognized that it would be of interest to correlate the behavioral findings with information as to histological changes in the peripheral organs or central pathways mediating audition, neuropathology on most of the animals must await the completion of other experiments and cannot be included in the present report. One asphyxiated animal (13) became acutely ill with pneumonia toward the end of the study, however, and was therefore rapidly perfused with saline, followed by 10% formalin. The brain, brain stem and upper cord were removed and embedded in paraffin. Representative sections from the cerebral cortex, cerebellum, basal ganglia and thalamus were stained with haematoxylin and eosin and by the Nissl method. Serial sections through the brain stem were similarly treated. The temporal bones were removed and are being processed for histological study of the cochleas, although this data will not be included in the present report.

The brain of a monkey (46) that had not been included in the behavioral study, but that had died twelve days after neonatal asphyxia, was also embedded and representative sections stained. This animal had been subjected to eight and a half minutes of secondary apnea at birth, as compared with seven minutes for monkey 13. It was therefore felt that it might present more histopathological findings than the one experimental animal available.

CHAPTER III

RESULTS

Neurological Findings

The two asphyxiated infants showing the most severe deficits in the neonatal period were 18 and 16. No. 18 was subjected to 10 min secondary apnea, or a total of 18.5 min from clamping of the cord to iniation of resuscitation. Resuscitation was difficult and, in addition to oxygen, 2 cc of sodium bicarbonate was administered intravenously. The infant remained flaccid for several hours and was not responsive to painful stimulation. When movements did appear, they were present initially only on the left side. Over the course of the first seven hours of life, the infant underwent several episodes of total respiratory failure. In three instances the apneic periods were brief, lasting only a few minutes, and required only mouth-to-mouth resuscitation. In one instance however, the apnea lasted two hours and it was necessary to intubate and maintain the animal on a respirator. By the following day the infant was breathing adequately but his arms were kept crossed over his chest, with the elbow, wrist, and fingers all sharply flexed. When lying in the prone position the hindlegs were kept extended rostrally up and over the forearms (Figure 9). There was no righting reflex; crying, sucking and rooting were very weak. Sucking and rooting emerged more strongly over the ensuing days and the postural abnormalities subsided by one week. Ambulation was delayed until about two weeks of age.

In the case of 16, secondary apnea had been of 9 min duration,

FIG. 9. Asphyxiated infant 18 at three days of age.

but resuscitation was effected without undue difficulty. During the first day of life this infant had several episodes or recurrent seizures each lasting about 30 sec and involving the head and forelimbs. It had difficulty in opening its mouth and sucking was elicited infrequently. When elicited, the suck was very weak. Sucking became normally strong by day four but difficulty in opening the mouth continued for one week. One forelimb, either right or left, was kept extended and the contralateral one flexed until day nine. The infant became active by day three, but its movements were clumsy and it fell often. For the first three weeks of life, when it walked, it kept its hands dorsiflexed with the forelimbs extended across the chest to the opposite side of the body. When the infant attempted to scratch itself with its forelimb it appeared to miss the trigger region and frequently succeeded only in scratching the floor. Scratching seemed more effective with the hindlimbs. After three weeks of age these symptoms had subsided and the animal appeared normal.

The tendency during the first few days of life to keep one forelimb flexed and the other extended was seen again in 13, an infant subjected to 7 min of secondary apnea. Sucking was not impaired in this animal, although its cry was somewhat high pitched and low in intensity for the first few days of life.

Of the remaining asphyxiates, one infant (84), subjected to 7 min of secondary apnea, showed muscular fasciculations and failed to support its head on the first day of life, but otherwise appeared normal. Another infant (83), subjected to $6\frac{1}{2}$ min of secondary apnea, showed

absence of suck and righting for three days but otherwise seemed normal. In this particular case, the mother had been anesthetized for caesarian section one week prior to actual delivery, but surgery had been cancelled because, on examination, the fetus appeared unduly small. The sixth asphyxiated infant (20), showed no abnormality, other than poor righting for the first days of life.

Of the eight control monkeys, six appeared normal in every respect. They were alert and responsive; sucking was vigorous, and rooting freely elicited during the first weeks of life. They cried loudly and vocalized freely. They moved actively from the first day of life, righted themselves with ease and could support themselves with the abdomen off the ground, often as early as the first day, never later than the fourth day of life. They walked within the first few days and often climbed, although they sometimes had difficulty getting down once they were up. One control animal (31) appeared to suck well, but did not display rooting and had some difficulty in feeding.

The last control (40), an infant weighing only 284 gm, was delivered by caesarian section under nembutal anesthesia. The infant was flaccid and did not suck well, or cry for the first two days of life. It is possible, however, that these symptoms were due to the depressant effects of the anesthetic. By the third day of life, the infant sucked well and became responsive and active. During the day, however, it developed respiratory distress and turned blue. It was treated with oxygen and antibiotics and appeared to make an uneventful recovery. There was, however, failure to gain weight, such that at 47 days of age

the infant remained at its birth weight. Subsequent to that time there was rapid weight gain, gradually tapering off, so that at four months of age the infant had reached an appropriate weight.

Acquisition

The intensity of shock used in this experiment did not exceed 10 ma for any animal, and was usually far lower. In no instance was there any indication of autonomic responses such as piloerection, urination or defecation, although a number of animals tended to 'freeze'. There was considerable variation between animals in the level of shock that was needed to maintain the response, but each individual animal showed day to day consistency. There was no difference between groups in the level of shock used.

The smooth surfaced tape lining the double-grill cage was effective in discouraging climbing in most animals. Two of the asphyxiated monkeys succeeding in escaping shock, however, by suspending themselves from the lid. In the first case (18), the animal hardly touched the grid during 11 sessions, after which the obvious solution was applied and the animal's forelimbs were tied behind its back during testing. In the second case (13), this remedy was applied after two free-swinging sessions.

Of the 14 animals in this study, five required shaping. Of these, two were neonatally asphyxiated (83 and 84) and three were controls (9, 31 and 42). The two asphyxiates and one of the controls (42) were among the youngest animals tested; the other two controls were among the oldest tested.

In four animals, two controls (40 and 42) and two asphyxiates (13 and 20), the addition of tone to the light CS did not alter the pattern of acquisition; in two animals, one control (9) and one

asphyxiate (16) there was a transient drop in rate, which recovered in 1-2 sessions. Two control animals (10 and 76) that had been working at a criterion of about 20% or less for 6 and 17 sessions respectively with light as the CS, jumped to about 80% within 4-5 sessions of light-plus-tone. One asphyxiate (83) that had required shaping for all of seven sessions with light as the CS, started to avoid within two sessions of light-plus-tone and reached 60% avoidance within nine sessions. Another asphyxiate (84) that had required extensive shaping for 21 sessions with light alone, continued to require shaping for a further 12 sessions with tone, but then "caught on" and reached the 80% criterion within six sessions. Similarly, a control monkey (31), that did not avoid, but escaped shock for 10 sessions with light as the CS, continued to escape for another eight sessions with tone-plus-light but then suddenly acquired the response and reached 80% criterion within four additional sessions. One asphyxiated monkey (18), that managed to escape shock for 11 sessions by hanging from the lid of the cage responded with tone as the CS on the first session that its hands were tied behind its back. Of the remaining two control animals, one (28) was switched directly from light to tone as the CS, without any decrement in performance, and one (71) was trained from the start with tone-plus-light.

There was considerable variation in the rate at which animals in both groups acquired the avoidance response (Table 4). There was no difference between groups, however, when mean number of sessions to reach 80% avoidance were compared (Mann-Whitney U, $p = .525$). The

TABLE 4

SESSIONS TO REACH 80% AVOIDANCE

Controls			Asphyxiates		
Subject	Age (mo)	# of sessions	Subject	Age (mo)	# of sessions
9	19	5	13	21	5
40	16	5	20	17	5
10	19	10	16	19	6
28	19	11	18	19	15
42	10	11	83	7	18
31	18	12	84	9	39
71	11	22			
76	7	22			
$\bar{X}$ = 12.3 SD = 6.58 r_s = -0.65 ($p < .05$)			$\bar{X}$ = 14.7 SD = 41.6 r_s = -0.62 (not sig., p = .05)		
Mann-Whitney U = .525 (not sig., p = .05) r_s (both groups) = -.853 ($p < .01$)					

monkeys showing the most rapid acquisition were among the oldest animals tested in both groups; those showing the slowest acquisition were, with one exception (31), the youngest animals tested. The Spearman rank correlation coefficient for both groups together and for the control group alone showed the negative correlation between age and training time to be significant ($p < .01$ and $< .05$ respectively). The correlation in the asphyxia group itself did not reach significance at the 5% level.

