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EFFECTS OF THE GENE Wh ON THE HEARING OF HAMSTERS USING AUDITORY BRAIN-STEM EVOKED RESPONSES

By

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ABSTRACT

EFFECTS OF Wh-GENE ON THE BSERs OF HAMSTERS
USING AUDITORY BRAIN-STEM EVOKED POTENTIALS

By

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The gene, anophthalmic white (Wh) in the Syrian hamster is an autosomal semidominant with pleiotropic effects leading to abnormalities eye development, pigmentation and hearing. While several investigations have been conducted on the morphologic, physiologic and behavioral abnormalities caused by this mutation, there is a complete lack of information on auditory brain-stem response of the genotypes currently available in the AN/As- Wh strain. Five sets of genotypes from the AN/As- Wh strain were used. Stimulus intensity was presented from 25-75 dB nHL (~ 50 to 100 dB peSPL). Filtered clicks with a major spectra at 2000 Hz and a repetition rate of 11.1/sec were presented to both ears of each animal. We demonstrate that genotypes differ in their responses to increasing intensity levels. As such, genotypes can be classified as normal, moderate-to-severe and profound with regard to hearing deficits.

DEDICATION

To my parents, Amegah (bereaved) and Kesevi, my wife, Doris, and children, Mawuli and Sitsophe.

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CHAPTER I

BACKGROUND AND PURPOSES

The golden Syrian hamster, (Mesocritus Auratus), was originally named Critus Auratus. It is believed that all Syrian hamsters currently being used as laboratory animals in Europe and the United States descended from three siblings, one male and two females, captured from Syria in 1930 (Adler, 1948). Since the time in which they were introduced to the laboratory setting, a number of mutations have been described and the genetics of several of these mutations have been investigated. As a result, at least two autosomal linkages have been established, in addition to sex-linkage. Several of these linkages are explained in Tables I-1, I-2 and I-3. It can be seen in Table I-1 that at least 16 mutations affecting coat color are known. Seven of these are of the recessive trait, while four are dominant. At least ten mutations affecting traits other than coat color are also known (see Tables I-2 and I-4). Of these, five affect hair, four appear to affect the nervous system and one affects the muscular system.

The gene anophthalmic white (Wh) (Table I-4) is one of the mutations that affect the nervous system. According to Asher (1968), this gene is perhaps one of the most potent in the Syrian hamster, as well as other mammals as related to disorders of the auditory system. This mutant gene was first described by Knapp and Polivanov (1958) as an autosomal recessive gene, inherited independently of the partial albino c^d mutation.

Beher and Beher (1959) demonstrated that the Wh mutation is dominant since the heterozygotes may be distinguished from the homozygotes. On this premise they suggested that the gene acted as a partial dominant. Based on this notion, they proposed the symbol Wh for the mutant gene. Heterozygous hamsters (Wh/wh) were found to be agouti, but posessed white belly fur as opposed to the pale cream fur of the wild type. Robinson (1962, 1964) described the Wh gene as incompletely dominant and indicated that animals homozygous for the mutant showed a complete absence of coat and skin pigmentation, while aplasia of the eyes resulted in extreme anophthalmia (see Appendix A for Definition of Terms).

Studies of the mutant Wh in the AN/AS-Wh strain (Asher, 1980) have revealed a strong interaction between Wh and cream (e). The expression of Wh is enhanced in the presence

of e and the homozygous cream (e/e) hamsters appear to be more active than the contrasting wild-type golden hamsters. In addition to the Wh and e, a mutation of the wild-type allele E, the extension locus show a strong epistatic interaction such that Wh/wh;E/E hamsters are white bellied agouti (the so-called imperial hamsters), while the Wh/wh; e/e hamsters are black-eyed whites (see table 4).

At the Biological Research Center at Michigan State University, inbred lines are maintained by full sibling matings. These inbred lines were designed to reveal several genetically-based differencies which provide several lines of experimental materials for bio-medical research. The strain contains the following genotypes segregating on a single genetic background: (1) wh/wh; E/E (agouti), (2) wh/wh;e/e (cream), (3) Wh/wh;E/e (white bellied agouti), (4) Wh/wh;e/e/ (black-eyed white) and (5) Wh/Wh; -- (anophthalmic white) (see table 4).

There are three major aspects of the gene that are well known: (1) The homozygotes Wh/Wh lack all melanocyte derived pigmentation, (2) they have severe optic degeneration but, for the purposes of this investigation, (3) they are deaf (Robinson, 1962, 1964; Yoon, 1973, 1975; Asher, 1981; and Asher and James, 1982). In fact, the gene Wh (anophthalmic white) of the Syrian hamster is known to be

Table 1. Mutations affecting coat color in the Syrian hamster (Yoon, 1973)

Gene Name	Symbol	Mode of inheritance	Description
Acromelanic white	cd	Autosomal recessive.	White pelage with dark pinna
Brown	b	Autosomal recessive	Amber in colour
CreaM	e	Autosomal recessive	Rich creamy yellow
Dark gray	dg	Autosomal recessive	Dark gray with less brown
Dermal pigmentation	-	-	Dermic melanism surpressed
Dominant spotting	Ds	Autosomal dominant	Irregular patches of white fur over the back and sides homozygotes are lethal
Frost eye	-	-	Gray hairs, occassional nomalies
Lethal gray	Lg	Autosomal dominant	Gray fur; lethal when homozygous,
Light undercolor	-	-	Whitish undercolour
Motled white	Mo	S-linked dominant.	heterozygous females have white areas
Piebald white	s	Autosomal recessive	Irregular patches
Ruby eye	ru	Autosomal recessive	Dilute coat colour
Rust	r	Autosomal recessive	Dark-brown
Tortoise shell	To	S-linked semidominant	Yellow in males and homozygous females, yellow patches in heterozygous females

Table 1 (continued)

Tawny White band	T Ba	- Autosomal dominant	Light agouti White band in trunk region

Table 2. Mutations Affecting Hair in the Syrian Hamster (Yoon, 1973)

Gene Name	Symbol	Mode of Inheritance	Description
Hairless	hr	Autosomal recessive	Sparse hair
Long hair	l	Autosomal recessive	Long hair
Naked	N	Autosomal semidominant	Heterozygous have sparse hair; homozygous are devoid of hair
Rex	rx	Autosomal recessive	Wavy hair
Satin has	Sa	Autosomal semidominant	Hair satiny sheen in hete-rozygous, and is satiny thin in homozygotes.

Table 3. Mutations affecting the nervous system (Yoon, 1973)

Gene Name	Symbol	Mode of Inheritance	Description
Anophthalmia	Wh	Autosomal semidominant	Homozygotes show acromia and anophthalmia and anomalous optic nerve and hearing; hetero-zygotes have diminished ventral hair
Hind-leg paralysis	pa	S-linked	recessive paralysed hind-legs
Hydrocephalus	hy	Autosomal recessive	Displacement of brain structures
Seizure	-	-	Frequent seizures

Table 4. The Syrian hamster from the AN/As- Wh and AN/As- E Strain

Gene Name	Symbol	Description
Agouti	wh/wh;E/E	Agouti on dorsal and pale yellow on ventrum
Agouti	wh/wh;E/e	Agouti on dorsal and pale yellow on ventrum
Cream	wh/wh;e/e	Dark yellow on dorsum and pale yellow on ventrum some white spots on ventrum
Imperial hamster	Wh/wh;E/E	White ventrum agouti on dorsal sprink white hairs
Imperial hamster	Wh/wh;E/e	White ventrum agouti on dorsal
Black-eyed hamster	Wh/wh;e/e	White with black eye
Anophthalmic	Wh/Wh;--	Lack all pigment, white blind and deaf.

a highly pleiotropic mutation, causing several morphologic, physiologic and behavioral abnormalities (Asher, 1968; Pratt, 1979, 1982; Hagen and Asher, 1983). The morphologic effects of the Wh mutation are to cause homozygotes to be deaf, blind and white. Further, a careful examination of the cochlea of the hamsters using light and electron microscopy reveal that the gene causes degeneration of the tectorial membrane, which becomes very apparent between 10 and 15 days of neonatal life (Asher, 1988). Other well known deleterious effects caused by this mutation include, infertility, small adrenal glands, growth retardation, reduced growth rates, increased metabolic rate with concomitant increase in food and water consumption, and altered plasma amino acid pools, (Asher, 1968, 1981).

While several studies have been conducted which reveal the morphologic, physiologic and behavioral abnormalities caused by the gene Wh, there is a complete lack of empirical data in the results of the audiology literature on the effects of the Wh gene on the hearing of hamsters. Mutations homologous to Wh are not unique to hamsters. That is, the Wh gene is believed to be homologous to the mouse mutant Mi^{Wh} and the Waardenberg syndrome WSI in man. Fundamental to this notion is that the Wh mutation showed a full range of phenotypic effects observed in the Waardenberg syndrome. Thus, Waardenberg patients are recognized by the following

characteristics: lateral displacement of inner canthi, a high broad nasal root, confluent eyebrows, heterochromia iridum, a white forelock or early greying, and congenital deafness of one or both ears. Despite the documented significance of the Wh gene as it relates to the Waardenberg syndrome in humans, however, an investigation to evaluate the hearing sensitivity of hamsters with this mutation using auditory brain-stem evoked responses has not been completed.

We know that the ABR can be used to effectively evaluate and monitor the hearing status of human infants (Hecox and Galambos, 1974; Salamy, Mckean and Buda, 1975; Schulman-Galambos and Galambos, 1975; Salamy and Mckean, 1976; Teas, 1982; among others). In the same vein, the ABR has also been investigated in other animal species including the cat (Jewett and Romano, 1972; Shipley, Buchwald and Norman, 1980; Laukli and Mair, 1982; Walsh, McGee and Javel, 1986a), rat (Jewett and Romano, 1972), mouse (Henry and Lepkowski, 1978; Shnerson and Pujol, 1982) and gerbil (Woolf and Ryan, 1985b). Thus, while the hamster has been chosen as a model for the study of the developing capabilities of the peripheral auditory apparatus (Pujol, Abonnenc and Rebillard, 1975; Pujol, Carlier and Lenoir, 1980; Stonek, 1977; Bock and Seiter, 1978; Relkin, Saunders and Konkle, 1979; Relkin and Saunders, 1980), very little is known about the hearing sensitivity of the hamster. A thorough review of

the results of the literature revealed that there has not been any investigations conducted to systematically evaluate the hearing of the hamsters. There is a need to conduct an investigation into the hearing of the hamsters using the ABR technique. Such an investigation may lead to a better understanding of the hearing patterns associated with the Wh gene and may provide insight into the possible commonality between the Wh gene and the Waardenberg syndrome in humans.

Therefore, the primary purpose of this investigation is to assess the hearing of hamsters in the AN/As-Wh strain. This study may assist subsequent studies currently being designed to clone and sequence the normal and mutant genes from humans (WS1) and hamsters (Asher, personal communication). In the long run, the genetic studies may determine how the primary defect of these genes cause the multitude of phenotypes. The logical sequel of this finding will be, perhaps, the development of a diagnostic procedure to identify affected Wh hamsters, and eventually, WS1 individuals in utero. With studies designed to assess the integrity of the auditory system of the AN/As hamster, one may determine the effect of the mutation on the hearing of the animals. Specifically, this experimental investigation was designed to test the following null hypothesis:

. The genotype wh/wh;E/e (Agouti) has no effect on the morphology, latency and amplitude of waves I-IV

of the auditory brain-stem response at varying intensities.

- The genotype wh/wh;e/e (Cream) has no effect on the morphology, latency and amplitude of waves I-IV of the auditory brain-stem response at varying intensities.
- The genotype Wh/wh;e/e (Black-eyed white) has no effect on the morphology, latency and amplitude of waves I-IV of the auditory brain-stem response at varying intensities.
- The genotype Wh/wh, E/e (White-belly Agouti) has no effect on the morphology, latency and amplitude of waves I-IV of the auditory brain-stem response at varying intensities.
- The genotype Wh/Wh;-- (Anophthalmic white) has no effect on the morphology, latency and amplitude of waves I-IV of the auditory brain-stem response at varying intensities.

CHAPTER II

REVIEW OF LITERATURE

Introduction

The Syrian hamster (Mesocritus Auratus) is a small rodent that has been used increasingly in biomedical research since its capture from Syria in 1930 (Adler and Theodore, 1931; Adler, 1948). One of the mutations that affect the nervous system, the Wh gene (anophthalmic white), of the Syrian hamster was discovered by Knapp and Polivanov (1958). Investigations (Robinson, 1962; Asher, 1968, 1981; James et al, 1980) have shown that the Wh mutation is a highly pleiotropic gene causing deleterious effects upon eye development, pigmentation and reproduction. While the Wh mutation causes deafness, apparently, very little is known about the details of the integrity of the auditory system of the genotypes. This is important since the Wh gene is believed to be homologous to the WS1 gene that causes deafness in humans associated with the Waardenberg syndrome. We do know that the ABR is an invaluable, non-invasive technique that has proved especially useful in assessing the hearing capabilities of both humans (Jewett and Williston, 1971) and animals (Jewett and Romano,

1972). As such, studies using ABR to evaluate the auditory system of the genotypes resulting from the Wh mutation may prove fruitful. In the following review, I will dicuss the phenotypic effects of the Wh mutation, and the use of the ABR in assessing hearing sensitivity in humans and animals.

Genotypes and Phenotypes of the Anophthalmic White (Wh) mutation and a mutation at a Second Locus Cream (e)

As already noted, the Wh mutation has three obvious characteristics, the complete suppression of pigment from the pelage so that the animal bears a remarkable resemblance to albinos, degeneration of the eye which results in anophthalmia (Robison, 1962; Asher, 1968), and the degeneration of the tectorial membrane (Asher, 1988). Thus, the homozygote, Wh/Wh; is devoid of pigment, is anophthalmic and is deaf. The eye structures are rudimentary and often the eyelids are sealed by an exudate (Robinson, 1964; Yoon, 1973; Asher and James, 1982).

The heterozygote, Wh/wh;E/- is superficially, a normal agouti. On a closer observation, however, the ventral surface is white instead of the usual pale yellow. The difference of pigmentation readily provides a distinguishing characteristic of the Wh/wh phenotype from the others and occurs between 8th and 12th day of life. The mutant gene epistatically interacts

with mutants at another locus e to produce other phenotypes such as wh/wh; e/e is cream colored, while Wh/wh;e/e is black-eyed white (Robinson, 1958, 1959, 1962, 1964; Asher, 1968) (see table 4). The e mutation is known to inhibit the formation of eumelanin (black pigment). Individuals homozygous for the mutant are referred to as "cream". The mutant is inherited as an autosomal recessive gene. The heterozygote, e/e, is of normal variability and fertility. The coat color varies from the straw-yellow to rich apricot yellow. Although eumelanin is removed from the hair, the eyes are dark and some pigment is observed around the genitalia of both sexes. The AN/As strain is homozygous for e. The normal allele E was then backcrossed onto the AN/As strain. The wh/wh;e/e, is a normal hamster with an agouti coloration on the dorsal fur and pale yellow on the ventrum. Dermal melanocytes are found in the hairy skin. The eyes are black (Robinson, 1958; Ghadially and Baker, 1960; Rapppaport, et al. 1963). The wh/wh; e/e, is similar to wh/wh;e/e, in phenotype, even though the combination of the extension genes E and e are different. The wh/wh; e/e, hamsters have dark yellow fur on the dorsum and pale yellow fur on the ventrum with ventral white spotting (Robinson, 1955; Illman and Ghadially, 1960; Pratt, 1979). The compound mutant hamsters, Wh/wh;e/e, on the contrary have almost white or completely white fur. Pigmentation, when it is present in the fur, is an extremely pale yellow and is usually noticeable on top

of the head and shoulders as descrete small patches. The ears have only patchy black pigmentation; while the eyes are dark but lighter in color than the eyes of wh/wh;e/e, or wh/wh; e/e (Pratt, 1979). Finally, Wh/wh;e/e have a white ventral fur, and a sprinkling of white hairs on the dorsum (Robinson, 1962, 1964). The Wh/wh;e/e, are similar to Wh/wh; e/e, in phenotype.