Thresholds

Individual audiograms obtained on the eight control monkeys are shown in Table 5 and Figure 10. At each of the five frequencies tested, readings varied from 9-17 dB across subjects, with the range of variation narrowest at the higher frequencies, 4 and 8 kHz. Threshold was lowest at 8 kHz in five subjects, 4 kHz in two subjects and was equally low at 8 and 1 kHz in one subject. Threshold was highest at 2 kHz in seven animals and at 0.5 kHz in one.

Data obtained on the six neonatally asphyxiated animals are shown in Table 5 and Figure 11. Variability between subjects ranged from 6 to 18 dB at the different frequencies, with the smallest variance at 0.5, 2 and 4 kHz. The averaged audiogram was similar in shape to that obtained on the control animals, but with an elevation of threshold at each of the five frequencies (Figure 12). There was no overlap between the distributions of the asphyxiate and control groups at 0.5 kHz and little overlap at 8 kHz.

Analysis of variance carried out with the control group reduced to six subjects by the elimination of the two animals with the lowest audiograms is shown in Table 6. Analysis of variance with the control group reduced by the elimination of the two animals with the highest audiograms is shown in Table 7. In both cases, treatment and frequency are shown to have highly significant effects on the results obtained. There was no significant interaction between these main effects. In other words, asphyxia affected auditory thresholds

TABLE 5

AUDITORY THRESHOLDS OF CONTROL AND ASPHYXIATED MONKEYS

(in dB re 0.0002 dyne/cm^2)

	Frequency (kHz)				
	0.5	1	2	4	8
Controls					
76	12.0	7.5	20.0	2.5	-0.5
42	5.0	3.5	7.0	-6.5	-2.5
71	3.5	3.5	7.0	2.0	1.0
40	6.5	1.5	12.5	-1.0	-4.0
31	6.5	3.5	16.5	2.0	3.0
9	-3.5	-6.5	3.5	-2.5	-6.5
10	6.5	-1.0	3.0	1.5	-1.5
28	3.5	-1.5	7.0	-2.5	-3.0
Mean	5.0	1.5	9.5	-1.0	-1.5
SD	4.3	4.3	6.2	3.1	3.1
Asphyxiates					
13	13.5	15.5	12.0	8.5	18.0
84	14.5	7.5	12.0	3.0	7.0
83	19.5	15.5	21.5	6.5	4.0
16	17.0	-2.0	18.0	2.0	0
18	17.5	13.5	11.5	-1.5	6.0
20	17.0	12.5	19.5	5.5	5.5
Mean	16.5	10.4	17.6	4.0	6.7
SD	2.2	6.8	4.6	3.6	6.0

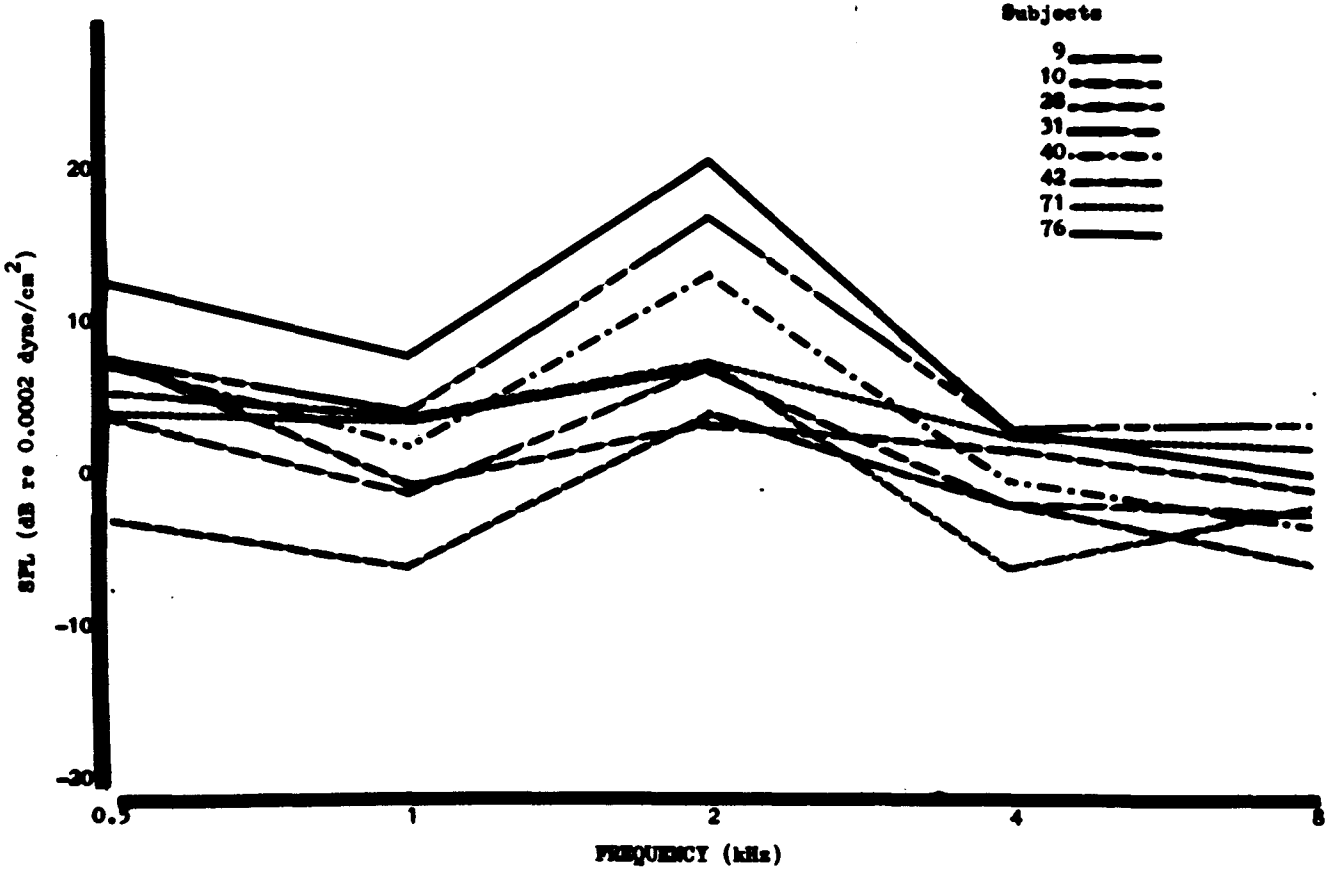


FIG. 10. Auditory thresholds of control monkeys.

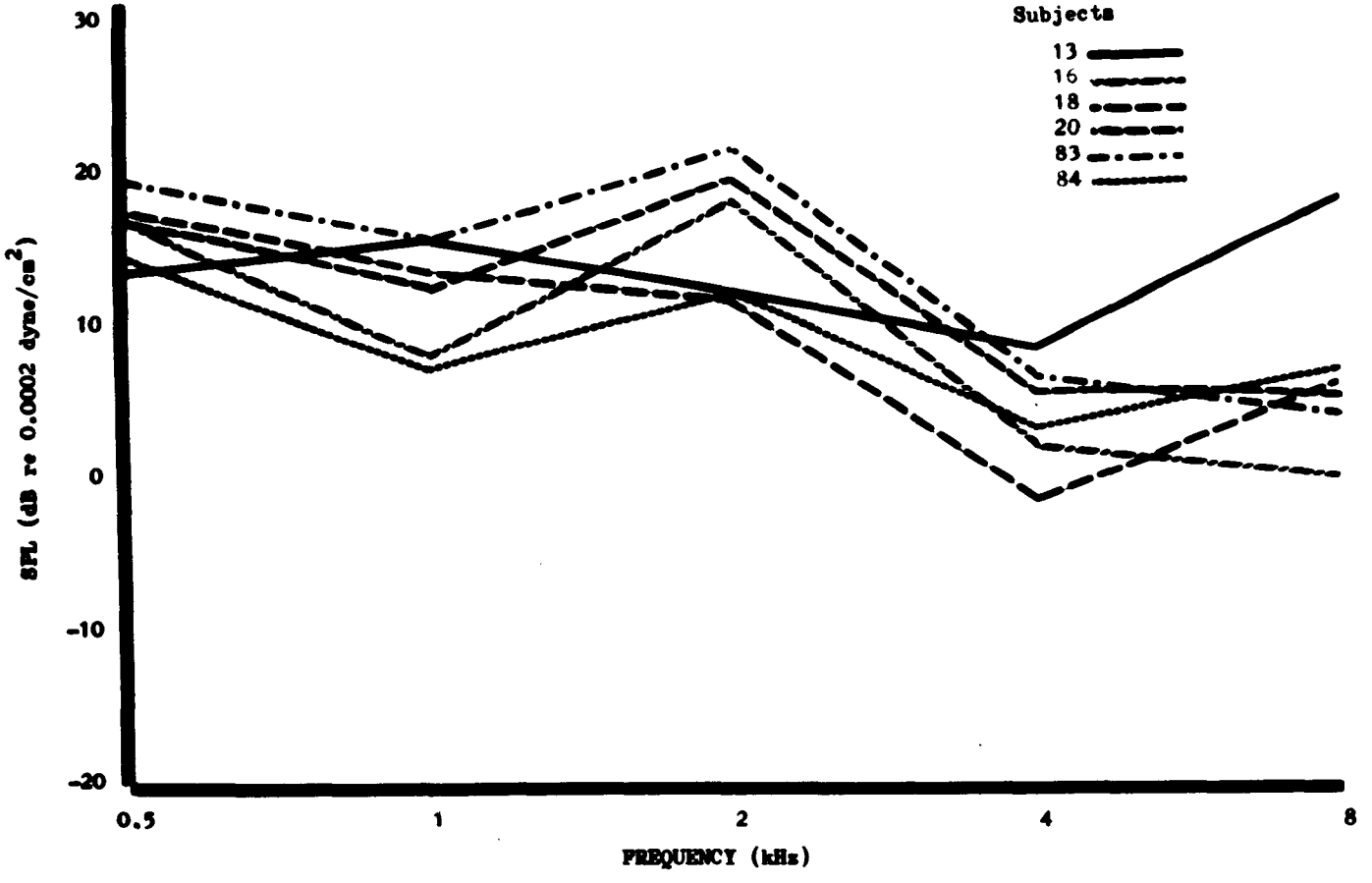


FIG. 11. Auditory thresholds of asphyxiated monkeys.

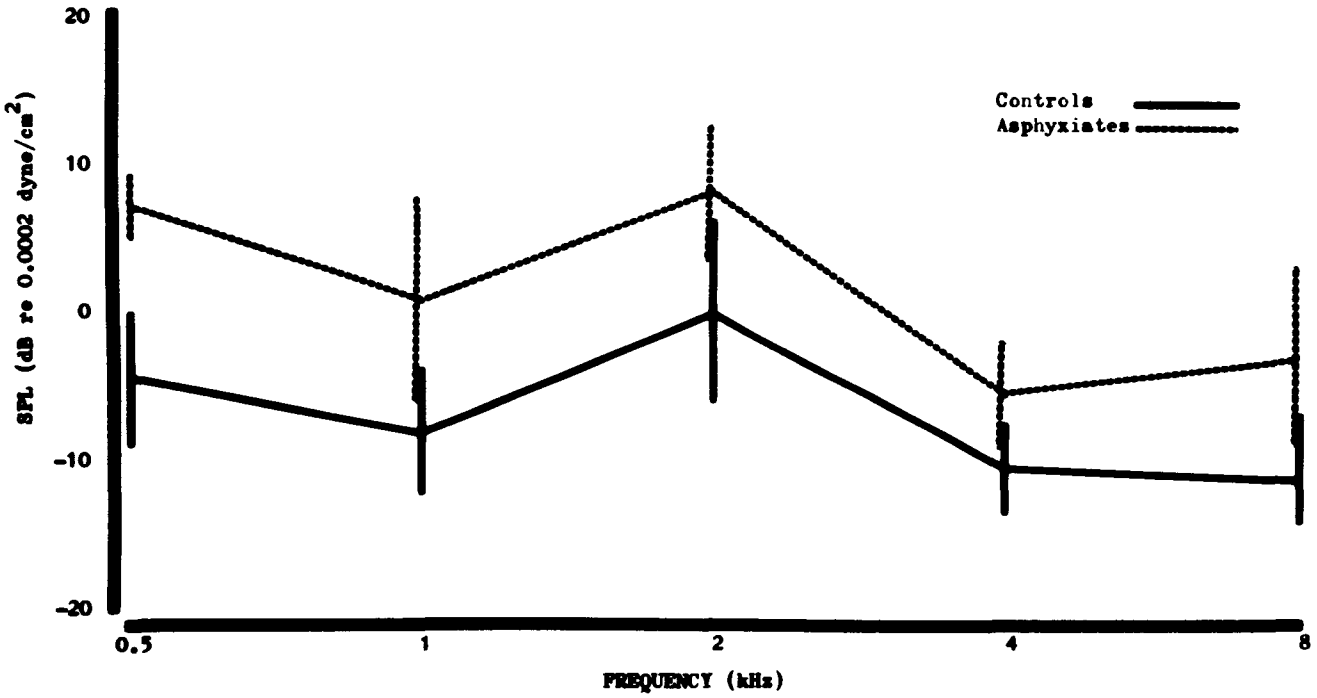


FIG. 12. Averaged auditory thresholds of control and asphyxiated monkeys.

TABLE 6

ANALYSIS OF VARIANCE WITH LOW THRESHOLD CONTROL MONKEYS (9 and 28) EXCLUDED

Source of variation	Sum of squares	df	Mean square	F	p
Between subjects	1111.16	11			
Treatment (T)	742.02	1	742.02	20.10	<.005
Subjects within groups (S)	369.14	10	36.91		
Within subjects	2030.28	48			
Frequency (F)	1360.82	4	340.20	22.12	<.005
T X F	54.18	4	13.54	0.88	
F X S	615.28	40	15.38		

TABLE 7

ANALYSIS OF VARIANCE WITH HIGH THRESHOLD CONTROL MONKEYS (76 and 31) EXCLUDED

Source of variation	Sum of squares	df	Mean square	F	p
Between subjects	1778.15	11			
Treatment (T)	1460.26	1	1460.26	45.93	<.005
Subjects within groups (S)	317.89	10	31.79		
Within subjects	1771.03	48			
Frequency (F)	1116.47	4	279.12	19.78	<.005
T X F	90.03	4	22.51	1.59	
F X S	564.53	40	14.11		

regardless of the frequency tested; thresholds differed across frequencies regardless of whether or not the animal had been asphyxiated.

The similarity in shape of the audiometric functions in the two groups of monkeys is represented in Table 8. For each animal, the frequency at which threshold was lowest was given a rank of 1, the frequency at which threshold was next lowest was given a rank of 2, and so on. This procedure permitted comparison of the shapes of different audiograms without reference to the absolute values involved. In general terms, the audiometric function could be described as decreasing from a high at 0.5 kHz to a low at 8 kHz, with a pronounced dip in acuity in the neighborhood of 2 kHz. The Kendall coefficient of concordance W, indicating the degree of association between subjects in the ranks assigned (Siegel, 1956), was significant at $p = < .01$ for each group.

Threshold data on control males and females, and on asphyxiate males and females, were compared for each frequency, using the Mann-Whitney U test. This analysis, summarized in Table 9, indicated that auditory thresholds of control males were significantly higher than those of control females at 2 kHz (5% level), but did not differ at the other frequencies. No significant differences in threshold were found between asphyxiated males and females.

TABLE 8

SCHEMATIC REPRESENTATION OF AUDIOMETRIC FUNCTIONS OF CONTROL AND ASPHYXIATED MONKEYS

(rank order of threshold values)

Subject	Frequency (kHz)				
	0.5	1	2	4	8
Controls					
76	4	3	5	2	1
42	4	3	5	1	2
71	3½	3½	5	2	1
40	4	3	5	2	1
31	4	3	5	1	2
9	3	1½	5	4	1½
10	5	2	4	3	1
28	4	3	5	2	1
	s = 596.5 Kendall W = 0.93 p < 0.01				
Asphyxiates					
13	3	4	2	1	5
84	5	3	4	1	2
83	4	3	5	2	1
16	4	1	5	3	2
18	4	3	5	1	2
20	4	3	5	1½	1½
	s = 194.5 Kendall W = 0.54 p < 0.01				

TABLE 9

AUDITORY THRESHOLDS IN MALE AND FEMALE MONKEYS
(in dB re 0.0002 dyne/cm^2)

	Frequency (kHz)				
	0.5	1	2	4	8
Controls					
Males (n=2)	9.3	5.5	18.2	2.3	1.3
Females (n=6)	3.6	-0.1	6.7	-1.5	2.8
Mann--Whitney U	1.0	1.0	0	0.5	1.0
p	0.07	0.07	0.036	0.07	0.07
Asphyxiates					
Males (n=4)	16.2	9.9	18.0	3.6	7.4
Females (n=2)	17.0	11.5	16.8	4.8	5.5
Mann-Whitney U	3	3.5	4.5	3	4
p	0.4	0.4	0.6	0.4	0.6

Audiograms obtained on three female control M. mulatta were compared with those obtained on three female control M. fascicularis. The results of the analysis are shown in Table 10, and it may be seen that there were no significant differences between the two species.