Effects of the Wh Gene in the Hamster

Attempts to examine the effects of the Wh gene in the hamster is not recent. The mutation was first described by Knapp and Polivanov (1958) as an autosomal recessive gene and was given the symbol Wh. Since that time, several investigators have attempted to describe the deleterious effects of the mutation in the hamster. Robinson (1962, 1964) described the Wh mutant as an incompletely dominant gene which produced in the homozygous condition a hamster which is completely devoid of pigment and is anophthalmic. Additionally, he observed that the gene appeared as an interspecies mutant similar to Mi^{wh} (microphthalmia white) of the house mouse.

Asher (1968) investigated the AN/As-Wh strain of hamsters to determine the phenotypic and genotypic characteristics of the Wh gene. Specifically, the purpose of his study was to describe the morphologic effects of the gene Wh and to

describe some of its biochemical activities. The characteristics that were measured on 45 adult (130 days) hamsters included total body weight, adrenal weights, uterine weights, plasma total proteins, plasma cholesterol and free plasma amino acid. General observations were made with respect to gross anatomy and behavior. Further, normal embryos were collected from day 11-0 through birth. Gross embryonic anatomy was examined along with total fetal weights.

Observations that were made on the embryos included number of normal embryos, number of abnormal embryos, relative position of each embryo in utero with respect to ovaries and genotypes of embryos. Histological examinations were also made of eyeless and normal embryos, as well as testicular material from eyeless and normal adult males. Finally, gross anatomical observation were made upon each adult hamster sacrificed.

It was observed that there were no apparent differences when the weights of the normal embryos (wh/wh) were compared with the weights of the eyeless embryos (Wh/Wh). This indicated that the Wh mutation appeared to act after birth. In addition, histological examination revealed an absence of pigment as well as an apparent detachment of the retina in a single eyeless embryo at days 13. It was observed that this defect may be related to the failure of the closure of the

choroid fissure. In adult hamsters, it was observed that the Wh gene affected pigmentation, eye development, degree of "nervousness", soundness of sleep, sexual differentiation, fur texture and the general growth. Pigmentation was absent from the fur and skin of the heterozygous Wh/wh hamsters with a slight reduction in retinal and ear pigmentation. On the contrary, pigmentation was completely absent from the Wh/Wh hamsters. Section of the orbital contents of a single adult eyeless Wh/Wh hamsters showed that adults had severe microthalmia rather than anophthalmia. Observation of a single slide of a normal (wh/wh) and eyeless (Wh/Wh) testicle revealed that the eyeless male lacked spermatozoa.

Data from the biochemical analysis suggested that the primary biochemical lesion caused by Wh is related to the urea cycle. Since eyeless Wh/Wh hamsters have elevated arginine levels and lowered citrulline levels, the "metabolic" bloc then appears at arginase (an enzyme in the liver) which converts arginine to ornithine and releases urea. It was proposed that the elevated levels of plasma arginine along with a possible NH_3 elevation could act as a metabolic inhibitor of fundamental biochemical and developmental processes. The differences noted in the adrenal size, sex differenciation and general growth between normal and eyeless hamsters could be explained by abnormal pituitary function. Malfunction of the pituitary could be explained by elevated levels of NH_3, arginine, or

citrulline in the cerebrospinal fluid. Pigmentation abnormalities could arise by two mechanisms, namely, inhibition of migration or differentiation of neural crest tissue. The abnormal eye development appeared to arise from improper closure of the choroid fissure.

James et al (1980) investigated the effects of the Wh mutation on reproduction in the Syrian hamster. The phenotypes used in the study included normal (wh/wh;e/e), heterozygous (Wh/wh;e/e), and homozygous (Wh/Wh;e/e) from the AN/As-Wh strain (Asher, 1968) (see table I-4). This strain was maintained by a full sibling mating where at least one parent of each generation was heterozygous for the Wh gene. Ten sets of the normal, homozygous and heterozygous hamsters were used. After the animals were anesthetized, the testicular tissue of both normal and eyeless hamsters were collected, and further processed for comparison by both light and electron microscopy. It was found that testicular tissue from several mutant animals approached the normal phenotype, due to variable expression of the gene. Most testes from homozygous mutant hamsters, however, were found to be hypoplastic and aspermic. Since the primary function of the gene was unknown at the time, it was suggested that the mutation either acted directly to alter pituitary function or that the abnormalities in reproduction were due to the failure of eye development and subsequent lack of function of the visual pathway.

Asher (1981) measured ten physiologic parameters controlled by the hypothalamic axis on males of three genotypes: wh/wh; e/e, Wh/wh; e/e, and Wh/Wh; e/e. The physiologic parameters measured included body weight, metabolic rate, fasting weight loss, body temperature, feed consumed, water consumed, urine volume, urinary pH, respiratory rate and heart rate. The results showed a reduction in body temperature and respiratory rate, and an elevation in metabolic rate, drinking rate, urinary pH and urinary volume. This indicated that the Wh/Wh mutation altered independently, six parameters known to be regulated by six different sites in the hypothalamus. The union of these six pleiotropic effects led to the conclusion that the development of the pituitary and hypothalamus (the location of the pituitary regulatory centers) in concert with the eyes are altered. In view of the fact that the biological intersection of the eye, the hypothalamus and the pituitary is the embryonic diencephalous, then, it was reasonable to propose that the primary action of the Wh gene is to alter all structures whose development and function are dependent on the embryonic diencephalon.

James and Asher (1981) compared the hypophysis from ten heterozygous, ten homozygous mutants and five normal enucleated hamsters from the AN/As-Wh strain using light and electron microscopy. The purpose of the study was to

determine whether morphologic abnormalities existed in the classes of hamsters. As expected, pituitaries from hamsters homozygous for the Wh gene showed far different morphologic characteristics from the normal heterozygous and normal enucleated animals. The investigators postulated that the Wh mutation either altered cellular differentiation of the embryonic hypophysis or caused an abnormal differentiation in the adult hamster.

Asher and James (1982) performed an ultrastructural analysis of embryonic eyes on three genotypes, namely, wh/wh;e/e, Wh/wh; e/e, and Wh/Wh;e/e, using scanning electron microscopy (SEM) and transmission electron microscopy (TEM). They found that the primary defect caused by the Wh mutation is the abnormal retention of cilia by embryonic cells. Since the normal sequence of events is to lose the cilia, Asher and James (1982) concluded that the Wh gene blocked the resorptive process. These retained cilia are proposed to interfere with normal cell-cell interactions and subsequent cell differentiations. It was then argued that the abnormal eye development in the Wh homozygous is associated with the presence of cilia on both epithelial cell layers of the optic cup and within the epithelium of the lense vessicle.

Hagen and Asher (1983) employed genetically normal (wh/wh), genetically normal and blinded (wh/wh- B) and mutant eyeless

hamsters (Wh/Wh) in order to determine whether the Wh gene by itself influences testicular differentiation, and also whether removal of the pineal gland will restore fertility in these classes of hamsters. Fundamental to this idea is that, male hamsters homozygous for this gene are usually sterile. Secondly, both Wh and the pineal organ are known to suppress reproductive functions. Here again, it was shown that testes from the experimentally blinded (wh/wh B) and 70% of the Wh/Wh hamsters degenerated to less than one-tenth of their normal size. With these properties known, it was surmised that the atrophy of the testes from Wh/Wh hamsters is a pineal-mediated phenomenon due to failure of eye development and the subsequent lack of a functional visual pathway. This hypothesis was demostrated to be true when genetically eyeless males had normal fertility after pinealectomy.

Asher (1988) explored the effect of the Wh mutation on the cochlea using light and electron microscopy. It was found that the Wh gene caused the degeneration of the tectorial membrane and that this became apparent between the tenth and fifteenth day of neonatal life. It turns out that this morphology is associated with the failure of the tectorial membrane to detach from the future inner sulcus cells of the organ of Corti. The tectorial membrane becomes hylane in nature and then disappears. The appearance of cilia, however, were not observed in any unusual places during inner ear

development. As such, the primary action of the gene Wh cannot be to inhibit resorption of cilia. Thus, the microscopic examinations demonstrated the effect of the Wh mutation on the cochlea of the hamster. No one at this point, however, has evaluated the auditory system of the genotypes found in the AN/As-Wh strain of hamsters using the ABR.

Deafness of Genetic Origin

Deafness of genetic origin may be present at birth or may manifest itself in infancy or later in life. Hereditary hearing loss may be classified into three types, namely, aplasia, hedero-degenerations and chromosomal abberations. Aplasia is described as incomplete or arrested development of the inner ear and is always congenital. There are various types of aplasia based on the degree of development. Thus, the time at which development was arrested determines the ultimate structural appearance of the ear (Ormerod, 1960). The various types of aplasia are named after those who first described them in the results of the literature. Thus, the most commonly occuring type is that described by Scheibe (1892). In this type of aplasia, the bony labyrinth is fully developed but the cochlea and the saccule are malformed. The next in order of frequency of occurence was described by Mondini (1791), and it is characterised by incomplete development of the bony and membraneous labyrinth. Another

group of aplasias is the Michel type (1863) and is characterized by lack of development of the petrous portion of the temporal bone. Heredogenerations is associated with deafness which may occur alone or in combination with other abnormalities (Johnson, 1952; Ormerod, 1960). Chromosomal abberations refer to anomalies due to the presence of an extra chromosome. It is responsible for a number of severe abnormalities (e.g. trisomy D), including deafness.

There is a controversy about the classification of the heredo-degenerations. Goodhill (1950) has classified this hereditary type of deafness into infantile and adult types, however, Cawthorn and Hinchcliff (1957) proposed a continuous distribution for the age of onset. Johnson (1952) reported that familial nerve deafness is transmited by a dominant gene. Ersner and Saltzman (1941) reporetd a family with sex-linked recessive inherited deafness, while, Ford (1952) described two families with progressive nerve deafness due to recessive inheritance. This type of deafness may occur alone or in combination with other abnormalities, in which case, they are known as syndromes.

Syndromes are classified into mesodermal, ectodermal and neuro-ectodermal syndromes. Mesodermal syndromes consist of varying, but generally consistent patterns of middle layer disorders of the central nervous sysytem, associated with

deafness. An example of this is the Marfans syndrome, usually characterized by dislocation of the lens, deafness and laxity of the joints. Ectodermal syndromes, on the other hand, consist of the Waardenberg syndrome, Usher's syndrome, which is characterized by retinitis pigmentosa, deafness and mental disorder. Neuroectodermal syndromes are characterized by subcutaneous tumors, auditory nerve tumors, pigmentary changes and deafness (Schuknecht, 1967).

Chromosomal abberations including trisomy (an extra chromosome) that causes anomalies associated with deafness. An example is trisomy D which has the following characteristics, absence of the external auditory canal, absence of the middle ear, microphthalmia and cataracts.

Interestingly, of all the syndromes discussed above, the Waardenberg syndrome was found to be homologous to the Wh gene; the focus of this study. Thus, the following review is a detailed description of the characteristics of the Waardenberg's syndrome.

The Waardenberg Syndrome

The Waardenberg syndrome is an inherited dominant condition. This mutant gene shows a marked pleiotrophy observed both between and within families (Waardenberg, 1951; Preus, Linstrom, Polomeno, et al, 1983; Arias, 1984; Mckusick, 1986). According to Wang, Karmodi, and Pashayan (1981), the syndrome affects 18 specific characteristics, six of which were listed in the original descriptions. The six original characteristics are: lateral displacement of medial canthi of the eyes and of inferior lacrymal puncta (distopia canthorum), a high broad nasal root, hyperplasia of the medial portion of the eye-brows and their confluence (confluence of the eye-brows), heterochromia iridis (different colored eyes), and congenital total or varying degrees of partial deafness (Bwibo and Mkono, 1970; Arias, 1971; Hageman and Delleman, 1977). According to Waardenberg (1951), deafness is the most obviours manifestation of the syndrome; occuring in about 20% of the 1,050 patients at institutes of the deaf in Holland.

Since the original description, over 1,200 cases of the syndrome have been reported, not only in patients of Dutch origin, but also in English, American, African, and Asian peoples (Wang, Karmodiet and Pashayani, 1981). These additional reports have helped to explain further the manifestations of the syndrome originally described by Waardenberg and have led to the description of additional

characteristics. These include abnormal pigmentation of the skin, premature graying of hair, peculiar facial appearance and pigmentary changes of the fundi, full lips, cleft palate, cardiac murmur and vestibular abnormality. Other isolated findings have been described, such as meningocele and atresia of the esophagus, but these have not been firmly established as characteristics of the syndrome (Zerlig, 1961; Bwibo and Mkono, 1970).

Fisch (1959) reported the audiologic findings of Waardenberg syndrome patients, describing two types of hearing loss. These are almost total bilateral deafness with some residual hearing in the low frequencies, and a unilateral moderate hearing loss with uniform loss for the lower and middle frequencies with an improvement of hearing in the higher frequencies. Fisch (1959) provided the only historic description of the temporal bone in a patient with the syndrome. He found total absence of the organ of Corti and atrophy of the spiral ganglion. The saccule and the utricle, however, were found to be normal.

Hageman (1977) studied 34 patients and noted that dominant hereditary deafness as part of the Waardenberg syndrome was found in 35% of the patients. Audiometric examination at the maximum output of the audiometer (110 dB HL) on eleven patients indicated that five of them had total bilateral

deafness, while six patients had unilateral hearing loss. From these examination, he described four types of hearing loss. These are profound bilateral hearing loss (type I), severe bilateral hearing loss (type II), profound unilateral hearing loss (III) and moderate unilateral hearing loss, particularly in the low frequencies (type IV).

Owing to the heterogenity of the Waardenberg syndrome, some investigators (Arias, 1971; Hageman and Delleman, 1977; Hageman, 1977) posited that the syndrome seemed to consist of two genetically distinct entities that can be differentiated clinically. These are type I Waardenberg syndrome with dystopia canthorum, and type II Waardenberg syndrome without dystopia canthorum. Both types have an autosomal dominant mode of transmission. Hageman and Delleman (1977) made an extensive literature review of more than 1,000 patients with the Waardenberg syndrome. They found that deafness in both ears (the most serious expression of the syndrome) occured in about 25% of patients with Waardenberg's syndrome type I and in 50% of patients with type II. This striking difference is connected with the difference between deafness and pigmentary disorders.

Klein (1983) provided evidence for another category of Waardenberg syndrome, known variously as, Waardenberg syndrome type III, Klein-Waardenberg syndrome, or Waardenberg syndrome

with upper limb abnormalities. In his studies on a French family, Klein (1983) reported a case in which the father had a complete Klein-Waardenberg syndrome including muscular and skeletal defects of the upper limbs; while his 12 year old son had the classical Waardenberg syndrome without upper limb abnormalities. It would appear that this mutation acts as an autosomal dominant type without linkage assignment. Patients with this disorder have the classical WSI phenotype an association with the abnormalities of upper limbs, hypoplasia of the musculoskeletal system and microcephally, at least in some patients.

Richieri-Costa, Gollop and Otto (1983) provided the description of another sub-division of patients initially classified by Waardenberg (1951) as WSI. He noted in his genetic study that five children of two Brazillian families had anophthalmia and multiple congenital abnormalities. Among the four affected children, three had bilateral while one had unilateral anophthalmia. He surmised that this mutation behaved as an autosomal recessive inheritance without linkage assignment. This syndrome was designated as Waardenberg Anophthalmia Syndrome (McKusick, 1986).

Another type of the Waardenberg syndrome (WSI) was described by Shah, Subhash, Desai et al (1981). In their study of twelve babies of five families in Bombay, India, they found

that these babies had intestinal obstructions in addition to having the classical WSI phenotype characteristics. On the basis of their study, they proposed that this combination may be inherited as an autosomal recessive trait. McKusick (1986) designated this variant of Waardenberg syndrome as Waardenberg-Shah-Syndrome.