Audiograms obtained on both control and asphyxiated monkeys proved to be remarkably stable over time. Once the level of training necessary for threshold determination had been reached, the audiogram could be reproduced session after session with no more than 1-2 dB change at any frequency.

TABLE 10

AUDITORY THRESHOLDS IN FEMALE CONTROL M. MULATTA & M. FASCICULARIS
(in dB re 0.0002 dyne/cm^2)

	Frequency (kHz)				
	0.5	1	2	4	8
M. mulatta (n=3)	2.7	-1.3	4.5	2.8	3.3
M. fascicularis (n=3)	4.8	1.2	6.6	4.2	3.7
Mann-Whitney U	4.5	3.5	1.0	2.5	4.0
p	0.5	0.35	0.1	0.2	0.5

This stability was retained after lapses of many weeks or even months. If, for some reason, it became necessary to test a previously trained animal after an interval of no training lasting as long as four months, it was seldom necessary to give more than 10-12 suprathreshold trials with shock on before threshold testing could be initiated.

Thresholds obtained by the method of constant stimuli proved similar to those obtained by the step method used in the body of this study. Audiograms were secured on four control and five asphyxiated monkeys using the method of constant stimuli. Despite the fact that one week to 10½ months had elapsed since the earlier testing by the step method, readings varied by but a few dB from previous values. In two of the control animals, two audiograms each were taken with the method of constant stimuli, one using 5 dB intervals and the other 1 dB intervals. The values obtained were alike, within a 2.5 dB range, except for one reading, which varied 5.5 dB (Table 11).

TABLE 11

AUDITORY THRESHOLDS OBTAINED BY TWO DIFFERENT PSYCHOPHYSICAL PROCEDURES

(in dB re 0.0002 dyne/cm^2)

Subject	Method	Date	Frequency (kHz)				
			0.5	1	2	4	8
Controls							
10	Step	12/22/69	6.5	-1.0	3.0	1.5	-1.5
	MCS-5[1]	2/23/71	4.5	-1.5	-0.5	-1.5	1.5
	MCS-1[2]	3/19/71	3.0	-4.0	-2.0	-3.0	1.5
42	Step	2/27/70	5.0	3.5	7.0	6.5	-2.5
	MCS-5	10/29/70	8.5	2.5	6.5	-7.5	-5.5
	MCS-1	3/23/71	6.0	0	5.5	-8.5	0
71	Step	10/20/70	3.5	3.5	7.0	2.0	1.0
	MCS-5	10/28/70	4.5	4.0	6.0	-2.0	1.5
76	Step	7/2/70	12.0	7.5	20.0	2.5	-0.5
	MCS-5	12/1/70	14.5	8.5	21.5	2.5	-4.0
Asphyxiates							
16	Step	1/4/70	17.0	-2.0	18.0	2.0	0
	MCS-5	11/5/70	14.5	-0.5	16.5	2.5	6.5
18	Step	3/3/70	17.5	13.5	22.5	-1.5	6.0
	MCS-5	11/4/70	14.5	14.5	22.5	-2.5	6.5
20	Step	2/13/70	17.0	12.5	19.5	5.5	5.5
	MCS-1	3/24/70	15.0	13.0	17.5	10.0	3.0
83	Step	8/18/70	19.5	15.5	21.5	6.5	4.0
	MCS-5	12/6/70	18.5	13.5	21.5	7.5	5.5
84	Step	10/12/70	14.5	7.5	12.0	3.0	7.0
	MCS-5	12/4/70	18.5	8.5	16.5	2.5	10.5

1. Method of constant stimuli, using 5 dB intervals.
2. Method of constant stimuli, using 1 dB intervals.

In two controls, readings were taken at 1.5 and 3 kHz in addition to the usual five frequencies (Figure 13). In one of these animals (42), the shape of the audiogram was virtually unchanged. In the other animal (10), however, the previously obtained, relatively flat audiogram was changed to one still similar to other controls, but with the point of greatest sensitivity moved from 1 to 1.5 kHz and the 'dip' shifted from 2 to 3 kHz.

Absolute thresholds could not be obtained on the human subjects, due to a hum in the amplifier (not the same one as used for monkey audiograms). Masked thresholds were measured, however, and are presented in Figure 14, together with the curve of normal hearing suggested as the basis for zero-reference levels of audiometers by the International Standards Organization (Davis & Kranz, 1964). The masked threshold parallels the standard curve, but is about 7 dB higher in the range from 1-4 kHz. It falls off somewhat at 0.5 and 8 kHz, but shows no dip in the region of 2 kHz such as is seen in the monkey audiogram.

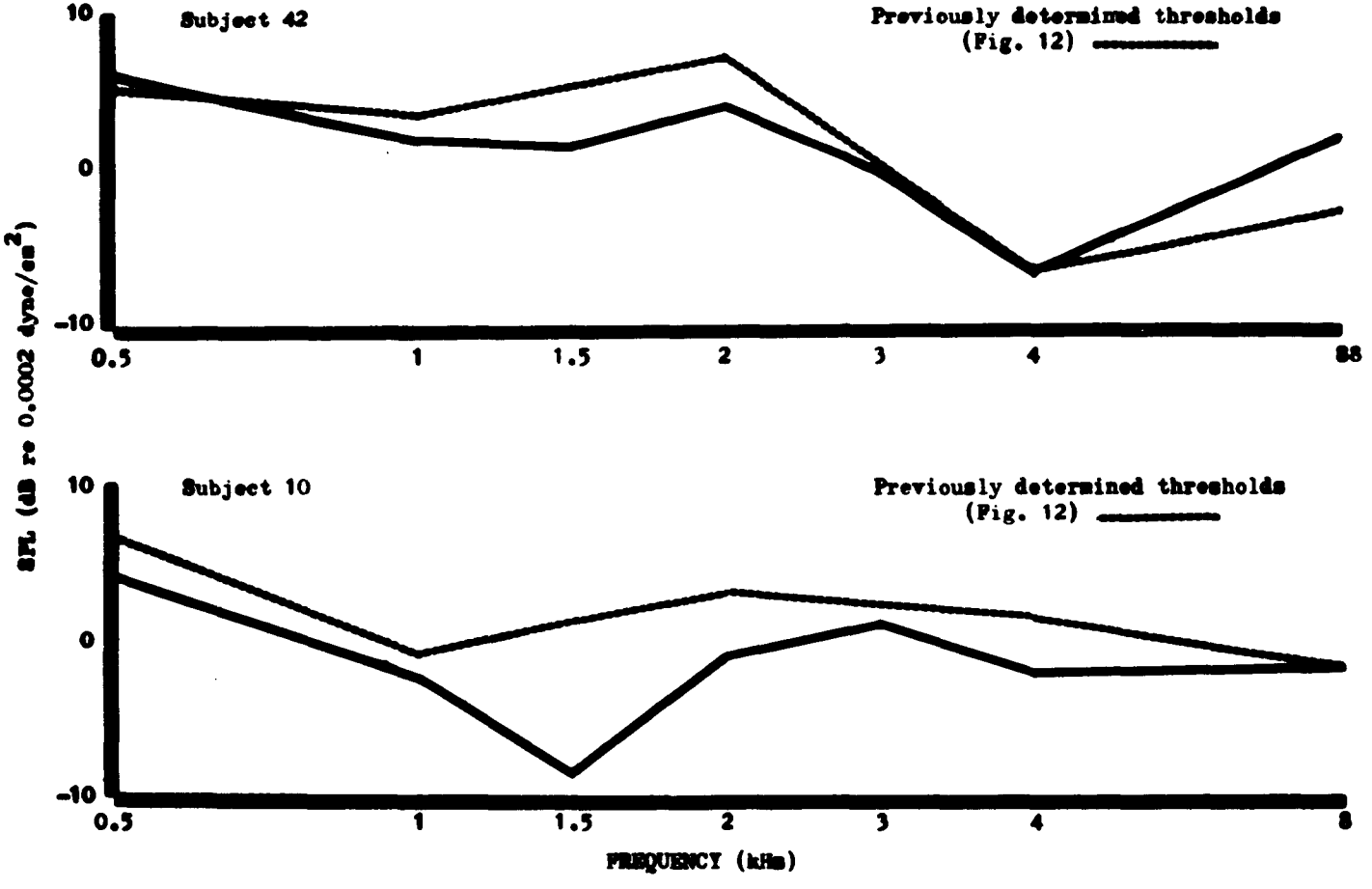


FIG. 13. Audiograms with interpolated readings at 1.5 and 3 kHz.