The foregoing review demonstrates that there is a complex heterogeneity in this syndrome both within and between families. Within this frame of reference, and in view of the fact that Waardenberg Syndrome is homologous to the Wh-mutation found in hamsters, Asher and Friedman (1988) gave the following three genetic explainations of the complex variation of phenotypes associated with the Waardenberg syndrome. These are (1) different mutant alleles at a single locus, (2) mutant alleles at more than one locus affecting the same developmental processes, and (3) a single mutant locus with alleles interacting with genes at other loci which vary among different families.

It can be argued that additional information is needed to enable investigators to continue with an analysis of the WSI phenotype. This information include (1) the number of loci responsible for the Waardenberg syndrome, (2) number of mutant alleles at a given locus, (3) the primary function of the loci involved, and (4) the mechanisms by which alterations of these

primary functions produce abnormalities of the eye, ear, pigmentation and skeleton. In order to obtain this information, it will be necessary to use an animal model which exhibit the phenotypic effects of the Waardenberg syndrome. We know that the Wh syndrome in the Syrian hamster covers an enormous range of phenotypic variations observed among the Waardenberg syndrome patients. As such, it would be possible to use the hamster model to clone the normal and mutant genes of WSI and Wh- so as to develop a diagnostic procedure for identifying affected individuals in utero. We also know that the major abnormality caused by the Waardenberg syndrome is total deafness (Hageman and Delleman, 1977). Therefore, a starting point for meaningful research that needs to be conducted in cloning the Wh gene would be to assess the hearing sensitivity of samples of the genotypes and phenotypes of Syrian hamsters from the AN/As- Wh strain using the ABR. These considerations constitute the underpinnings of the present study.

The Use of ABR in Hearing Evaluation

In 1970, Jewett and Williston recorded far-field auditory evoked potentials from the human brain stem (brain stem auditory evoked response, BAER) by using scalp electrodes (Jewett and Williston, 1971). BAERs are produced by the electrical activity in the peripheral and central nervous system in response to sound stimuli. In recording these

potentials, the electrodes are affixed to the scalp at three positions. The positive electrode is placed at the vertex (Cz), the indifferent electrode over the test ear's mastoid and the ground electrode over the forehead (FPz). The components of the auditory brain-stem response waves have been shown by dissection studies (Buchwald and Huang, 1975) to be associated with the following anatomical locations: wave I cochlear nerve; wave II cochlear nucleus; wave III, superior olivary complex; wave IV, ventral nucleus of the lateral lemniscus, and V, the inferior colliculus. The exact location of wave IV is disputed (Stockard and Rossiter, 1977) and often appears to be fused with wave V (Starr and Achor, 1975). The location of wave VI may be the medial geniculate body (Stockard and Rossiter, 1977) but its location along with that of wave VII is still disputed (Brackman and Selters, 1978). On the other hand, Hashimoto et al (1981) claim that wave I originates from the distal portion of the cochlear nerve, while wave II originates from the proximal portion of the cochlear nerve and perhaps the pons. Waves III and IV are from the pons and the lateral lemniscus, and wave V from the lateral lemniscus. Møller and Janetta (1985) agreed with Hashimoto et al (1981) on the generators of waves I and II; and noted further that wave III originates from the cochlear nucleus, while wave IV originates from the superior olivary complex and the lateral lemniscus. Møller and Janetta (1985), posited, however, that the neural generators of waves V, VI

and VII are exceedingly complex in that more than one anatomical structure contributes to each peak and that each anatomical structure contributes to more than one peak (see table 5). The possibility that the ABR might provide useful information for estimating thresholds in man and animals has been suggested (Jewett and Williston, 1971). Thus, for children and adults unable to cooperate during standard audiological procedures, such an objective physiologic measure could provide invaluable information about the integrity of the peripheral system. We also know that the ABR procedure can be reliably used in assessing the hearing sensitivity of animals (Jewett and Romano, 1972), and particularly in the hamster, and under anesthesia (Schweitzer, 1987). It has been shown that the threshold of the ABR is near the behavioral threshold for the same signal in human subjects. In addition, it has been posited that as the intensity decreases, the amplitude of the ABR waves decreases, while latency increases (Picton, Hillyard, and Kraus et al, 1974; Hecox and Galambos, 1974; Yamada, Yamane and Kodera, 1977). These promising results enouraged clinicians to include the ABR test in the clinical armamentarium, as will be justified in the following review of the results of the literature in both humans and animals.

Table 5. Generator sources of ABR in humans and in the rat

Human	Possible Generator	Rat	Possible Generator
I	Distal portion of Cochlea Nerve	P1	Auditory Nerve
II	Proximal portion of Cochlea Nerve	P2	Cochlear Nucleus
III	Cochlear Nucleus	P3	Superior Olivary Complex
IV	Superior Olivary Complex	P4	Lateral Lemniscus
V	Lateral Lemniscus	P5	Inferior Colliculus

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Human Studies of ABR

Hecox and Galambos (1974) recorded the ABR in infants (3 weeks to 3 years of age) and adults, using three intensity levels (60, 40 and 20 dB SL) in varying order for the former and six levels of attenuation (-60, -50, -40, -30, -20, and -10 dB SL) for the latter. Tracings were made on all subjects by summing responses presented to the monoaural ear using a 0.1 msec square wave generated at a rate of 30 per second. They found that the amplitudes and latencies of ABR waves vary with stimulus intensity. This demonstrated reliability and limited variability of the ABR waves, providing the basis for an optimistic estimate of their usefulness as an objective method of assessing hearing in infants and adults.

Stillman (1976) employed 500 Hz tone bursts to record ABR from 49 subjects. Twenty one of these subjects had normal hearing, ten were hearing-impaired, while eighteen were suspected of having a hearing impairment. He used a tone busrt of 4 msec duration, 1 msec rise/fall and a repetition rate of 15/sec. Intensity was varied to the point where threshold could be determined. Here, again, it was found that successive intensity increments resulted in an increase in amplitude and a decrease in latency. Additionally, ABR threshold for all normal hearing subjects occured between 10 and 30 dB SL, with most subjects exhibiting threshold at 20 dB SL. For the

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hearing-impaired individuals, however, response thresholds were found to be higher than for normals, and that specific values were related to the extent of the hearing loss. Further, changes in the amplitude and waveform responses as a function of stimulus intensity were generally unlike changes in the response for normal subjects. As expected, mean latency for normal subjects was found to decrease as a function of stimulus intensity. For hearing-impaired subjects, (all of whom had moderate to severe hearing loss at 500 Hz) the latency-intensity functions were displaced to the right on the abscissa; and at this point, an intense stimulus was required to evoke a response.

Møller and Blegvad (1976) used unflitered clicks to study 60 patients with sensorineural hearing loss. Of this number, 48 had symmetrical bilateral hearing loss, while 12 had assymetrical hearing-impairment. Among subjects with symmetrical hearing loss, 25% had a flat audiogram, 25% had a gradually slopping audiogram, 25% had a steeply slopping audiogram while the remaining 25% consisted of a mixed group with diverse types of audiograms. Filtered clicks at varying repetition rates of 12 and 16/sec were presented at 10 to 90 dB HL in 10 dB steps. The results showed that patients with a flat hearing loss produced ABR responses that are closer to the subjective threshold than was the case in subjects with gradually slopping or steeply slopping audiograms. Interest-

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ingly, in patients with flat hearing loss, all of whom had better hearing in the higher frequencies, latency was found to be shorter than in patients with pronounced high frequency hearing loss (that is, groups with gradually slopping and steeply slopping audiograms).

Coats and Martin (1977) studied 16 normal subjects and 35 high frequency hearing loss subjects, using filtered clicks presented at varying intensity levels of 10 to 90 dB HL in 10 dB steps. At each intensity level, responses to rarefaction and condensation clicks were plotted seperately. The procedure adopted to obtain thresholds was a straight line projected to visually fit to the lowest three suprathreshold points on the input-output curve. It was found that subjects with high-frequency hearing loss affected ABR waveforms. In addition, such subjects showed prolonged latency of wave V of the ABR. Further, ABR peaks prior to wave V appear out of phase for condensation and rarefaction stimuli. Interestingly, the wave I to IV condensation -rarefaction polarity reversals were not present in all patients with high-frequency hearing loss.

Yamada et al (1979) examined the effects of inner ear pathology on wave V of the ABR in 12 patients with flat, low frequency, severe high frequency and gradual high frequency sensorineural hearing loss. Auditory stimuli consisted of

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clicks presented at 90 to 10 dB SL in 10 dB steps. The results showed that in patients with flat and low frequency sensorineural hearing loss, the latencies of wave V at intensities of 4-10 dB greater than their response thresholds were approximately the same as those in normal subjects. It was also noted that in patients with high frequency sensorineural hearing loss, the latencies were always delayed; compared with those with normal hearing. Finally, in patients with gradually slopping high-frequency hearing loss, the latency of wave V was delayed according to the degree of hearing loss, as determined by the pure-tone audiogram.

Jerger and Mauldin (1978) measured ABR threshold and latency from 275 ears of 185 patients with sensorineural hearing loss. The acoustic signal used was a half cycle of a 3000 Hz sinusoid at the rate of 20/sec. Each subject was tested at the intensity levels of 100 dB HL in 10 to 20 dB decrements until ABR waves were no longer discernible. The lowest intensity level at which a repeatable response was observed was defined as ABR threshold. In addition to the ABR thresholds and latency measures, a number of pure tone indices were also obtained from the patients' clinical audiograms. These consisted of pure tone averages at 500, 1000 and 2000 Hz; 1000, 2000 and 4000 Hz; and thresholds at 2000 and 4000 Hz. Correlational analysis of the ABR thresholds with the audiometric indices of sensorineural hearing loss indicated

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the highest correlation in the 2000 to 4000 Hz threshold region. The highest correlation was with the 4000 Hz threshold (r = 0.49), but the correlation with the PTA (r = 0.48) was almost as high. It was also shown that the pure tone thresholds at 1000, 2000 and 4000 Hz was most accurately predicted by multiplying ABR threshold by 0.6. The study also reaffirmed the prolongation of ABR latency with down-slopping audiometric configurations.

Kavanagh and Beardsley (1979) tested 33 subjects, 24 of who had sensorineural hearing loss, eight had conductive hearing loss and one had a mixed type of hearing impairment. Filtered clicks were presented at a rate of 31/sec and at varying intensity levels. The threshold for ABRs was defined as the stimulus intensity at which waves were first discernible. In evaluating the ABR to determine hearing sensitivity, three measurements were made. These included the threshold of the ABR, latency and amplitude of wave V. It turned out that in determining the degree of hearing loss, wave V threshold was the best index. Wave latency at high intensity levels was found to have little correspondence to degree of sensorineural hearing loss. Wave V amplitude was found to be highly variable among subjects, but still a useful indicator for detecting pathology.

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Fria and Sabo (1980) compared ABR and audiometric findings in 10 patients using latency intensity function (LIF) techniques. Tone pips varied from 10 to 90 dB HL were used as stimuli. Wave V latency was plotted and compared with the normal LIF. A horizontal line was drawn at 20 dB from the normal latency to the patients LIF. The point where this line intersected the LIF was the estimated conductive hearing loss component. From this analysis, it was found that seven of the ten patients had predictable conductive hearing loss that were less than 15 dB different from the 4000 Hz pure tone threshold.

Rosenhammer et al (1980) recorded ABR from 62 normal hearing subjects, grouped according to gender and age. The groups comprised young females (mean age of 26.8 years), old females (56.1 years), young males (59.3 years) and old males (59.3 years). Filtered clicks were presented at attenuation levels of -80, -60, and -40 dB SPL, and at a repetition rate of 22.5/sec. The ABRs were measured with respect to peak latencies and interpeak intervals. With regard to peak latencies, highly significant differencies were established between the group of young females on the one hand, and the other three groups on the other; with females exhibiting shorter latencies of the order of 0.2 msec. Differencies between the other three groups were less significant. Furthermore, in old subjetcs, but not in the young ones, the

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individual III-V interval exhibited a significant increase with reduction of click intensity from 80 to 60 dB SL of the order of 0.1 msec. Variances of interpeak interval measures were seen not to differ significantly.

Bauch and Olsen (1986) investigated the effects of 2000, 3000 and 4000 Hz hearing sensitivity on the ABR from 458 patients with cochlear hearing loss. Rarefaction clicks with 100 μs duration and a repetition rate of 30/sec were used. The criteria used for abnormal ABR responses were defined as: (1) absolute latency of wave V exceeding 6.20 msec (i.e. delayed latency of wave V > 6.2 msec), and (2) absence of repeatable ABR waveform. As expected, hearing sensitivity at 2000, 3000 and 4000 Hz was found to have an influence on ABR waveforms. It was observed that the percentage of abnormal ABR results increased with the severity of the hearing loss. This was due, presumably, to cochlear dysfunction and reduced cochlear response. The salient features of the results were as follows. First, hearing sensitivity at 2000 Hz influenced responses more than sensitivity at 4000 Hz, when both were compared at similar intensity levels. When 2000 Hz hearing sensitivity was normal, and 3000 and 4000 Hz thrsholds were at 35 dB HL or better, ABR results were normal for cochlear hearing loss patients, at least 94% of the time. When 2000 Hz sensitivity was normal, but thresholds for 3000 and 4000 Hz were at 40 dB HL or poorer, ABR results were abnormal for

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15 to 80% of the patients with cochlear hearing losses, depending on the severity of the hearing deficit. The occurence of the abnormal ABR reuslts increased markedly when 2000 Hz thresholds reached 65 to 70 dB HL and 3000 and 4000 Hz thresholds were in the 50 to 70 dB HL range.

Bauch and Olsen (1987) further analyzed the influence of 2000 to 4000 Hz hearing sensitivity on ABRs by averaging thresholds at 2000, 3000 and 4000 Hz derived from a previous study (Bauch and Olsen, 1986). They found that the percent of normal ABR results decreased quite systematically as the averaged three-frequency hearing loss increased.

Prosser and Arslan (1987) collected three sets of data on wave V of ABR on normal subjects and those with sensorineural hearing loss. The three sets of data were comprised of 10 normal, 56 patients with sensori-neural hearing loss and 32 patients suffering from surgically confirmed CPA tumors. Tone pulses of 0.1 msec duration, 75 msec interstimulus interval (ISI) and alternating polarity was used. The normal hearing subjects were stimulated at intensities from 90 to 0 dB nHL in 5 dB steps. The hearing-impaired patients were stimulated at a fixed intensity of 90 dB nHL. The V index was calculated according to the formula V = LP(90) - Ln(90-X), where LP(90) represents wave V latency of the pathological ear at 90 dB nHL; Ln(90-X) represents wave V latency as predicted

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from normal intensity -latency function; and X represents the patients pure-tone hearing loss as the average of the frequencies at 2000 and 4000 Hz. It was found that for cochlear patients, V was negative. When the absolute values were plotted against the PTA (2000 and 4000 Hz) hearing threshold, a linear relationship was found ($r = 0.94$). For retrocochlear patients, however, the V was mostly positive, with no significant ralationship with the PTA (2000 and 4000 Hz) threshold ($r = 0.61$).

Jerger and Johnson (1988) studied the interactive effects of gender, age, and degree of sensorineural hearing on the absolute latency of wave V in 325 subjects with cochlear hearing loss and 87 subjets with surgically confirmed retrocochlear disease. Filtered clicks with alternating polarity and a repetition rate of 21.1/sec were used as auditory stimului. The click presentation level was determined by the degree of high frequency hearing loss. This was quantified as the average of the pure-tone threshold hearing levels (HTLs) at 1000, 2000 and 4000 Hz. Here again, the results showed that wave V latency increased systematically as high frequency hearing loss increased. The trend was not, however, linear. For example, wave V latency showed little change with degree of hearing loss up to the 50 to 60 dB region, but increased in a linear fashion with a further increase in hearing loss.