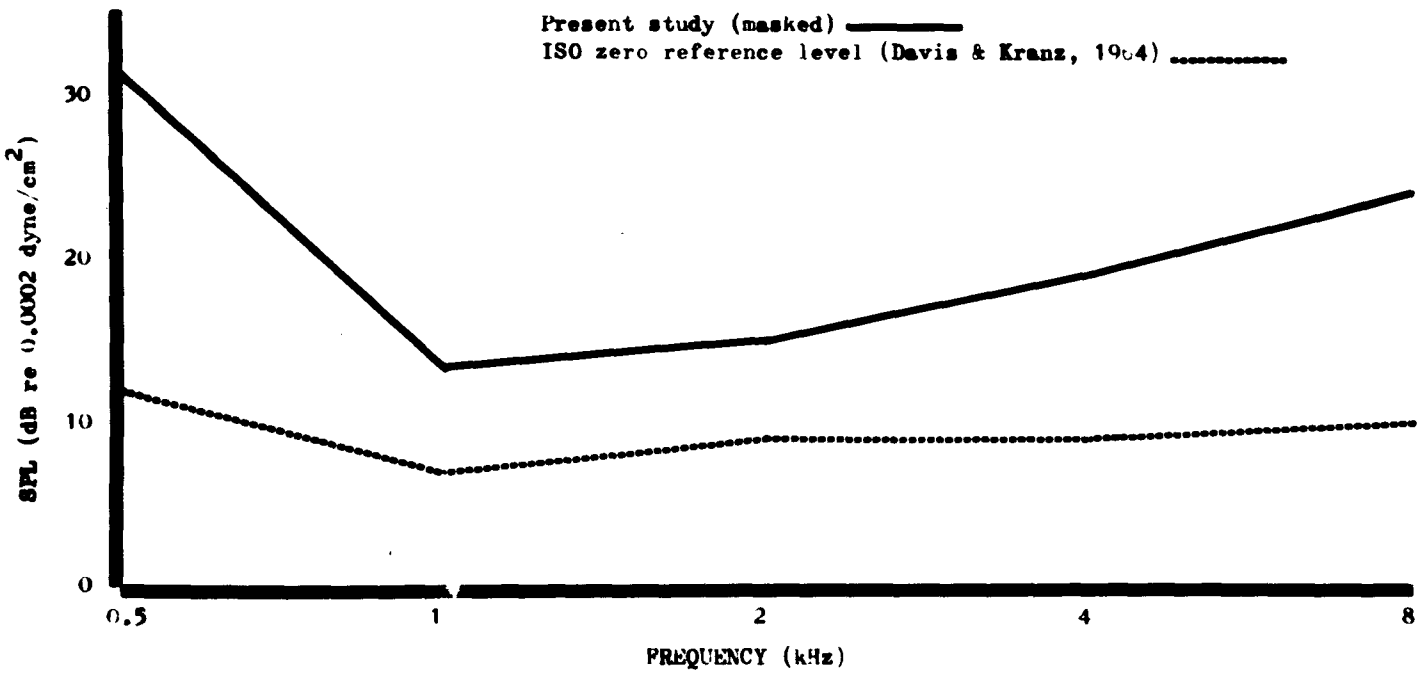


FIG. 14. Human audiograms.

Pathology

Histopathological examination was carried out on the brain and spinal cord of monkey 13, who was subjected to seven minutes of secondary apnea at birth, and died of pneumonia at the age of two and a half years.

No focal lesions were present in the cerebral cortex, basal ganglia or thalamus, and the cerebellum appeared normal in respect to all folia and nuclei. Neuronal loss was evident in the inferior colliculi, but there was no focal lesion. Neuronal loss was also seen in the trigeminal afferent and medial cuneate nuclei, while the superior olivary complex showed some atrophy. Because of the lack of uniformity in cell dispersion through the cochlear nuclei, the question as to whether there was cellular loss in this instance could not be answered without actual cell counts. The spinal cord showed no abnormalities.

In order to provide an example of the effects of a period of neonatal asphyxia more prolonged than that used for monkey 13, examination of the brain was also carried out on monkey 46. This animal, a female M. mulatta, was not included in the behavioral study. She was subjected to eight and a half minutes of asphyxia at birth, and died of pneumonia at 12 days of age. In this case focal lesions were present bilaterally in the inferior collicular, secondary trigeminal, medial vestibular, superior olivary and medial cuneate nuclei, as well as in the ventrolateral nuclei of the thalamus. No lesions were seen in the cerebral or cerebellar cortex or in the hippocampus.

CHAPTER IV

DISCUSSION

Methodology

The ambient SPL in the testing cage was higher than might be considered optimal for auditory work, although previous investigators (e.g. Bragg & Dreher, 1969) have worked with an equally high or higher ambient even within audiometric rooms. As it happens, the spectrum of the ambient was such that it was composed primarily of frequencies below those used in the study. The masking effect of background noise, if significant at all, would then have been limited to the lowest frequency tested.

Throughout the experiment, consideration was given to the possibility that artefacts in the sound field, such as would be produced by standing waves, might confound the findings. Frequent calibration of the field, however, revealed little variation from one location to another and considerable stability over time. Miller, Watson and Covell (1963), working with cats, pointed out that each threshold is an average based on several sessions and this tends to obviate sampling problems. Even had there been small peaks or troughs in the sound field, it seems unlikely that different animals would position themselves in such an identical manner that each would be responding to the same peculiarities of the sound pattern. Yet the threshold data on the eight control animals in the present study are in close agreement, both as to the range of hearing acuity and the shape of the audiogram.

The initial reading at each session was taken from a central

location because it was found that animals do not generally sample the entire field. By the time they are avoiding at the criterion required for threshold testing, they have developed idiosyncratic patterns of response such that each subject remains within a small area of the total field, most usually close to the hurdle. Miller et al. (1963) used the median of sound pressure levels recorded at different locations in the double-grill cage as the basis for their calculations of threshold intensity, using the argument that the cat sampled the entire sound field. In another context, however, they mentioned that their animals developed stereotyped behaviors in the double-grill cage.

The psychophysical procedure used was loosely based on the only previous study of monkey audiograms using the double-grill cage, that of Fujita and Elliott (1965), with several significant deviations from their methodology. Fujita and Elliott defined a successful trial as one in which the animal avoided to the first tone presented or, if it failed to do this, avoided on the next two presentations of the same intensity. In the present study, a successful trial was defined as one in which the animal avoided on two out of three presentations of a given intensity. In both studies, an unsuccessful trial was one in which the animal failed to respond on two out of three presentations of a given intensity. Our use of a slightly more stringent criterion might have been expected to result in somewhat higher thresholds than those of Fujita and Elliott, but, in fact, did not.

Fujita and Elliott presented trials in a descending order, starting 20-30 dB above estimated threshold, and increasing the attenuation by 10 dB steps. Following an unsuccessful trial, the intensity of tone for the next trial was increased by 5 dB. In the present study, similar size steps were used but stimuli were presented in ascending order of intensity and, after an unsuccessful trial, the intensity was decreased. The reason for this was that pilot work had indicated that the young animals used in this study tired before all frequencies could be tested if stimuli were presented in descending order. This was particularly true as the starting intensity needed to be quite high in order to accomodate anticipated differences in threshold between asphyxiated and normal animals.

In this study, in contradistinction to that of Fujita and Elliott, shock was turned off during threshold testing. It was found that by the time an animal was ready for threshold testing, he was performing at such an efficient level that he rarely, if ever, received shock. Furthermore, with the use of an ascending series, the animal would not detect the stimulus for as many trials as it took to approach threshold. If shock were left on, then the animal would receive what would be to him unsignalled shocks. Farrer and Prim (1965), working with chimpanzees, also found it necessary to turn off shock at the first failure to respond of a well trained animal. The interposition of suprathreshold trials with shock turned on, after a series of no-response trials, obviated the possibility of extinction. It also assured the animal's continued attention.

The fact that the audiograms obtained by the method of constant stimuli did not differ significantly from those obtained by the modified method of limits vindicates, in a sense, the choice of procedure. With the method of limits, the possibility exists for the animal to develop a pattern of responding, or not responding, which is guided by the ordered stimulus presentation. In the present case, the 'habit' of not responding during ascending trials may continue until there is a relatively high probability of signal detection. On descending trials, on the other hand, the 'habit' of responding may continue until there is a relatively low probability of detection. The randomization procedure incorporated in the method of constant stimuli assures that extraneous factors affecting signal detection will operate equally at all stimulus intensities and thus not influence threshold values. The greatest difference between readings taken by the two methods was 6.5 dB, and this was found with an interval of 10 mos between the two tests. Békésy (1947) pointed out that variations in threshold of even well motivated human subjects can be as great as 5 dB from one half minute to the next.