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In summary, it can be observed that the ABR is a useful technique to use in the differential diagnosis of both conductive and sensorineural hearing loss. It provides data which objectively evaluates the site-of-the-lesions, whether cochlear or retrocochlear. We do note that this diagnostic information could be drawn from three main sources. These include the threshold of the ABR waves and the amplitudes and latencies of waves I, III and V. With regard to threshold, the results of the literature showed that threshold for normal hearing subjects occured between 10 to 30 dB SL. For hearing-impaired subjects, response thresholds were higher than for normal subjects, and the specific values are related to the extent of the hearing loss. In the case of latency measures, the mean latency of normal hearing subjects decreased as a function of stimulus intensity. As expected, the latency input-output functions for hearing-impaired subjects were displaced to the right on the abscissa, as greater stimuli were required to evoke a response (Stillman et al, 1976). By the same token, diagnostic information was also provided by interpeak latency intervals, such as, I-III, III-V and I-V. Thus it was clearly shown that in patients with cochlear hearing loss, the ABR evoked by high intensity stimuli showed a I-V interval quite similar to those measurable in individuals with normal hearing. Amplitude of the ABR was shown to be the least useful index in determining

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hearing sensitivity, due to its large standard deviation. The usefulness of amplitude in establishing threshold was noted by Kavanagh and Beardsley (1979), who reported that at high intensities, when the latency of wave V is normal, the amplitude is often abnormal. Thus, these fundamental observations formed the basis of the criteria to be utilzed in measuring the hearing sensitivity of the hamsters in the AN/As- Wh strain.

Animal Studies of ABR

The use of ABR in animal studies over the past 25 years has paralled the significant increase in experimental and clinical use of this procedure in humans. To be true, the techniques that are used to non-invasively record ABRs from animals are similar in most respects to those used for recordings in humans. Under typical operating conditions, however, some notable differences can be found in the procedures commonly used in recording ABRs from animals. In the first place, conventional earphone headsets, routinely used to record human ABRs are obviously inappropriate for animal use. As such, when recording ABRs from animals, sound stimuli are either presented free-field or, more often, through a closed ear-tube system. Fundamental to the use of the ear-tube is the fact that it can be inserted in the external auditory canal with high reliability of placement so that the test and re-test differences in absolute stimulation levels are minimal.

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There is another point that bears mentioning. While cup-shaped, or flat disc electrodes typically used in recording human ABRs can also be used with animals, needle electrodes are preferred because they insure a more stable recording with animals. In addition, human ABR recordings are typically done with the subject either awake or sleep. A general anesthesia is administered only when the patient cannot be successfuly tested in any other state. As a rule, however, ABR recordings in animals are almost never: never made with the animal awake or in natural sleep (unless in some way restrained). As such, animals are always sedated or anesthetized during testing.

In another vein, in laboratory animals, the smaller absolute head size and the concomitant brain size result in a more favorable situation for recording the responses elicited by auditory stimuli. Therefore, ABRs detected at the scalp in non-primate laboratory animals tend to have relatively large amplitude waves as compared to humans. Still another reason is that a more favorable signal-to-noise ratio is observed in the signal detected from an animal's scalp as compared to that detected from the human scalp. The logical sequel for these two differencies is that, the overall amount of gain needed in the biological amplifier system and the amount of averaging

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necesssary to evoke a clear response may be lower than that commonly used for recordings in humans.

The recording of ABRs in laboratory animals is not of recent origin. In point of fact, Jewett (1970) was the first investigator to record ABR from the scalp of the cat using filtered clicks. Since then, ABRs have been extensively recorded in guinea pigs (Dobie and Berlin, 1979b; Gardi and Berlin, 1981; Dun, 1984), rats (Jewett and Romano, 1972; Schorn et al, 1976; Tokimoto et al, 1977; Iwasa and Potsil, 1982; Church et al, 1984), cats (Jewett and Romano, 1972; Buchwald and Huang, 1975; Achor and Starr, 1980a, 1980b; Shipley et al, 1980; Laukli and Mair, 1982; Walsh et al, 1986a, 1986b, 1986c), monkeys (Allen and Starr, 1978) mouse (Henry and Lepkowski, 1978; Henry and Haythorn, 1978; Henry, 1979; Henry and Chole, 1979a, 1979b; Shnerson and Pujol, 1982) gerbil (Wolf and Ryan, 1985b; Smith and Kraus, 1987) and hamster (Schweitzer, 1987; Moore et al, 1988). It can be argued that most of the studies noted above were not directed specifically to the use of ABR in determining the thresholds of the animals investigated. As such, we will not review these studies. In view of this fact, however, the following review of the literature will focus on the use of ABR in evaluating hearing sensitivity in animals.

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Jewett and Romano (1972) explored neonatal development of the ABR from the scalp of 12 adult rats and 124 rat pups and three adult cats together with eight kittens. Animals were anesthetized using sodium pentobarbital. The pentobarbital dosage in cats was 45 mg/kg body weight, and in rats it was 30 mg/kg body weight up to 30 days of age; 45 mg/kg body weight up to 45 days of age; and 60 mg/kg body weight up to 60 days of age. Halothane oxygene was administered to the rats, rat pups, kitten and the adult cats. Stimuli consisted of clicks that were varied from 30 to 85 dB SL for the rats and from 40 to 70 dB for the kittens and cats. It was found that for all the animals, a reduced intensity resulted in an increased latency. At younger ages, the absolute increase in latency was found to be greater.

Henry and Haythorn (1978) studied the effects of age and intensity on ABR in the laboratory mouse. Twenty eight 16-day old and twenty eight 35-day old mice of the C/575BL/6 strain were used in the study. Subjects were anesthetized with 60 mg/kg sodium petobarbitol and were presented with four series of clicks at a repetition rate of 5/sec. Intensity of the stimulus was varied at the attenuation levels of -70, -50, -30 and -10 dB HL. Here again, an inverse relationship between stimulus intensity and latency of the components of the ABR was observed in the mature 35-day old mice. By contrast, the

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lantencies of the ABR waves of the 16-day old mice did not decline as the stimulus intensity was increased.

Henry and Lepkowski (1978) investigated ABR correlates of genetic progressive hearing loss in the mouse. They compared the C/57BL/6 mice which displayed genetic sensorineural progressive hearing loss with the CBA/J strain of mice. From 8 to 11 mice of each genotype were tested at 50, 100 and 200 days of age. The animals were anesthetized with 60 mg/kg sodium pentobarbitol and maintained at a room temperature of 37°C, as measured by a microthermister placed against the bulla. Acoustic stimuli consisted of clicks presented at the rate of 20/sec and at the atteuation levels of -100, -60 and -40 dB SPL. It turns out that there were no genotypic differences in the amplitudes and the latencies of the ABR waves from the two groups of mice at 50 and 100 days of age. Viewing the amplitudes of the two genotypes at 60 dB SPL revealed that over 150 day span, amplitude of the ABR waves decreased for the two genotypes. In addition, no significant age-related latency changes in the genotypes were observed at 60 dB SPL. Further, at 50 days, no genotypic differencies were noted in relative amplitude of the ABR waves as a function of stimulus intensity. Interestingly, by 100 days, the amplitude-intensity relationship which was observed began to change, in that at this age the C/57BL/6 strain of mice had a smaller dynamic range for the amplitude of ABR waves.

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Again, at 200 days of age, pronounced genotypic differences were observed in the rate of relative amplitude change to increasing click intensity.

Henry (1979) examined ABR amplitudes and latencies in 18 inbred CBA/J laboratory mice. The animals were anesthetized with chlorphrothixine (0.60 mg/kg) and pre-anesthetized with sodium pentobarbital (60 mg/kg). Body temperature was maintained at 37.8°C. Tone pips at nine frequencies from 4000 to 64000 Hz produced at a repetition rate of 20/sec, 200 us rise/decay time and 1.0 msec duration were used. The results revealed that the amplitude and latency of the ABR waves varied as a function of stimulus frequency. In addition, input-output functions were found to be related to frequency, with the 4000 Hz curves having longer latencies than 8000 Hz curves.

Osako et al (1979) explored the effects of kanamycin on the ABR during post-natal development of the hearing of 105 rats. Anesthesia was applied (25 mg/kg sodium pentobarbital) after a daily dosage of 400 mg/kg of kanamycin. Tone pips of varying intensities and frequencies were used as input signals. A pronounced suppression of ABR waves and thresholds of the auditory responses were observed in the group of rats to which kanamycin was administered.

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Church et al (1984) employed clicks presented at attenuation levels of -0, -20, -40 and -60 dB and a repetition rate of 8/sec to evaluate variations in ABRs of normal laboratory rats as a function of stimulus intensity. They found that the latencies of waves I, II, III and IV of the ABRs decreased with increasing intensity, while amplitudes increased with increasing stimulus intensity levels. At threshold (0 and 10 dB levels) wave II of the ABR was the most prominent component.

Dun (1984) studied the postnatal development of ABR in 10 guinea pigs anesthetized with sodium pentobarbital (3 mg/100 kg). Acoustic clicks were presented at a repetition rate of 10/sec and at the attenuation levels of -20, -40 and -60 dB SPL, ranging in frequency from 500 to 15000 Hz. It was found that at birth, thresholds of the ABR in the experimental group of animals corresponded with those of the adult animals in the control group. Shortly after birth, however, the evoked potentials appeared at significantly longer latencies at all three intensities.

Smith and Kraus (1987) investigated the postnatal development of the ABR in 71 unanesthetized mongolian gerbils. Filtered clicks varied from 0 to 100 dB HL in 10 dB steps, were delivered through an ear speculum with an attached sound tube. The sound tube was glued into the external auditory meatus to

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provide monoaural stimulation. Threshold was defined as the lowest intensity level yielding a replicable ABR. At the highest intensity level used (100 dB HL) no replicable responses were obtained prior to 12th day after birth. An ABR threshold was however, obtained after the 12th day of birth. During this period, the response remained variable in sensitivity, and by day 20, all ABR waves were detectable. In contrast to the adult response in which wave IV was the most consistently detected component at threshold, wave I was the most sensitive indicator of threshold in the developing gerbil.

ABR in the Syrian hamster

It was reported earlier in this review that the Syrian hamster provides an excellent opportunity for the study of developmental biology because of its relative maturity at the time of birth (Boyer, 1953; Hoffman et al, 1968). Thus, it would appear that the hamster is a suitable model for the study of developmental capabilities of the peripheral auditory system (Pujol et al, 1975; Reilkin and Saunders, 1980). Although the hamster has been regarded as a model of research on the peripheral auditory system, not much is known about the central auditory system responses of the hamster. Thus, the only available study on the hamster that employed the ABR technique was that of Schweitzer (1987).

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Schweitzer (1987) studied 40 golden Syrian hamsters using a mixed within and between subject design. Forty infant hamsters (13 days after birth) were assigned to an acute condition (between subjects). These were tested once on either postnatal day 14, 16, 18, 20, 22, 24, 30 or 40. Sixteen infant hamsters (used in the acute condition) were again assigned to a chronic condition (within subjects) and were tested on day 16, 30, and four of the other ages studied in the acute condition. Five normal adult hamsters at least 60 days old were used as a control group. The animals were anesthetized with chloropent, a mixture of chloral hydrate (dosage, 150 mg/kg) and sodium pentobarbital (dosage, 30 mg/kg). A body temperature of 37°C was maintained by a heating pad. Threshold was determined with descending and ascending series of click intensities at 5 dB intervals and at a repetition rate of 20/sec. Latencies were measured using the wave form that was evoked at 50 dB SPL; an intensity that was sufficiently above threshold for all animals 18 days and older.

The salient features of the results were that at day 14, no reliable ABRs could be evoked even by clicks as high as 94 dB SPL. By day 16, however, all of the hamsters showed a reliable response to clicks at 94 dB SPL. A significant decrease in threshold was noted between days 18 and 20. Peak-to-peak amplitudes were found to be variable.

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Nevertheless, a progressive increase in the amplitude of the later peaks (waves III, IV and V) occured with increasing age. As expected, the latencies of all waves decreased with age.

In summary, several studies have consistently explored the effects of the Wh mutation on the hamster. Indeed, it has been demostrated that the Wh gene is a highly pleiotropic mutation causing numerous morphologic, physiologic and behavioral abnormalities. To be true, the more obviours morphological effects of this gene as noted was to cause homozygotes to be deaf, blind and white. Still another observation was that the Wh gene in the Syrian hamster is homologous to the Waardenberg syndrome in humans, in that they both appear to affect the same developmental processes. We do note that deafness is the most serious defect of the Wh mutation (in the hamster), and the Waardenberg syndrome (in humans), of the individuals affected. It is well known that the ABR as a non-invasive measure has proved invaluable in monitoring the hearing sensitivities of infants and a variety of animals. To be true, studies of the evoked potential correlates of genetic progressive hearing loss in two genotypes of mice (C57BL/6 and CBA/J) have shown that hearing thresholds, amplitudes and latencies differed for these two genotypes. We also know that at this point in time, there has not been any investigation conducted to determine the hearing sensitivity of the five sets of genotypic hamsters now

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available in the AN/As-Wh strain. Thus, an ABR study designed to determine the hearing levels of these Wh genotypes is deemed important, in that it may pave the way for the long term objective of using the hamster as a model for the normal and mutant genes of WS1 and Wh deafness. Data generated from this study may assist in the development of a diagnostic procedure to identify individuals affected by this gene in utero, so that hearing loss might be detected and perhaps prevented via genetic engineering (Asher and Friedman, 1988) at the earliest possible age. There is the need, then, to investigate the hearing sensitivity of the Wh genotypes and phenotypes in the AN/As-Wh strain, hence, the impetus for the present study.

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CHAPTER III

INSTRUMENTATION AND PROCEDURE

Stimulus generating System

The basic experimental apparatus employed in the presentation and control of the clicks was similar to the one described by Moore et al (1988) and is shown in the block diagram in Figure III-I. The specific components were the following:

- .One power source ($MI^2$100)
- .One function generator (MI^2 208)
- .One dual attenuator (MI^2 108)
- .One amplifier (Grass P5 Series)
- .One level discriminator (MI^2 104)
- .One data controller timer (MI^2 214)

Clicks generated by a computer program were routed manually to the ears of each hamster from the function generator (M208), dual attenuator (M108), the amplifier and the filter.

Figure III-1. Block diagram of instrumentation.

FIGURE III—1 INSTRUMENTATION

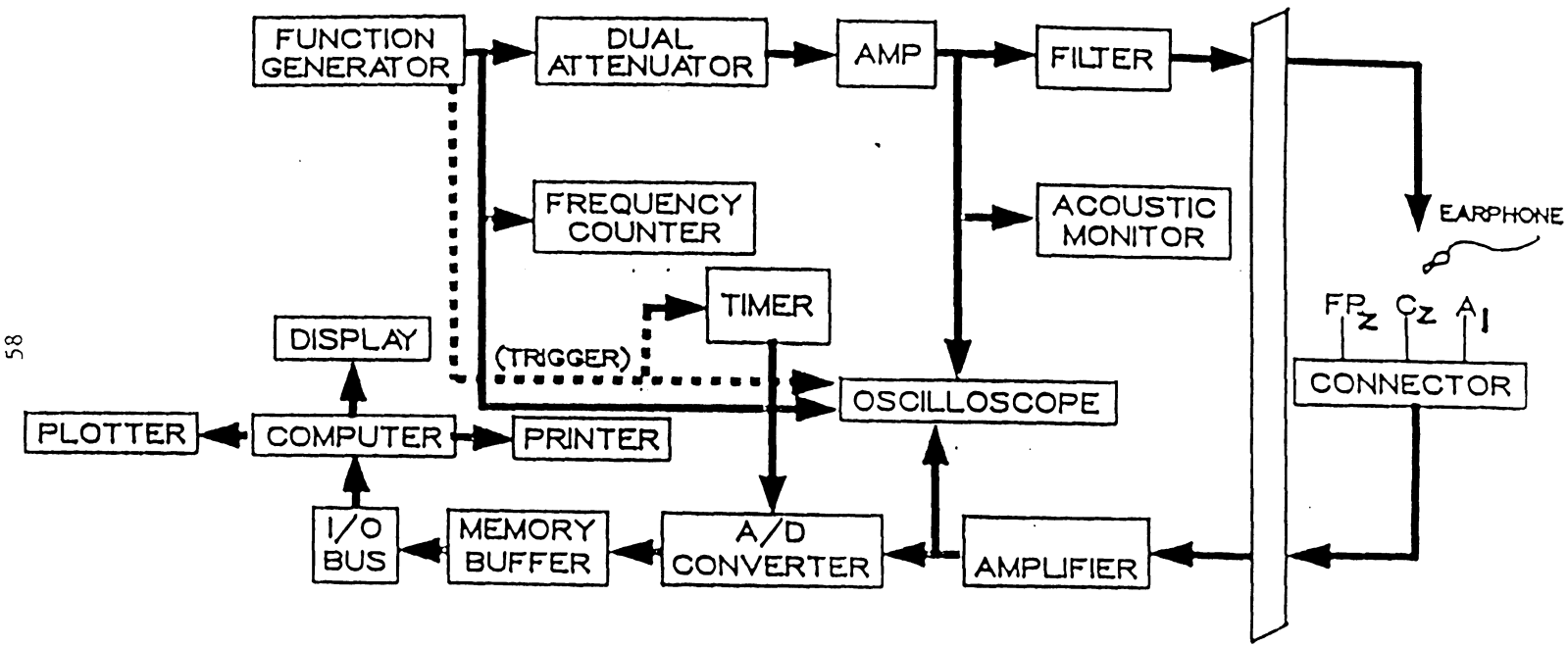

FIGURE III—1 INSTRUMENTATION

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Electrophysiologic Recording Sysytem

The experimental equipment employed in the recording of electrophysiologic activity is also shown in figure III-I. The specific components were the following:

.Three needle elctrodes (AkAI)

.One grass amplifier (Grass P5 Series)

.One A/D converter (MI2 202)

.One I/O Bus (MI2 101)

.One computer (IBM AT)

.One plotter (IBM 6180)

.One oscilloscope (Textronix D15)

.One frequency counter (Hewlett Packard 5314A)

Each electrode was connected to the grass amplifier which amplifies the small electrophysiologic signal from the animal. The amplified signal was then filtered with a band pass from 100-3 KHz and the gain was set at 200 K. This setting, of course, resulted in a gain of 106 dB. The electrophysiologic activity was then passed through an averager (A/D converter, memory buffer and I/O Bus) and summed 2048 times, using a sweep time of 10 msec, a dwell time of 10 μs, a 100,000 KHz sample rate and 1000 data points. The monitor oscilloscope was used to display the clicks from the function generator, clicks before attenuation, clicks prior to earphone and electrophysiologic

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Figure III-2

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activity. The summed responses were printed in an analog form by the printer which was connected to the computer.

Calibration System

Figure III-2 shows the block diagram of the equipment used in the calibration of the earphones. As a first step, the microphone was calibrated using a pistophone. Secondly, a click was sent through the function generator and to the attenuator, the amplifier, and to the headphones. The output from the earphone was routed through the 2cc coupler to the sound level meter. The oscilloscope that is connected to the sound level meter was used to observe the output signal. In order to establish a peak equivalent SPL, a calibrated sine wave with the same amplitude and frequency as the filterd click was generated using a second function generator. The idea was to make certain that the SPL reading of the click was equivalent to the SPL reading of the sine wave (pure tone). The duration of the pulse (filtered click) was 0.2 msec. We know that for a 0.2 msec pulse, the first cycle must be about 2 kHz. Accordingly, a 111 dB SPL tone burst from the function generator at 2 kHz produced a 1650 Hz signal at 111 dB SPL measured on the sound level meter. The 1650 Hz signal gave us less than 0.5 msec which means that it was the best stimulus to use. A computer program was written so as to make certain that the

Figure III-2. Block diagram of calibration equipment

FIGURE III—2 CALIBRATION SYSTEM

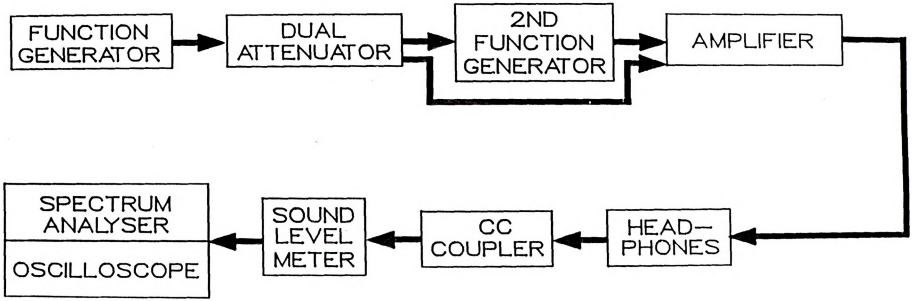

FIGURE III—2 CALIBRATION SYSTEM

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111 dB SPL generated at 2 kHz at the input level was equivalent to the 1650 Hz at 111 dB SPL at the output level.

Durrant (1983) posited that in order to establish the peak equivalent SPL of a click stimulus, the comparison tone must have a frequency whose period is approximately the same as that of the first cycle of the click, i.e., about 3 KHz, or a frequency equal to the resonant frequency of the earphone/speaker, i.e., around 2 KHz. The filtered click was calibrated by first passing it through an oscilloscope and its waveform observed visually. We also monitored the filtered clicks routed to the earphones using the oscilloscope and spectrum analyzer. This judgement about the nature of the filtered click was based on the theory that computer generated filtered clicks are created by the formula:

$$\text{Sin } w \cdot \text{Sin } \phi \quad \text{when } \phi = 0 \text{ to } 180 \text{ , } w = 0 \text{ to } X \cdot 360$$

where X is the number of cycles desired.

We do know that an infinite 2000 Hz sine wave would give us a straight line frequency spectrum. We also know that as the number of cycles per second decrease from infinity, then, the frequency bandwidth of the spectrum also decreases.

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Materials

The hamsters employed in this study were desinated AN/As-Wh by Asher (1968), and are currently maintained by full-sibling mating (congenic strain maintained by crossing) at the Biology Research Center Michigan State University. This designation was done according to the standard nomenclature for inbred strains of hamster (Asher, 1968). Thus, by appropriate selection, the strain contained the gene Wh and its normal allele wh on a common genetic background. Since the expression of Wh was enhanced by the presence of cream e, the strain was made homozygous for e. The gene e, by itself, prevents the production of eumelanin without apparently altering any other aspect of the phenotype. The normal gene E was later incorporated back into the AN/As through repeated backcrossing. This normal allele was placed back into the strain to promote a more complete understanding of the impact of the Wh gene. The rational for adopting the congenic strain in developing the AN/As-Wh strain and the repeated backcrossing of E mutant into the inbred strain was to eliminate genetic variability, and also, to ensure the full manifestation of the Wh gene.

Animals with different alleles of the E gene and the Wh gene, in this investigation, were of the seventh generation, backcrossing. All other animals in the AN/As strain are at their 24th generation with at least one parent at each

generation being a heterozygote. This degree of inbreeding insures that hamsters from this strain are 99.9% identical for genes other than the Wh. Hamsters were housed in polycarbonate cages with galvanized stainless steel tops, cleaned weekly and provided with water. Lighting of the animals was on a regime of 14 hours of light and ten hours of darkness. Thus, all hamsters were exposed to the same environment so as to control environmental factors that may influence the full expression of our mutation of interest, the Wh gene. The above genetic combinations resulted in the five genotypes of hamsters utilized in this study. Thus, the five groups of hamsters used in the study were selected from the following genotypes: wh/wh;E/e (agouti), wh/wh; e/e (cream), Wh/wh;E/e (white bellied agouti), Wh/wh;e/e (black-eyed white), and Wh/Wh; (anophthalmic white).

Procedure

As a first step in the experiment, the animals were anesthetized with rompun (dose = 10mg/kg) and sodium petobarbital (dose = 30 mg/kg). A heating pad was used to maintain body temperature at 37°C. Supplemental dosages of rompun were administered as necessary to maitain a constant background EEG level. Three stainless steel electrodes were inserted subcutaneously, with active on the vertex (Cz), ground at the center of the forehead (FPz) and reference to the pinna ipsilateral to the side of the recording. The

Akai "Walkman type" of earphone was used as a transducer (Ai). A 5cc syringe tip was cut and attached to the earpiece with parafin. The click was directed through the syringe tip to the ear canal of each animal. Each animal was then put into a specially prepared animal holder made of molded foam and then placed in an stereotaxic restrainer in a sound restricted room. A quiescent state was desirable since it promoted a quieter physiologic background and reduced on-going noise levels (Moore, 1971). Clicks of 0.2 msec duration were presented to the both ears of each hamster. The clicks with rarefaction, condensation and alternating polarity were presented at a repetition rate of 11.1/sec at an intensity level of 100 dB, p.e. SPL, and decreased by increaments of 10 dB until ABR waves were abscent. Threshold was defined as a 1.0 μV between the minimum negative and maximum positive peak amplitudes for waves I, II, III and IV.

Data Reduction and Statistical Analysis

ABR threshold was defined as the stimulus intensity at which waves can be detected and was measured as a 1.0 uV difference between the minimum negative and maximum positive amplitudes. This definition has been used by several investigators (Møller and Blegvad, 1976; Henry, 1979; Kavanagh and Beardsley, 1979; Osako, Tokimoto and Matsura, 1979; Kodera and Yagi, 1979; Jerger and Mauldin, 1978; Dun,

1984; Church, Williams and Holloway, 1984; Smith and Kraus, 1987; Schweitzer, 1987). The latencies were measured from the onset of the stimulus to the most prominent peaks of waves I, II, III and IV. Further, peak-to-peak amplitude measurements were made from the first positive peak to the next negative troughs of waves I, II, III and IV. The mean, standard deviation, slope and intercepts were calculated for the threshold data as well as the amplitude and latency values of waves I, II, III and IV across animal genotypes. Composite data were computed for amplitudes and latencies of waves I, II, III and IV as a function of stimulus intensity. By the same token, amplitude and latency data for waves I, III and IV were compared across genotypes at a specific intensity level.

The statistical procedure selected to test for the differencies between the means of the thresholds, amplitude and latency of waves I, II, III, and IV of the various genotypes was the analysis of variance. According to several investigators (Myers, 1979; Winer, 1971; Borg and Gall, 1979) ANOVA statistics are powerful tools that can be used even with small samples. Thus, the two-factor ANOVA design was used to determine the simultaneous effects of intensity and genotype (independent variables), and their combined effects on the dependent measures (amplitude and latency of waves I, II, III and IV of the ABR). The ANOVA

is very robust (Silverman, 1985), and thus, if we violate the normality assumption in terms of the sample size in each genotype, we can still be assured of the accuracy of our analysis (Glass and Hopkins, 1984). We also employed the Chi-square to test for differences between the Wh-locus and the E-locus. The Duncan's Multiple Range Test was used to determine which class means contribute significantly to the differences detected by the F-test. The Duncan's test utilizes the error mean square from the analysis of variance as the best estimate of variance of the populations. From the error mean square, the standard error of the mean is obtained. Before this test is performed, one must see that the number of replications for each cell are equal. This post hoc test also utilizes "protected significant studentized ranges". These values represent significant student-t values at the five and one percent levels of significance which have been calculated considering the degree of freedom for the error mean square and the number of means spanned in the comparison. Duncan (1955) has accordingly tabulated values of the "significant studentized ranges" with respect to level of significance (five or one percent), degrees of freedom of the error mean square, and number of means spanned in the comparison. Finally, the "shortest significant range" (Rp) is obtained by multiplying the "significant studentized range" by standard error of the mean. Rp = S.E.m X significant studentized

range. In performing the test, all means compared are placed on a single line in ascending order. The values of Rp are then calculated from data available in the analysis of variance table. A different value of Rp exists for different numbers of means spanned. Thus, by comparing the differences between any two means, and the appropriate value of Rp, one may determine whether the means are significantly different at the five or one percent level. That is, by a few simple calculations, one may compare any number of means and determine which are high, low, or intermediate. The "significant studentized ranges" extrapolated from Duncan's (1955) table at P = .05 with 40 degrees of freedom and for 2, 3, 4, 5, 6 means spanned are: 2.86, 3.01, 3.10, 3.17 and 3.22. These values were used in determining differences between means of various intensity levels. By multiplying these values by the appropriate standard error, the shortest significant ranges (R) are obtained. Thus, by performing the Duncan's (1955) test, the effect of treatments are determined when such differences were indicated by the analysis of variance.

CHAPTER 4

RESULTS

This chapter presents the BSER findings obtained from 20 hamsters. Wherever applicable, descriptive and inferential statistics which were performed so as to provide answers to the various research questions posed. A visual representation of data is also provided by use of tables and figures. The study employed a 5 x 6 and a 2 x 6 factorial design as it sought to determine thresholds, latency and amplitude of BSERs of five sets of genotypes at six intensity levels of 25, 35, 45, 55, 65 and 75 dB nHL for both right and left ears. Specifically, the study was designed to answer the following null hypothesis:

(1) The genotype wh/wh, E/e (Agouti) has no effect on the morphology, latency and amplitude of waves I - IV of the auditory brain-stem response at varying intensities.

(2) The genotype wh/wh, e/e (Cream) has no effect on the morphology, latency and amplitude of waves I-IV of auditory brain-stem response at varying

intensities

(3) The genotype Wh/wh, e/e (Black-eyed white) has no effect on the morphology, latency and amplitude of waves I - IV of the auditory brain-stem response at varying intensities.

(4) The genotype Wh/wh, E/e (White-belly Agouti) has no effect on the morphology, latency and amplitude of auditory brain-stem response at varying intensities

(5) The genotype Wh/wh-- has no effect on the morphology, latency and amplitude of auditory brain-stem response at varying intensities.

Twenty animals designated as AN/As-Wh by Asher (1968) were used in the study. They were chosen from a total of 30 hamsters so as to constitute a final sample. Animals were anesthetized with rompun (dose = 10 mg/kg) and sodium pentobarbital (dose = 30 mg/kg). Three stainless steel electrodes were inserted subcutaneously, with active on the vertex (C_z), ground at the center of the forehead (FP^z) and reference at the pinna ipsilateral to the side of the recording. Stimulus intensity was presented from 25 - 75 dB nHL (~ 50 - 100 dB p.e. SPL). Clicks with a major spectral peak at 2000 Hz and a repetition rate of 11.1/sec were presented to both ears of each animal.

The typical responses were a series of positive-negative waves. The waves obtained from two sets of genotypes, Agouti (wh/wh, E/e) and the Cream (wh/wh, e/e) exhibit the same distinct morphological characteristics and the same time of occurence as reported by earlier investigators in several other species (Jewett, 1970; Jewett and Romano, 1972; Dobie and Berlin, 1976; Church et al, 1976; Schweitzer, 1987; and Ahmadizadeh et al, 1987). The peaks have been designated in this study as waves I-IV (Jewett, 1970). We note that wave III is the most stable, especially at lower intensity levels in most animals; wave II is often fused with wave III and is often clear at higher intensity levels. By contrast, Black-eyed white (Wh/wh e/e), White-belly agouti (Wh/wh, E/e) and Anophthalmic white (Wh/Wh--) present a different morphology of BSER waves. Thus, with the Black-eyed white (BEW) and the White-belly agouti (WBA), we see that at high intensity levels, waves I - IV followed the same morphological patterns as the normal genotypes. However, the waves have a narrow dynamic range compared with the normal genotypes. Additionally, at high intensity levels of 55 - 75 dB nHL waves I-IV are mostly discernible. Below this intensity level, however, the waves often become indistinguishable. We see that in some animals there is a potential preceding wave I. This potential is referred to as I'. Again in certain traces, one can discern wave V. In the case of the Anophthalmic white (AW), waves I - IV were

totally absent. The analog wave forms for the twenty genotypes can be found in Appendix B and the numerical values are listed in Appendix C through F.