Neurological and Pathological Findings

An important difference between the procedure for the production of neonatal asphyxia used here and that used by Windle and Myers lies in the choice of anesthetic agent. Windle used only local analgesia; Myers used nembutal; in the present study halothane was employed. It would be anticipated, and the results tend to confirm, that anesthesia would interact to mitigate the effects of asphyxia. Moreover, anesthesia would, in large part, eliminate the emotional factors that must be present in the awake experimental subject, and that may in themselves affect the variables under study. Thus, the present results should be compared with those of Windle more in terms of the behavioral and neuropathological aftermath than in terms of precise durations of the inflicted asphyxia.

Animals in the present study displayed a degree of neurological deficit less than that reported by Windle for similar durations of asphyxia (de Arellano, McCroskey, Dennery, & Windle, 1959). The nature of the deficits were similar in the two cases, consisting mainly of loss of sucking and rooting reflexes and various manifestations of muscular incoordination. As with the animals studied by Windle (1968) these symptoms were transitory, and tended to disappear after the first few days of life. After one month of age, usually earlier, no animal could be identified as asphyxiated on the basis of its appearance, neurological examination, or cage behavior.

Further indication that asphyxia of comparable duration was less damaging to animals in this study than to those reported by Windle is found in the pathology on monkey 13. This animal, which had been subjected to seven minutes of secondary apnea, or a total of $15\frac{1}{2}$ minutes asphyxiation, showed lesions in the brain stem comparable to those reported by Windle (1971) in animals asphyxiated for a total of six to seven minutes. Similarly, brain stem pathology on monkey 46, asphyxiated for 17 minutes, was comparable to that seen by Windle in monkeys asphyxiated for 12 minutes (Windle, 1971).

It would therefore appear that halothane anesthesia is protective against the brain damage induced by neonatal asphyxia. Whether this protection is an effect of the anesthetic agent *per se*, or is the result of the concomitant lowering of body temperature can only be answered by further experimentation.

Acquisition

Because shaping procedures were used with some of the animals, the acquisition of avoidance responding of normal and asphyxiated monkeys is not precisely defined by the present study. Nevertheless, certain questions were answered. One of these concerned the possibility that neonatally asphyxiated monkeys might have had more difficulty than normal animals in acquiring the avoidance response. There is no indication in the results that this was the case. The average number of sessions to reach criterion was no greater for asphyxiated than for control animals. This was true even though one of the asphyxiated animals (18) removed himself from the learning situation, so to speak, for the first 10 sessions of training. Intrasubject variability did not differ between the experimental and control animals.

A tendency for the rate of acquisition of avoidance responding in the double-grill cage to increase as a function of age is strongly indicated by the data, particularly that on the control monkeys. The use of shaping does not permit firm conclusions to be drawn, but, nevertheless, a significant negative correlation was obtained. No previous report could be found concerned with the ontogeny of avoidance behavior in primates, presumably due to the tendency of infant monkeys, noted both here and elsewhere (Zimmerman & Torrey, 1965), to freeze in response to shock. This tendency would make any more rigorous study than the one conducted here difficult to accomplish with monkeys. It

would seem of interest, however, to pursue this point with some other more tractable species.

The possibility had been considered that sound might prove to be a less effective CS for the experimental than for the control monkeys, because of the anticipated damage to auditory structures as a result of asphyxia at birth. The results indicated that this was not the case; suprathreshold tone stimuli were at least as effective a signal as was light for both groups of subjects. In no case did an animal fail to respond to tone who had previously responded to light and, in some cases, a response was obtained to tone after no response had occurred with light.

Threshold Measurements

Normative data

It is difficult to explain the wide variation in monkey audiograms reported by previous investigators. Methodological differences can account for discrepancies in absolute auditory thresholds, but can hardly explain differences in the shape of the audiogram. Even if consideration is limited to those studies using negative reinforcers, review of Table 1 shows that five of nine previous studies are in substantial agreement with the present one in finding the maximum sensitivity at 7-8 kHz and the minimum sensitivity at 0.5-1 kHz. The other four studies reported opposite findings, with hearing maximal at 2 kHz and minimal at 8 kHz.

Because of the fact that four previous investigations had reported hearing to be most acute in the monkey at 2 kHz, whereas the present study found hearing to be depressed at this frequency, audiograms were obtained on human subjects, using the same sound field as for the monkey subjects. If there were some artefact in the field that was being missed by the calibration procedures, this should reveal itself in the human audiogram. For humans, the shape of the audiometric function is well established, and any deviation from it would have to be attributed to apparatus or procedural error. No proud claims can be made with regard to the data obtained, other than to point out that the shape of the human audiogram was as anticipated. No unexpected dip in sensitivity was found at 2 kHz; hearing was, in fact, optimal in this frequency range. This finding reinforced the belief that the

dip in sensitivity found in the monkeys represented a real aspect of the hearing function, not an unsuspected attribute of the sound field.

The conviction that the decreased sensitivity at 2 kHz was a genuine finding was further supported by the results obtained with frequencies bordering the one in question. If the decrease in sensitivity were due to a trough in a standing wave pattern, then responses to nearby frequencies, with their different periods, should reveal a sharp increase in apparent sensitivity on each side of 2 kHz. Only two animals were tested with the necessary extra frequencies, but these showed no such drop-off. In fact, one showed a broadening of the band of relative insensitivity from 2 kHz to 3 kHz.

Tonal dips have been reported in both humans and experimental animals. The dips in the human audiogram occur anywhere between 1 and 5 kHz, but are most common in the region of 4 kHz (Wever, 1942). Tonal dips at frequencies close to 4 kHz have also been found in cats (Miller, et al., 1963), marmosets (Seiden, 1958) and chimpanzees (Elder, 1934), as well as in monkeys (Wendt, 1934). Only two previous studies of monkeys reported a dip at 2 kHz. One of these was by Fujita and Elliott (1965), using a single-bar avoidance paradigm; the other by Stebbins, Green and Miller (1966), using a positively reinforced key-press response. A number of studies, however, have shown that it may occur at this frequency in individual animals.

It is unlikely that the dip in the audiometric function reflects resonant or absorbtive characteristics of the middle ear, since it is

found in species with widely differing sizes and shapes of ear. It also seems improbable that it is cochlear in origin. Wever (1942) attempted to correlate the presence of such dips in the human audiogram with the occurence of cochlear pathology but, with the techniques available to him, was unable to establish any such relationship. Furthermore, cochlear potentials in the marmosets used by Seiden (1958) did not show the same decrease in sensitivity at 4 kHz as did the behavioral audiogram (Wever, & Vernon, 1961). It is of interest to note, however, that when cats are exposed to broad-band noise at an overall SPL of 114 dB for $\frac{1}{4}$ - $\frac{1}{2}$ hour, their audiograms show a dip including one octave in the region of 1, 2 or 4 kHz, depending on the individual cat (Miller, et al., 1963).

The finding of a difference in threshold at one frequency (2 kHz) between male and female subjects was not altogether unexpected. Although the international standard for audiometers (Davis, & Kranz, 1964) establishes the same reference-zero level for men and for women, Corso (1963) reported that 18-24 year old women had somewhat better hearing, and less intersubject variability, than did men of the same age. Previous investigators of auditory acuity in monkeys have not mentioned sex differences, but, on the other hand, no study has been designed to parcel out this factor. Harris (1943) used four male and two females, but reported a complete audiogram on only one of the females. The thresholds for this animal were within the range of the male readings except at 8 kHz, where the threshold was higher for the

female than for the males. Seiden (1958) used one male and five female marmosets and, in this instance, the thresholds for the males were higher at 5, 7 and 10 kHz. 8 kHz, *per se*, was not tested. The only conclusion that can be drawn, of course, is that too few animals have been tested to make a valid comparison, but that there is no *a priori* reason to anticipate a sex difference. In the present study, although the statistical test chosen is appropriate for small numbers of subjects and has considerable power, the possibility cannot be excluded that there may have been some extraneous factor operating to affect the auditory thresholds of the two control males. Principal among such possibilities would be that these animals had undergone a period of asphyxia before, during or after birth, of which the investigator had no knowledge. Negating this interpretation, however, is the fact that the thresholds were elevated at only one frequency in the control males, as compared to the control females, whereas thresholds in the asphyxiated animals, male and female, were elevated at all the frequencies tested.