Latency for Normal Hearing Genotypes

This section describes latency values for the Agouti and the Cream. The latency data from these genotypes for waves I - IV are presented in Appendix G; input - output latency functions can be found in figures 1 and 2. Inspection of Appendix G and figures 1 and 2 revealed that as the intensity of the stimulus is increased from 25-75 dB nHL, all four waves showed a systematic decrease in absolute latency as a function of increasing stimulus intensity. Exceptions were noted however, in the individual data, in that in some animals, latency remained constant as a function of stimulus intensity. This was noted for wave II in the right ear of HM33 (Agouti) (p. 238) at intensity levels of 35 and 45 dB nHL and for wave I of HM21 (Cream) (p. 245) at the same intensity levels. Observe also that as the intensity is increased, the standard deviation (SD) of the waves decrease. In certain definite regions, however, there is also an increase in SD as intensity is increased. We know that the magnitude of the SD indicates the degree of variability of measures from which it is computed. We also know that the larger the standard deviation, the more variable the measures. The smallest possible SD as we know,

is zero, which of course, indicates no variability. Thus, the smaller the SD the more representative the mean. It can be observed in our data that represented in figures 1 and 2 that at each intensity level, the latency values are heterogenous. The factorial analysis revealed that the coefficient of variation in latency for the Agouti and the Cream for waves I - IV is less than 15% This indicates lower variability among the animals.

The two-way analysis of variance (ANOVA) was calculated for waves I, II, III and IV to determine whether the genotypes wh/wh E/e (Agouti) and wh/wh e/e (Cream) have any significant effect on latency. It turns out that the latencies of waves I - IV are dependent on genotype. Thus, the two-way ANOVA indicated that there is a genotypic difference with respect to latency. There was no interaction between genotype and latency (Appendices H1 and H2). The ANOVA values for the right ear are: Wave II, [$F(1,5) = 7.16 = P < .05$], wave III, [$F(1,5) = 8.32$ $P < .05$] wave IV, [$F(1,5) = 6.42$ $P < .05$]. For the left ear, the two-way ANOVA results are: Wave I, [$F(1,5) = .967$ $P > .05$], wave II, [$F(1,5) = 19.06$ $P < .05$], wave III, [$F(1,5) = 3.46$ $P > .05$] and wave IV, [$F(1,5) = 6.52$ $P < .05$]. We know that the null hypothesis states that no differences will be found between the means compared. If the null hypothesis were true, we will find this large differences

Figure IV-1. Input-output latency functions for Agouti (wh/wh, E/e).

AGOUTI - LATENCY

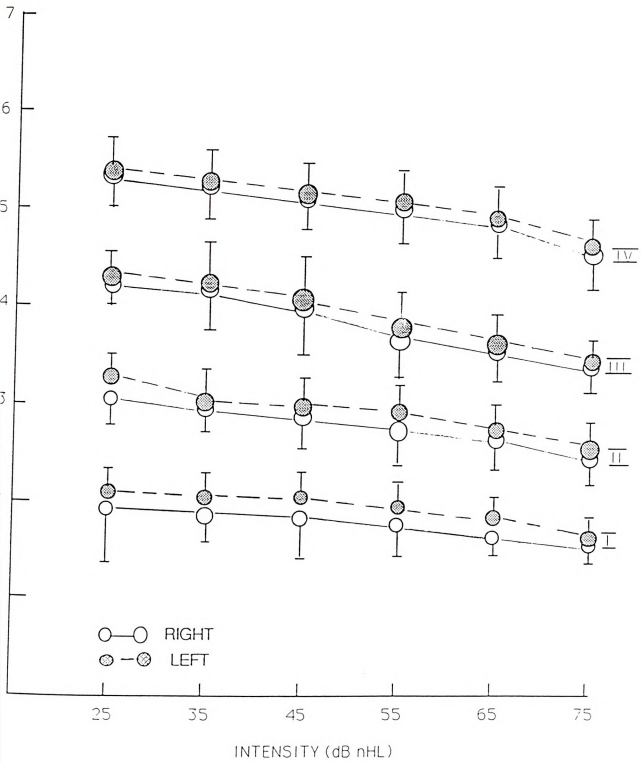
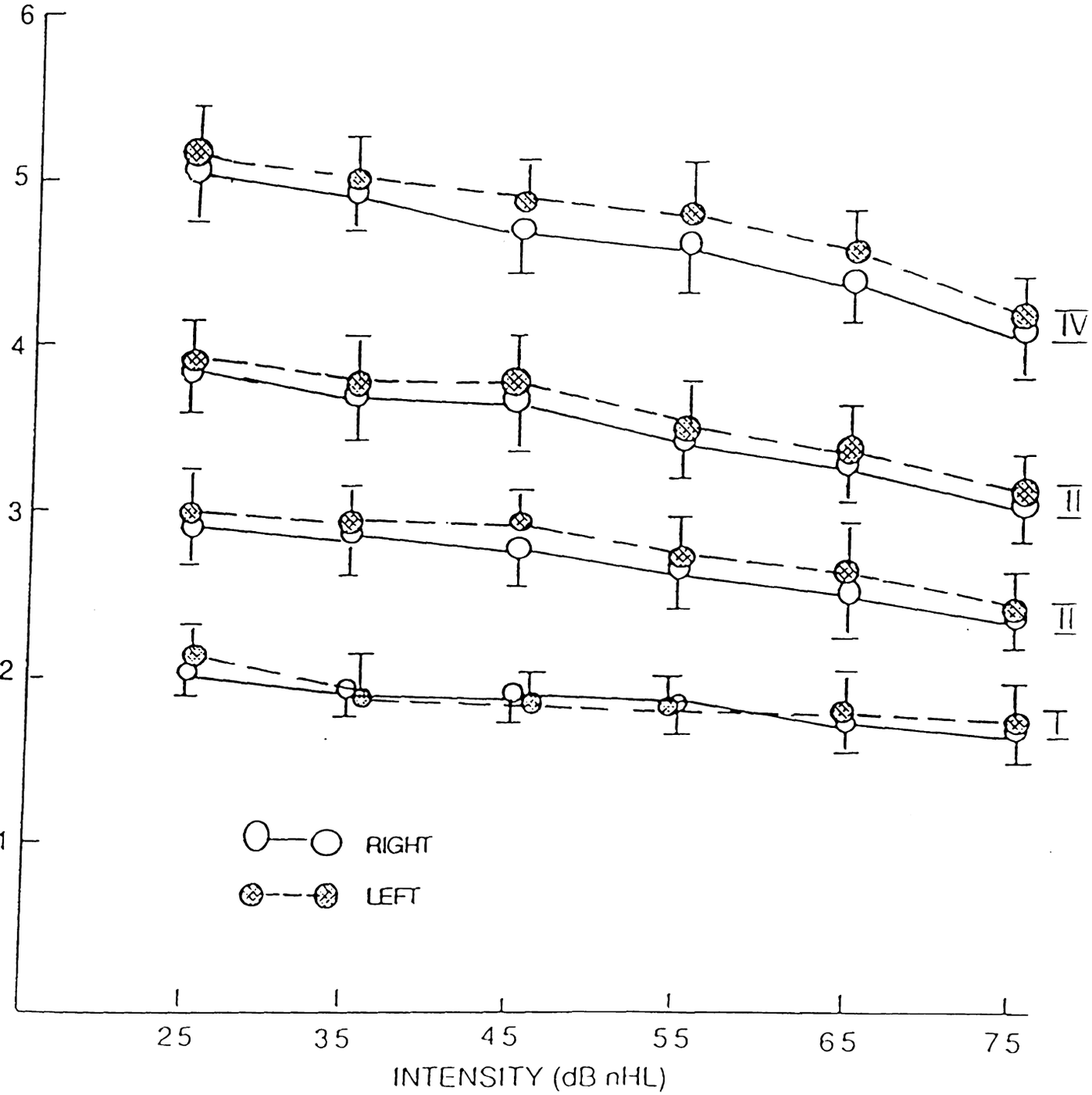


Figure IV-2. Input-output latency functions for the Cream (wh/wh, e/e).

CREAM - LATENCY



(H1 and H2) between the sample means only once in twenty experiments. Since we have found this large difference, it is probable that the null hypothesis of no difference between the sample means is false. Therefore, we reject the null hypothesis for waves II, III and IV for the right ear, and waves II and IV in the left ear and conclude that genotype wh/wh E/e and wh/wh e/e affect latency differently. Observe in Appendix G that the various intensity levels have significant effect on the latency of the twogenotypes. Duncan's (1955) test was used to determine differences in latency values caused by varying intensity levels (Appendix I).

In another vein, it can be observed that at a click intensity level of 75 dB nHL, the inter-aural latency (ILD) was not the same for Agouti and the Cream (Appendix J). In the case of the Agouti, the ILD were: wave I .06, wave II .01, wave III .07 and wave IV .08. The ILD values for the Cream were: wave I .04, wave II .01, wave III .17 and wave IV .21. It can be noted that ILD ranged from .01 ms to .08 for waves I - IV, whereas in the case of the Cream, ILD ranged from .01 ms to .21 ms. Observe that ILD were closer for the two genotypes for waves I and II. The two-way ANOVA showed no significant difference between right and left ears for the Agouti and the Cream across all intensity levels for waves I-IV (Appendix K).

Latency for Hearing-Impaired

This section describes latency responses for genotypes Wh/wh e/e (BEW), Wh/wh E/e (WBA) and Wh/Wh-- (AW), for whom some or all of the waves are absent. We see in Appendix G and figures IV-3 through IV-5 the composite data and the input-output plots respectively. Inspection of the Appendix and the figures revealed gross differences in normal and hearing-imaired genotypes. It can be seen that for the BEW and WBA the latencies are displaced to the right and as expected, intense stimuli are required to evoke a response. The global picture is that at intensity levels in which responses were obtained for all animals (65-75 dB nHL) lantency decreased as a function of stimulus intensity. Below 65 dB nHL, the pattern appeared different, in that the mean latency values from which points on the graph are derived are from responses of fewer ears. Observe also that SD bars were not ploted on the graphs at some intensity levels. This was the case since a response was obtained from only one ear or where the mean values cancel each other, resulting in zero SD and thus, indicating no variability. Observe also that latency values were not calculated for genotype Wh/Wh-- in that recordable responses were not obtained even at the highest intensity level of 90 dB nHL. We also note that latency changes differed for the two genotypes. For example, a a gradual-slopping

Figure IV-3. Input-output functions for waves I and II for the Black-eyed white (Wh/wh, e/e).

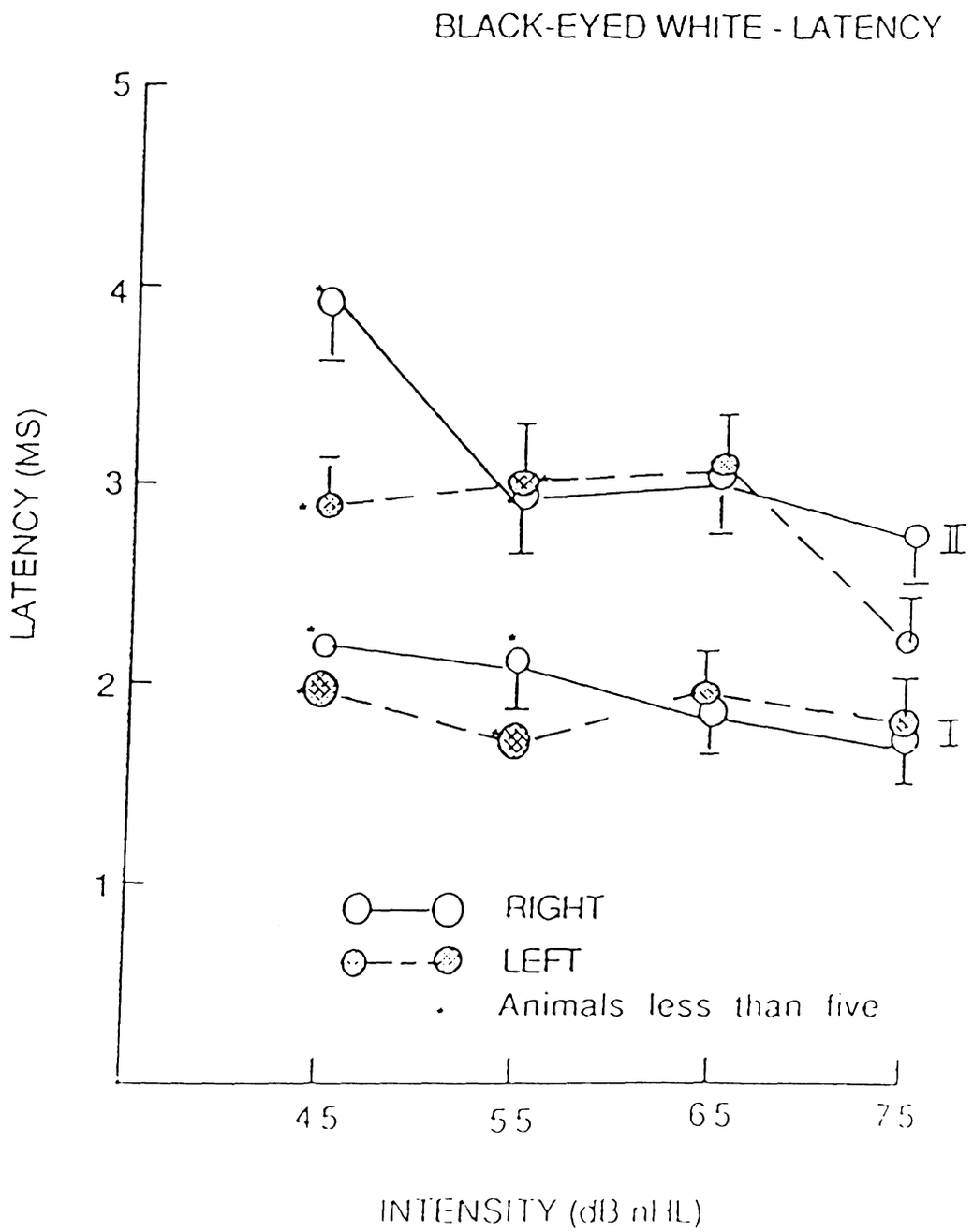

BLACK-EYED WHITE - LATENCY
LATENCY (MS)
5
4
3
2
1
II
I
RIGHT
LEFT
Animals less than five
45
55
65
75
INTENSITY (dB nHL)

Figure IV-4. Input-output functions for waves III and IV for the Black-eyed white (Wh/wh, e/e).