The lack of difference found between the auditory thresholds of *M. mulatta* and *M. fascicularis* is of some interest. Most previous studies have used only one species. Harris (1943), however, used two species of macaque, *M. mulatta* and *M. sinica*. His results for the two species were compared, using the Mann-Whitney U test, and are shown in Table 12. Clearly, no differences between the species were found. Stebbins, Green and Miller (1966) studied one *M. nemestrina* and three

M. fascicularis, and, again, the audiogram on M. nemestrina did not appear to differ from that of M. fascicularis.

TABLE 12

AUDITORY THRESHOLDS OF M. MULATTA AND M. SINICA (TAKEN FROM HARRIS, 1943) (in dB re 0.0002 dyne/cm^2

	Frequency (kHz)				
	0.5	1	2	4	8
M. mulatta (n=5)	7.1	-3.2	-4.2	2.0	only 1 reading
M. sinica (n=2)	5.7	-1.3	-6.4	2.7	
Mann-Whitney U	4.0	3.0	2.0	5.0	
p	0.429	0.286	0.4	0.571	

These results, and those obtained in the present study, differ from those of Fujita and Elliott (1965), who were the only previous investigators to look for species differences specifically. They obtained audiograms on four M. mulatta and three M. fascicularis, using a single-bar avoidance technique. Their data, extrapolated as best could be done from the published figures, are shown in Table 13. M. mulatta thresholds were significantly higher than those of M. fascicularis at 2 kHz, lower at 8 kHz.

The contribution of prematurity to the production of asphyxial brain damage cannot be evaluated in the present study, as all the experimental animals were delivered within nine days of term. One infant (13), however, was markedly small for age, weighing considerably

less than is usually considered necessary for survival. Despite the fact that this animal was subjected to one of the shortest periods of asphyxia used in this study, and did not display any marked neurological deficit, it had the most elevated thresholds of any animal at 4 and 8 kHz. High frequency losses of this type are typical of human infants with kernicterus (Crabtree, & Gerrard, 1950; Fisch, & Norman, 1961), but not of those born prematurely.

TABLE 13

AUDITORY THRESHOLDS OF M. MULATTA AND M. FASCICULARIS

(TAKEN FROM FUJITA AND ELLIOTT, 1965)

(in dB re 0.0002 dyne/cm^2)

	Frequency (kHz)				
	0.5	1	2	4	8
M. mulatta (n=4)	6.3	4.8	6.3	-1.3	-6.5
M. fascicularis (n=3)	2.3	0.7	2.3	-0.7	1.3
Mann-Whitney U	2.5	3.0	0	5.0	0.0
p	0.114	0.2	0.028	0.429	0.028

Among the controls, one infant (40) was significantly underweight. This animal was born to a mother which conceived in the feral state, and such pregnancies frequently result in premature births. The infant developed respiratory distress on the third day of life, much as human prematures are prone to do. There was, in this case, no elevation of auditory threshold at any frequency.

Auditory thresholds in neonatally asphyxiated monkeys

Consideration was given to the possibility that the altered thresholds in asphyxiated subjects might be the result, not of a deficit in sensory capacity, but of a deficit in some aspect of behavior that biased the results. Such an alternative interpretation seemed particularly relevant as differences in reactivity and emotionality between normal and neonatally asphyxiated monkeys have been reported (Saxon, 1961a, 1961b). Windle (1963) found that nuclei in the somatosensory pathways were almost as sensitive to neonatal anoxia as were auditory relay nuclei, so that it could be argued that asphyxiated monkeys might have an altered appreciation of pain. If this were so, then the experimental animals might be less responsive to shock and, consequently, more willing to 'risk' a false negative response than would be a control animal. It would follow then that such animals should take longer to reach a given criterion of avoidance responding than control animals. In the present experiment, however, no differences were found between groups in the level of shock required to maintain avoidance responding, or in the training necessary to acquire the conditioned avoidance behavior. This latter observation is in accord with the finding, using a Sidman avoidance technique, that asphyxiated animals avoid as well as normals if an auditory warning stimulus is provided (Ryman, Berman, & Berman, 1971). Furthermore, all animals were trained to an equally high criterion of performance before threshold testing was begun and, in many cases, were overtrained

for many sessions during which they avoided 100% of the time, taking no shock whatsoever.

The overall elevation of auditory thresholds found in neonatally asphyxiated monkeys does not parallel the audiological findings typical of children suffering the sequelae of perinatal asphyxia. Mentally retarded, kernicteric and cerebral palsied children who have hearing deficits usually show a loss chiefly for high frequency tones (Crabtree, & Gerrard, 1950; Fisch, 1956; Flottorp, et al., 1957; Markle, & Miller, 1963; Perlstein, 1955), although an occasional case is mentioned in which the loss is uniform across frequencies (Crabtree, & Gerrard, 1950; Goodhill, 1950).

Theoretical considerations would suggest that the symmetrical loss of hearing for high tones found in humans after neonatal asphyxia has its origin in a central, rather than peripheral, location. Certain investigators, nevertheless, favor the possibility of cochlear involvement, because of the presence of recruitment and, occasionally, diplacusis in patients with asphyxia-induced deficits (Flottorp, et al., 1957). These symptoms are usually considered indicative of end-organ damage, but study of the cochlear apparatus in such cases has revealed no evidence of damage to the organ of Corti, the ganglion cells or nerve fibers (Crabtree, & Gerrard, 1950; Hall, 1964). Hall, in fact, found preservation of the outer hair cells, the most sensitive parts of the auditory transducer, even when, for one reason or another, structural elements within the inner ear were damaged.

In order for the lesion to be cochlear in origin, the area of damage within the two ears would have to be identical to account for the symmetrical nature of the deficit and would need to affect only the basal turn to affect only high frequencies. Experimentally induced lesions of the cochlea, resulting from exposure to noise or to exogenously administered ototoxic substances, affect the basal regions of the cochlea first. As the severity of the insult increases, however, there is spread of the area of damage toward the apex (Kiang, Moxon, & Levine, 1970). Such lesions, therefore, do not offer a parallel to asphyxia induced lesions in the human.

Extreme anoxia is known to reduce the amplitude of the cochlear microphonic in the adult cat and guinea pig (Falbe-Hansen, Christensen, Gisselsson, Hansen, & Permin, 1958) but its effect is not frequency specific (Lawrence, & Wever, 1952). Chodynicki and Matwijewicz (1968) reported that asphyxia resulted in a diminution, usually transient, in the cochlear microphonic of the guinea pig fetus. They used only a 1 kHz test tone, however, so that no conclusions can be drawn as to the interaction of anoxia and frequency related cochlear structures. The reduction in amplitude of the response to this frequency, nevertheless, eliminates the possibility that the effect could be confined to high frequency sensitive components.

Pathological examination of the inner ears of experimental animals has shown that the hair cells are remarkably resistant to the effects of anoxia; this, despite the fact that the cochlear microphonic

is presumed to originate in the hair cells. If anything, it is the supporting structures of the organ of Corti that are affected by anoxia (Lawrence, & Wever, 1952). According to Falbe-Hansen, et al. (1958), even these elements remain unaffected. These results, all obtained on adult animals, tend to confirm the finding of a lack of damage to cochlear structures in human infants subjected to neonatal asphyxia.

The overall elevation of auditory thresholds in monkeys asphyxiated at birth might be attributed to loss of outer hair cells along the length of the cochlea or degeneration of the stria vascularis. A relationship has been demonstrated between outer hair cell density and magnitude of threshold elevation (Schuknecht, 1970), and the stria is believed to be the source of nutrients for the hair cells (Vosteen, 1963). Microscopic examination of the inner ears of monkeys asphyxiated at birth has not, however, yet been carried out. The reasons for this, presumably, are threefold; the technical difficulties associated with adequate preparation of cochlear material, the evidence that central pathways are themselves affected, and reports that no damage has been found in the inner ear structures of human infants asphyxiated at birth. In addition, it is conceivable that the degree of damage to hair cells necessary to account for a hearing loss as small as that found in the present study would necessitate the use of techniques more sophisticated than that of light microscopy for its detection. Electron microscopy, scanning electron microscopy or phase-contrast microscopy might be required to reveal minimal changes

in the organ of Corti (Engström, Ades, & Bredberg, 1970)

If the elevation in thresholds in asphyxiated monkeys were due to loss of outer hair cells or to changes in the stria vascularis, then it should be possible to demonstrate recruitment in these animals. This could be done, possibly, by using the stapedius muscle reflex as an objective test of recruitment (Lidén, 1970). In the absence of a positive test of recruitment, however, sufficient pathology has already been demonstrated to adequately explain the findings. It is conceivable that both peripheral and central auditory mechanisms could be affected by neonatal asphyxia, but there is at the moment no convincing reason to believe that end-organ damage is the principal cause of the resultant hearing deficit. In the interest of parsimony, two etiological factors should not be postulated when one will suffice.