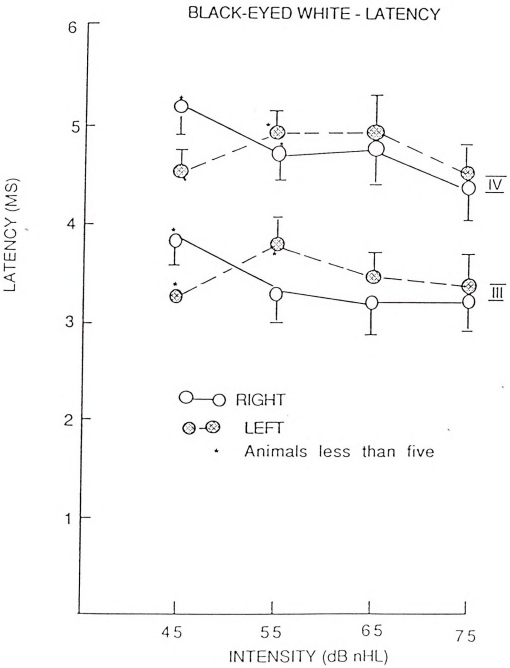
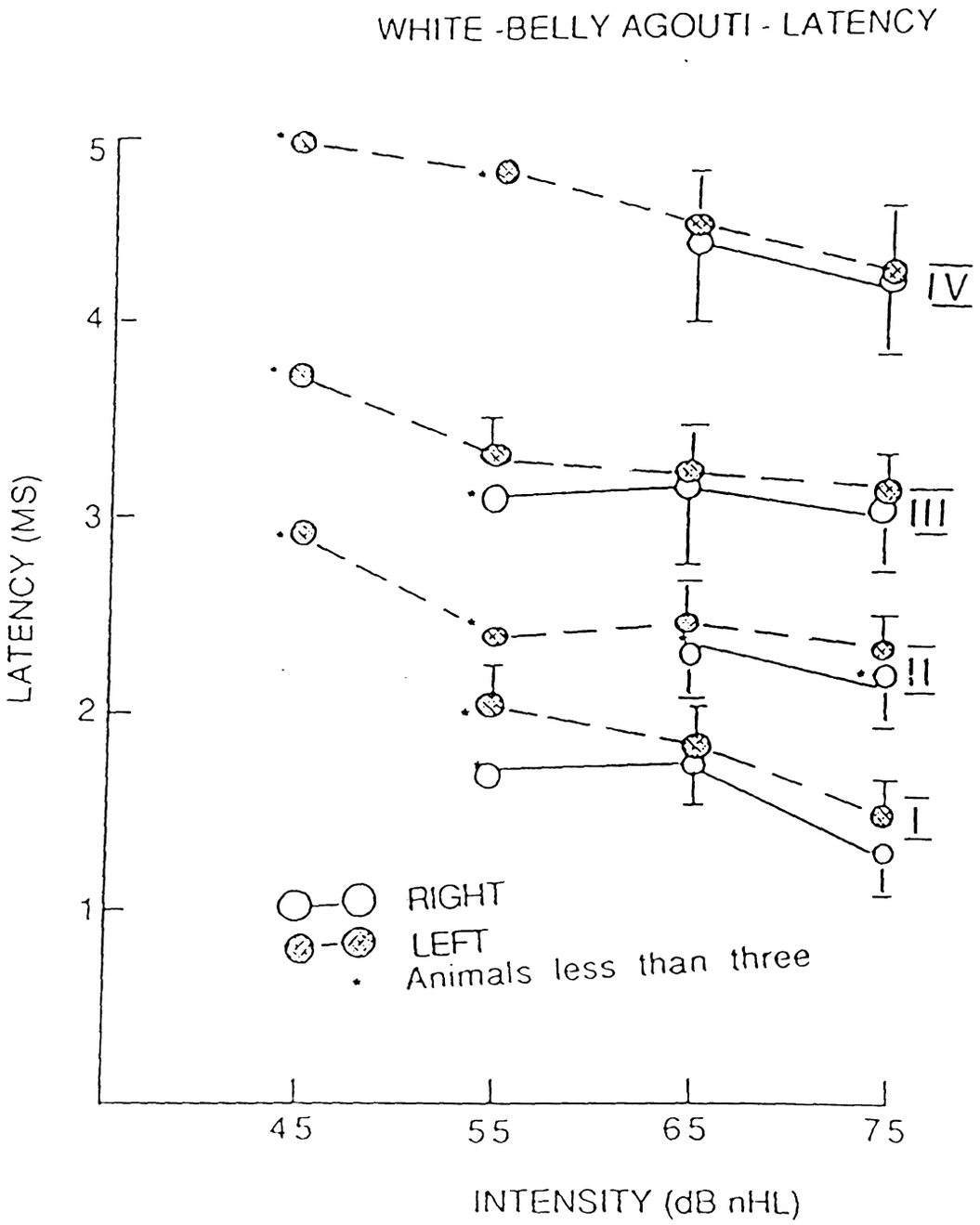

BLACK-EYED WHITE - LATENCY
LATENCY (MS)
6
5
4
3
2
1
IV
III
RIGHT
LEFT
Animals less than five
45
55
65
75
INTENSITY (dB nHL)

Figure IV-5. Input-output for the White-belly Agouti (Wh/wh, E/e).


WHITE -BELLY AGOUTI - LATENCY
LATENCY (MS)
5
4
3
2
1
IV
III
II
I
RIGHT
LEFT
Animals less than three
45
55
65
75
INTENSITY (dB nHL)

configuration of the input-output plot was noted for the BEW for the right ear. The left ear for the same genotype showed somewhat of a flat input-output configuration. With regard to the WBA, the input-output functions for both ears had a gradual slope. The exception noted was for wave I in which the functions became steeper at 65-75 dB nHL for both ears. A non-orthogonal ANOVA was performed comparing mean latency measures for the BEW and the WBA for waves I-IV (Appendice L). From this analysis, we observed that there was no significant difference between BEW and WBA in latency for all waves, except wave II (left ear) and wave III (right ear). There was no genotype-intensity interaction for the two waves. No significant difference was observed between the genotypes for the remaining waves.

Appendix M shows the ILD for the BEW and the WBA at 75 dB nHL. It can be observed that ILD for the two genotypes ranged from -.05 to .59 ms. Thus, for the BEW, the ILD values were: Wave I, -.05 ms, wave II, .59 ms, wave III, .28 ms, and wave IV, .15 ms. In the case of the WBA, the ILDs were: Wave I, .34 ms, wave II, 1.4, wave III, .35 ms and wave IV, .11 ms. The two-way ANOVA was calculated for waves I-IV to determine whether there are differencies in latency values between the two ears for each genotype across the intensity levels of 55-75 dB nHL. As expected, the

results were not statistically significant (Appendix N). intensity levels of 55-75 dB nHL. As expected, the were not statistically significant (Appendix N). resultsthe

In another vein, figures IV-6 through IV-9 reveal the composite latency data for all genotypes depicting the degree of interaction among all genotypes across the various intensity levels. In the statistical literature, a distinction is made between two types of interaction, ordinal and disordinal. In the ordinal case, the rank order of one factor on the basis of their dependent variable values is the same within each level of the independent variable. Our latency data as can be seen in figure IV-6 depicts an ordinalinteraction among the genotypes. As can be seen, superiority exists for the Cream in relation to other genotypes at all intensity levels. That is, even though superiority exists, a single statement about the superior treatment can be madewithout qualification or reference to the other genotypes. On the other hand, we see in figures IV-7 through IV-9 a graphic representation of disordinal interaction among genotypes. As can be seen, superiority did not exist for any genotype across the various intensity levels. In this regard, we cannot make a single statement about the superior treatment without qualification. Therefore, the observations regarding a better treatment depends on the response obtained by a

Figure IV-6. Composite data points of all genotypes for latency as a function of stimulus intensity for wave I.

RT AND LT EAR WAVE I

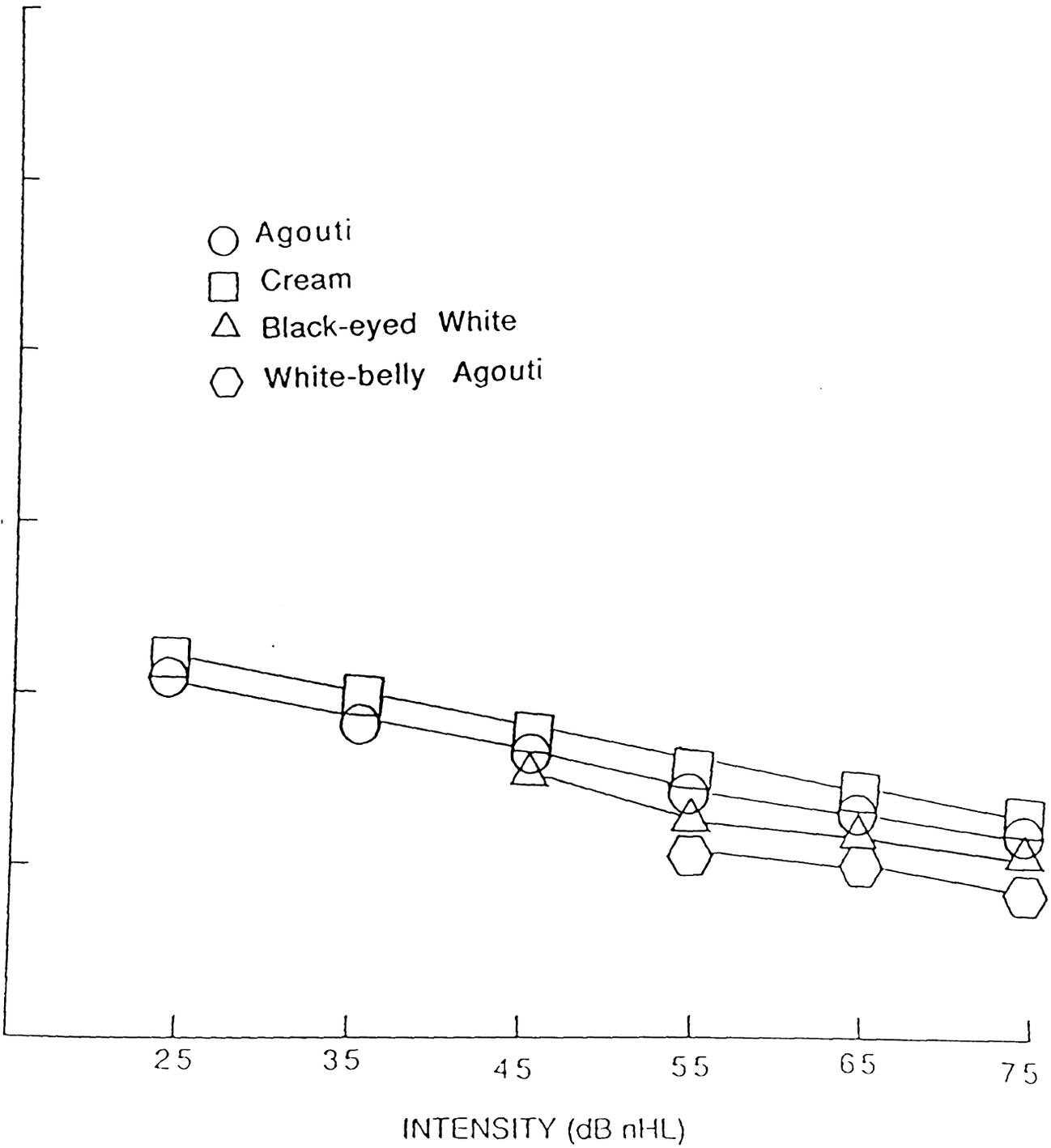


Figure IV-7. Composite data points of all genotypes for latency as a function of stimulus intensity for wave II.


LATENCY (MS)

RT AND LT EAR WAVE II

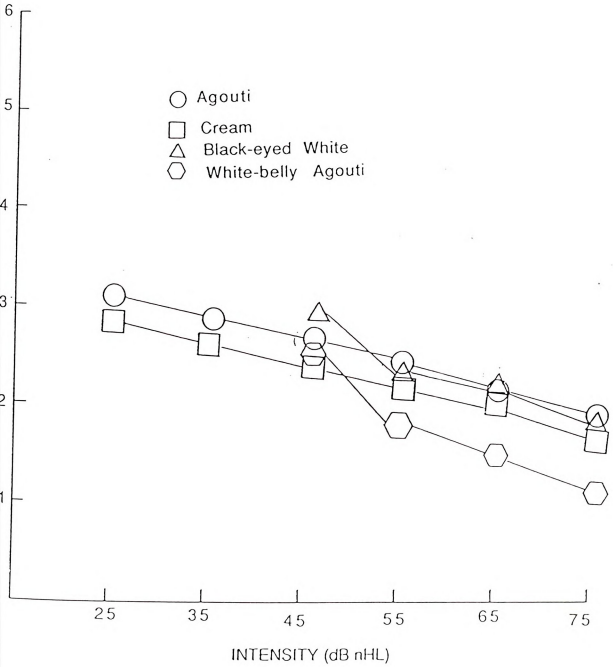


Figure IV-8. Composite data points of all genotypes for latency as a function of stimulus intensity for wave III.


LATENCY (MS)

RT AND LT EAR WAVE III

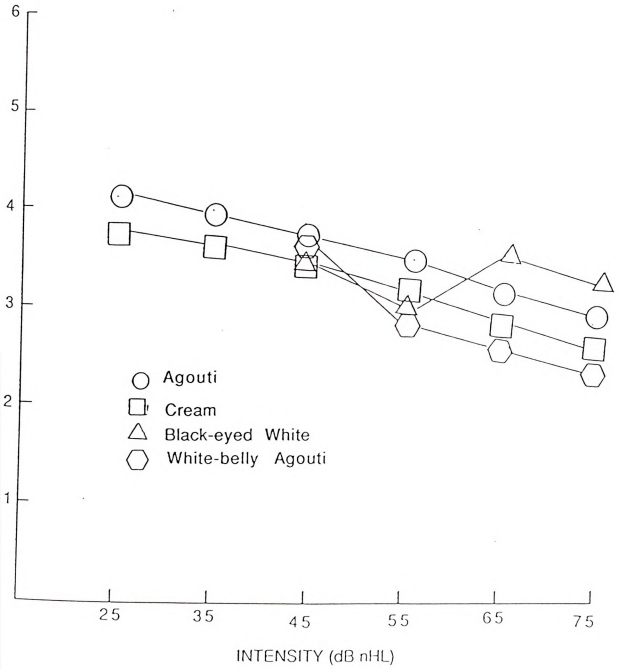
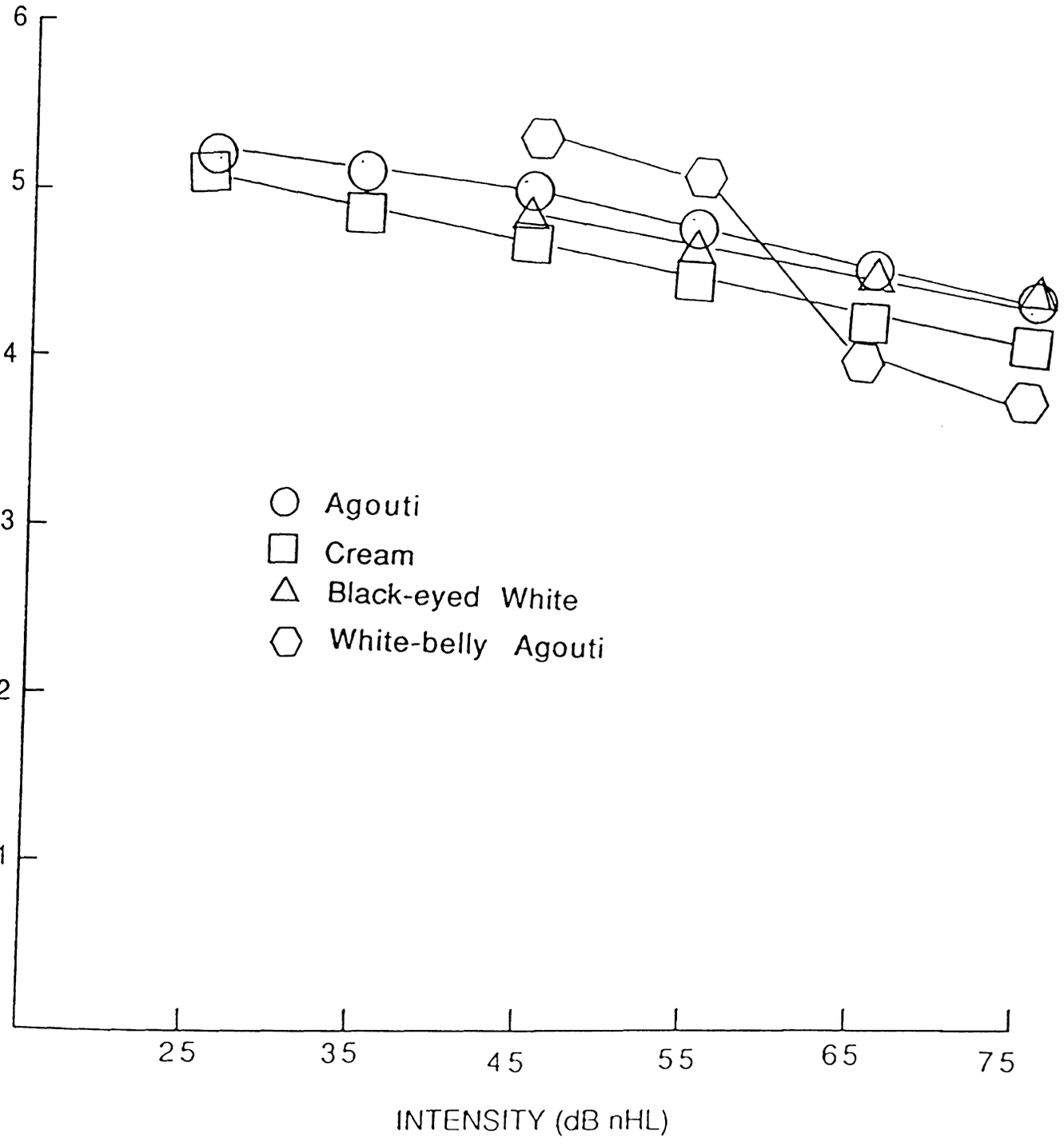


Figure IV-9. Composite data points of all genotypes for latency as a function of stimulus intensity for wave IV.