Windle (1963, 1968, 1969, 1970) has found lesions in the inferior colliculi, as well as other structures along the central auditory pathways of monkeys asphyxiated at birth. There may be cellular loss in the cochlear nuclei, though the variability in size of the nuclei and the lack of uniformity of cell dispersion through their mass makes cell counts necessary before a positive statement to this effect could be made (Windle, 1971). The pathology found in the present study is completely consistent with these findings. Hall (1962, 1963, 1964) has found degeneration in the cochlear nuclei and also, to a lesser extent, in the inferior colliculi of children asphyxiated in the neonatal period.

Even though the locus of damage after neonatal asphyxia may be logically inferred to be retrocochlear in both the human and the monkey, the problem of explaining the difference in the audiological findings between the two still remains. One possibility is that there is a variation between the species in the distribution of frequency sensitive units through the affected nuclei. If the tonotopic organization in the human were such that cells responsive to high frequencies were in a position more vulnerable to the effects of anoxia than in the case of the monkeys, then the particular susceptibility to high tone hearing loss would be understandable. A second possibility is that the flat hearing loss found in the monkeys was but the first stage in a process which, if allowed to continue, would result in the sloping audiogram characteristic of the human. It is possible that the halothane used in the present study, which could not be blown off by the fetus because of the occlusion of the respiratory passages, exerted a protective action or interacted with the effects of lowered body temperature to prevent the degree of cochlear nuclear destruction found in the human. Reduction in body temperature has been shown to prolong the duration of gasping in asphyxia in several species (Miller, & Miller, 1965).

The third, and most plausible, explanation for the fact that neonatally asphyxiated monkeys show an overall diminution of sensitivity to tonal stimuli, whereas asphyxiated human infants show a loss primarily for high frequencies would be based on the evidence that there

is a difference between the two species in the relative vulnerability to anoxia of the inferior colliculi and the cochlear nuclei. In the human infant, the principal sites of pathology after asphyxia appear to be the cochlear nuclei, and lesions here are generally held to be associated with a high frequency hearing loss (Fisch, 1956, 1970; Barr, & Klockhoff, 1959). An explanation for this can be found in the observation (Katsuki, 1961) that cells in the cochlear nuclei have a fairly sharp high frequency cut off, but respond to a wide range of low frequencies. Thus, any partial lesion of the nucleus would have to spare the low frequencies.

In the monkey, the structure most vulnerable to neonatal asphyxia is the inferior colliculus (Myers, 1970; Windle, 1963, 1968). At this level of the auditory pathway, cells that are frequency sensitive have narrow tuning curves, without asymmetry favoring the low frequencies. Any lesion in the nucleus, therefore, might be expected to affect cells with different best-frequencies in a more or less uniform manner, so that any functional loss would not be frequency specific. In this regard, Raab and Ades (1946) and Oesterreich and Neff (1961) found that bilateral ablation of the principal cortical auditory areas in the cat did not affect the threshold for discrimination of an intensity difference, but that the additional ablation of both inferior colliculi resulted in an increase of the difference limen of 5-10 dB.

There are indications then that asphyxia at birth affects brain

stem nuclei in both the human and the monkey, but that the locus of greatest damage differs in the two cases. In the human, the cochlear nuclei are most severely affected and their involvement results in a loss of acuity for high frequency tones. In the monkey, the inferior colliculi are the most vulnerable of the brain stem nuclei and the damage inflicted upon them is reflected in an overall elevation of auditory thresholds. As asphyxiated animals typically show multiple pathological lesions, however, the final determination of the structure critically affected cannot be made on the basis of histopathology alone. Other experimental approaches, such as lesion studies of individual brain stem nuclei would be necessary before definitive conclusions could be drawn as to the neural substrate for the observed elevation of auditory thresholds in neonatally asphyxiated monkeys.

CHAPTER V

SUMMARY AND CONCLUSIONS

The vulnerability of auditory structures to the effects of acute neonatal asphyxia has been demonstrated by Windle (1963) and confirmed by Myers (1970) in the rhesus monkey. An episode of total anoxia, experimentally induced at birth, results in a pattern of pathology characterized by bilateral involvement of brain stem nuclei. Of these, the most sensitive to oxygen lack are the inferior colliculi, with other afferent nuclei of the auditory, vestibular and somatosensory systems becoming involved after longer periods of asphyxia. While previous studies on the behavioral capacity of neonatally asphyxiated monkeys had revealed deficits in reactivity (Saxon, 1961b), delayed response performance (Hyman, Parker, Berman, & Berman, 1970) and unsignalled avoidance behavior (Hyman, et al., 1971), there had been no previous investigation of the sensory capacity of such animals, although the predisposition of afferent relay nuclei to anoxic damage suggested that sensory deficits should exist.

In the present study, pure tone thresholds were obtained on six neonatally asphyxiated rhesus monkeys and were compared with those obtained on eight control monkeys. The asphyxiated animals had significantly higher thresholds at each frequency tested, 0.5, 1, 2, 4 and 8 kHz. The procedure used, shuttle box avoidance, provided reliable data on both groups of animals and no differences were noted between asphyxiates and normals in their ability to acquire the required avoidance behavior. Deficits in auditory thresholds were revealed

in animals that, despite a history of neonatal anoxia, showed few signs of damage on neurological examination.

With lesions in numerous subcortical auditory nuclei, deficits in auditory function in addition to the elevation of pure-tone thresholds would be expected. The possible areas of dysfunction are legion but, in particular, deficits in frequency or intensity discrimination or in auditory localization should be anticipated. It would seem appropriate, therefore, that neonatally asphyxiated monkeys be further tested to establish a profile for these behaviors, and that the obtained results be applied in the evaluation of human infants who have undergone anoxia during the neonatal period.

APPENDIX I

Glossary

Abruptio placentae	Premature detachment of the placenta[1].
Anoxia	Absence, or lack of oxygen.
Apnea	Transient cessation of respiration.
Asphyxia	Anoxia with lowered blood oxygen, increased blood carbon dioxide and lactic acid accumulation[2].
Atelectasis	Incomplete expansion of the lungs at birth[1].
Athetosis, athetoid	Slow, writhing, involuntary movements, especially of the hands.
Diplacusis	The perception of a single auditory stimulus as two sounds.
Hypoxemia	Lack of oxygen in blood.
Hypoxia	Lack of oxygen.
Kernicterus	Condition characterized by elevated serum bilirubin, with yellow staining of brain nuclei[2].
Leukomalacia	Softening of the white matter[4].
Microgyria	Condition of the brain characterized by numerous small convolutions[1].
Oligemia	Deficiency in the volume of blood[1].
Placenta praevia	Placenta developed in the lower uterine segment, covering or adjoining the internal os[1].
Polyhydramnios	Presence of excessive volume of amniotic fluid.
Porencephalic cyst	Cavity in brain developed during fetal life or infancy[1].
Primary apnea	Period of respiratory arrest following initial respiratory movements of asphyxia, followed by asphyxial gasping[3].
Secondary apnea	Respiratory arrest following asphyxial gasping. Also known as terminal apnea[3].

Status marmoratus	Lesion involving primarily the corpus striatum and thalamus with fiber networks ramifying through basal ganglia [5].
Ulegyria	Sclerotic or pseudo-microgyria, characterized by focal small, flat or retracted convolutions, with narrow sulci[5].
Watershed lesion	Asphyxial lesion in zone bordering two arterial sources.

1. Dorland, 1965
2. Windle, 1968
3. Windle, 1970
4. Banker, 1966
5. Malamud, 1963.

APPENDIX II

ABBREVIATED CLASSIFICATION OF INFRAHUMAN PRIMATES: INTERRELATIONSHIPS BETWEEN SPECIES REFERRED TO IN TEST

Suborder	Superfamily	Family	Genus	Species	Vernacular names(s)
Simiae	Ceboidea (New World monkeys, platyrrhine monkeys)	Callithricidae	Callithrix	Hapale Jacchus	Marmoset
		Cebidae	Saimiri	Sciureus	Squirrel monkey
			Cebus	Capuchinus	Capuchin
	Cercopithecoidea	Cercopithecidae	Macaca	Mulatta, rhesus Radiata Nemestrina Fascicularis, irus cynomolgus Sinica	Macaque Bonnet macaque Pig-tailed macaque Java, crab-eating macaque Toque macaque
			Cercocebus		Mangabey
			Papio		Baboon
	Hominoidea (apes and man)	Pongidae (great apes)	Pan		Chimpanzee

Adapted in part from Schrier, Harlow, & Stollnitz, 1965

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