LATIMER (MS)

RT AND LT EAR WAVE IV



particular genotype at a given intensity level. For example, for wave II in figure IV-7 the BEW showed a higher latency value, whereas at 75 dB nHL the Agouti showed a higher latency value. Similarly, waves III and IV also exhibit a disordinal interaction.

We also see in figure IV-6 through IV-9 how the independent variable of intensity and the dependent variable of latency are related for the various genotypes. It can be observed that the relationship between intensity and latency is an inverse function since an increase in intensity tend to be associated with a decrease in latency. Correlation coefficients were calculated to determine the strength of the relationship between intensity and latency values so that we could determine whether a reliable prediction could be made for latency given a particular intensity level and for a particular genotype. The correlation coefficient was done using the formula:

$$r = \frac{nxy - 2xy}{nx2 - (x)2\ ny2 - (y)2}$$

As expected, the correlation values for the latency values of waves I, II, III, and IV showed a strong negative correlation (Appendix O). The notable exception was the BEW in which it was observed that the latency first decreased and then increased as a function of stimulus intensity.

Viewing Appendix P and the figures described above, one finds that thresholds for the Agouti and the Cream (25 dB nHL) are lower than for the BEW and WBA (45 dB nHL and above). It can also be observed that mean thresholds for the right ear are slightly greater for the WBA than the BEW for waves II, III, and IV. The exception noted was for wave I in which mean thresholds are slightly greater for BEW than for WBA. Interestingly, opposite findings were noted in the left ear. As it turned out, mean threshold values were higher for the BEW than for the WBA. The exception noted was for wave IV in which average thresholds are the same for both BEW and WBA. It is noted, of course, that thresholds were not recorded for genotype Wh/Wh--, even at the highest intensity level of 75 dB nHL.

It was of interest to determine the effect of each gene locus and their possible interaction on latency. Accordingly, the Chi-square was performed comparing the effect of Wh-locus and E/e-locus on latency. Interestingly, there were no statistically significant differences between Wh-locus and E/e-locus with respect to latency (Appendix Q). There was no interaction between the two genes with respect to latency.

Amplitude in Normal Genotypes

We see in Appendix R the mean amplitude values for the Agouti and Cream. In addition, figures IV-10 through IV-17 depicts input-output plots for the Agouti and Cream. It was observed that as intensity increased, the amplitude of waves I-IV increased with increasing SDs. Exceptions were, noted however, in some individuals for whom amplitude remained constant and even reduced as a function of stimulus intensity. This was noted for wave II in the right ear of the Agouti (HM012) at the intensity levels of 45, 55 and 65 dB nHL. Similar observations were noted in the Cream (HM021). The two-way ANOVA was calculated for the mean amplitudes of waves I, II, III and IV essentially to determine whether there is a significant difference in the Agouti and the Cream with regard to amplitude and whether the various intensity levels have any significant effects on amplitude of both genotypes. The results indicated significant differences between the Agouti and the Cream (Appendices S1 and S2). Appendix S1 summarises the results giving the F-values determined. For example, the statistical differences between the mean values for wave IIIwas as follows: Right ear, [$F(1,5) = 4.30$ $P < .05$] and left ear [$F(1,5) = 50 = P < .05$]. No significant difference was noted between genotypes for wave I in the

Figure IV-10. Input-output amplitude functions for wave I of the Agouti (wh/wh, E/e).

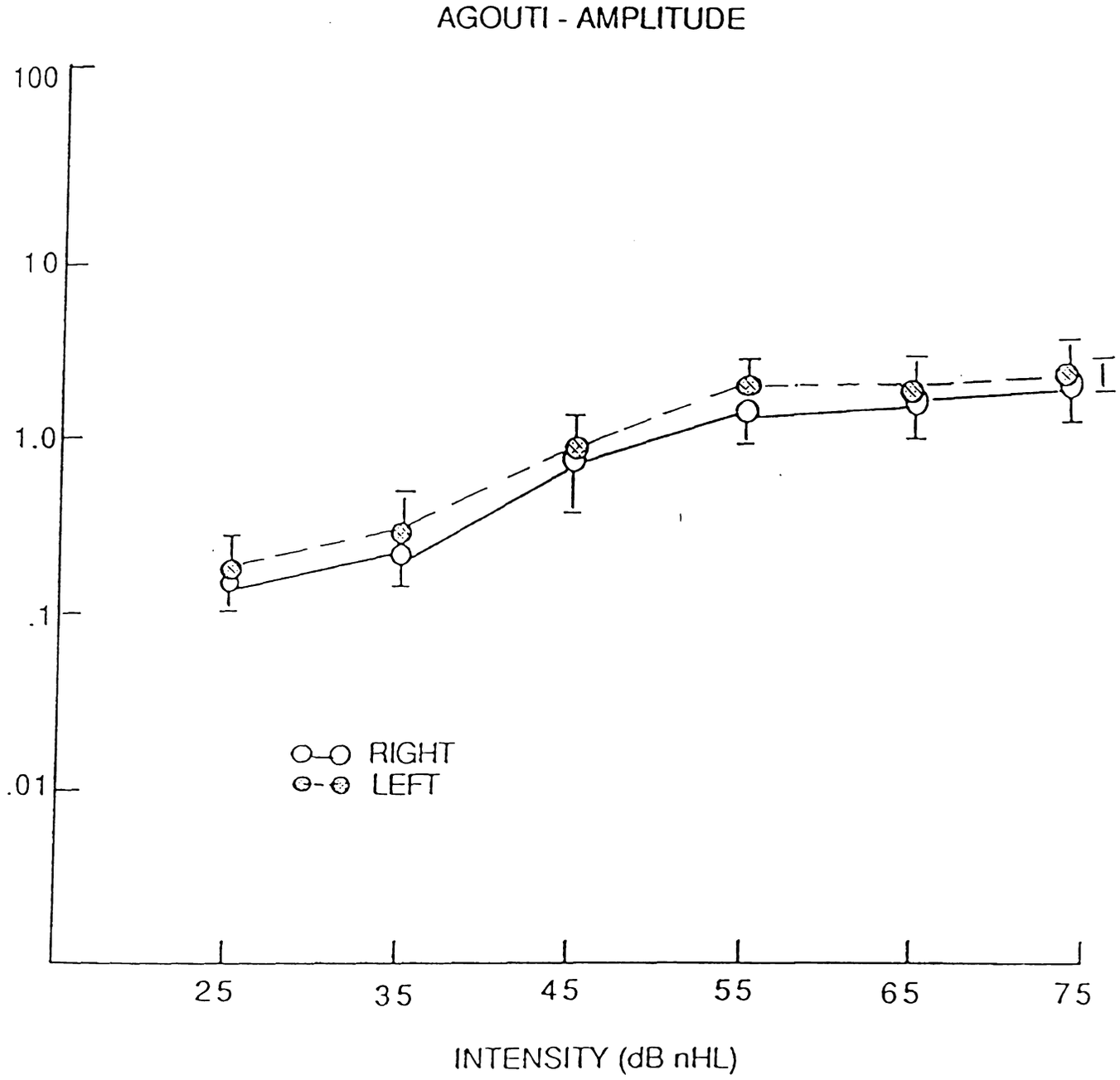

AGOUTI - AMPLITUDE
100
10
1.0
.1
.01
RIGHT
LEFT
25
35
45
55
65
75
INTENSITY (dB nHL)

Figure IV-11. Input-output amplitude functions for wave II of the Agouti (wh/wh, E/e).

AGOUTI - AMPLITUDE

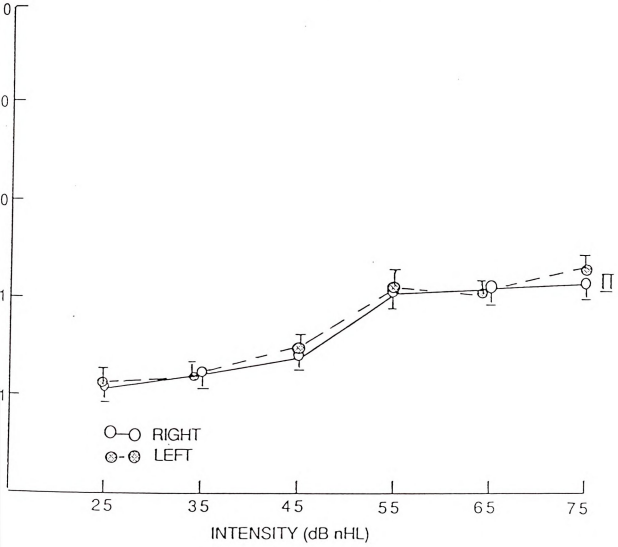


Figure IV-12. Input-output amplitude functions for wave III of the Agouti (wh/wh, E/e).

AMPLITUDE (NV) X 100

AGOUTI - AMPLITUDE

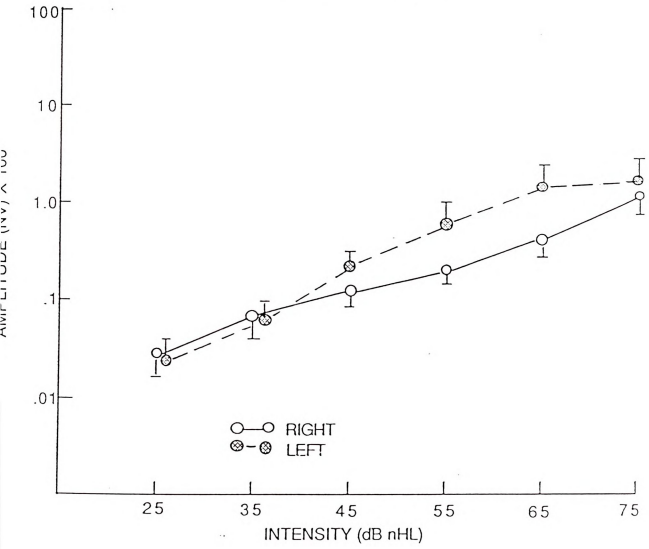


Figure IV-13. Input-output amplitude functions for wave IV of the Agouti (wh/wh, E/e).

AMPLITUDE (mV) x 100

10

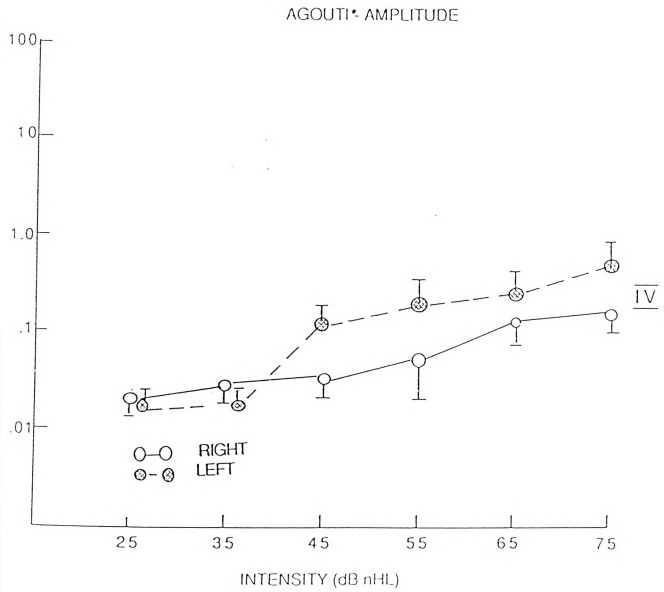

AGOUTI - AMPLITUDE
100
10
1.0
.1
.01
IV
O—O RIGHT
⊗-⊗ LEFT
25
35
45
55
65
75
INTENSITY (dB nHL)

Figure IV-14. Input-output amplitude functions for wave I of the Cream (wh/wh, e/e).

CREAM - AMPLITUDE

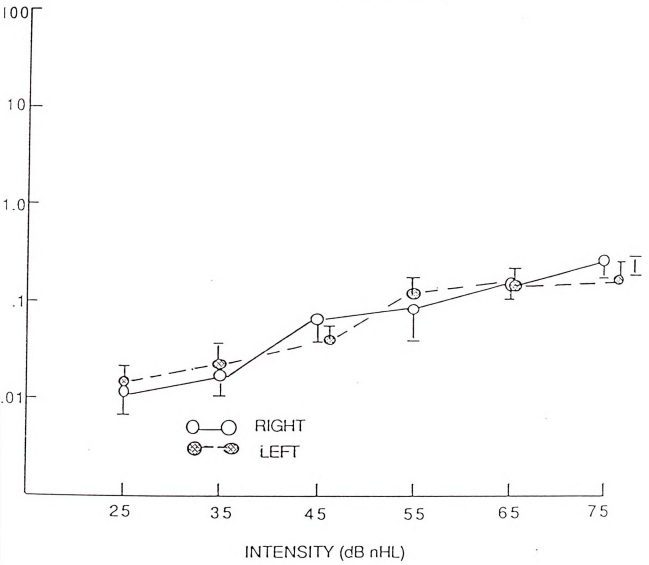


Figure IV-15. Input-output amplitude functions for wave II of the Cream (wh/wh, e/e).

CREAM - AMPLITUDE

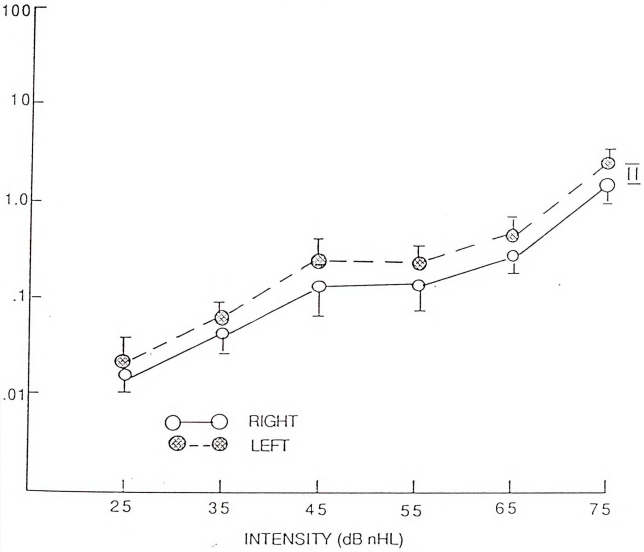


Figure IV-16. Input-output amplitude functions for wave III of the Cream (wh/wh, e/e).

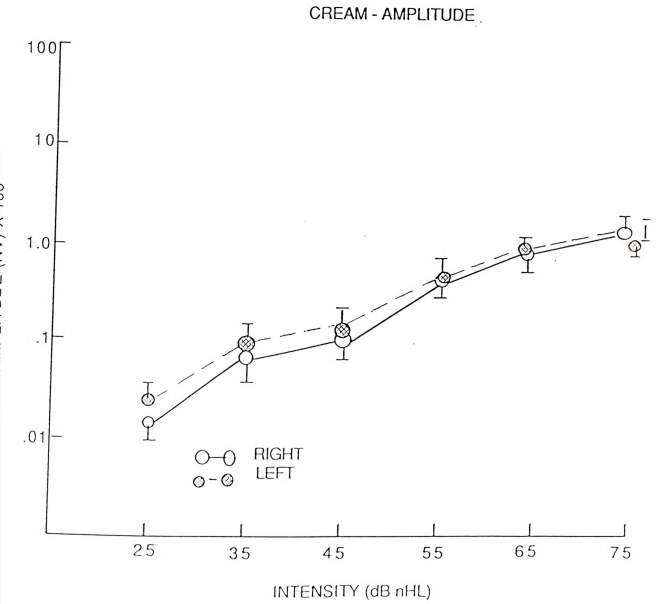
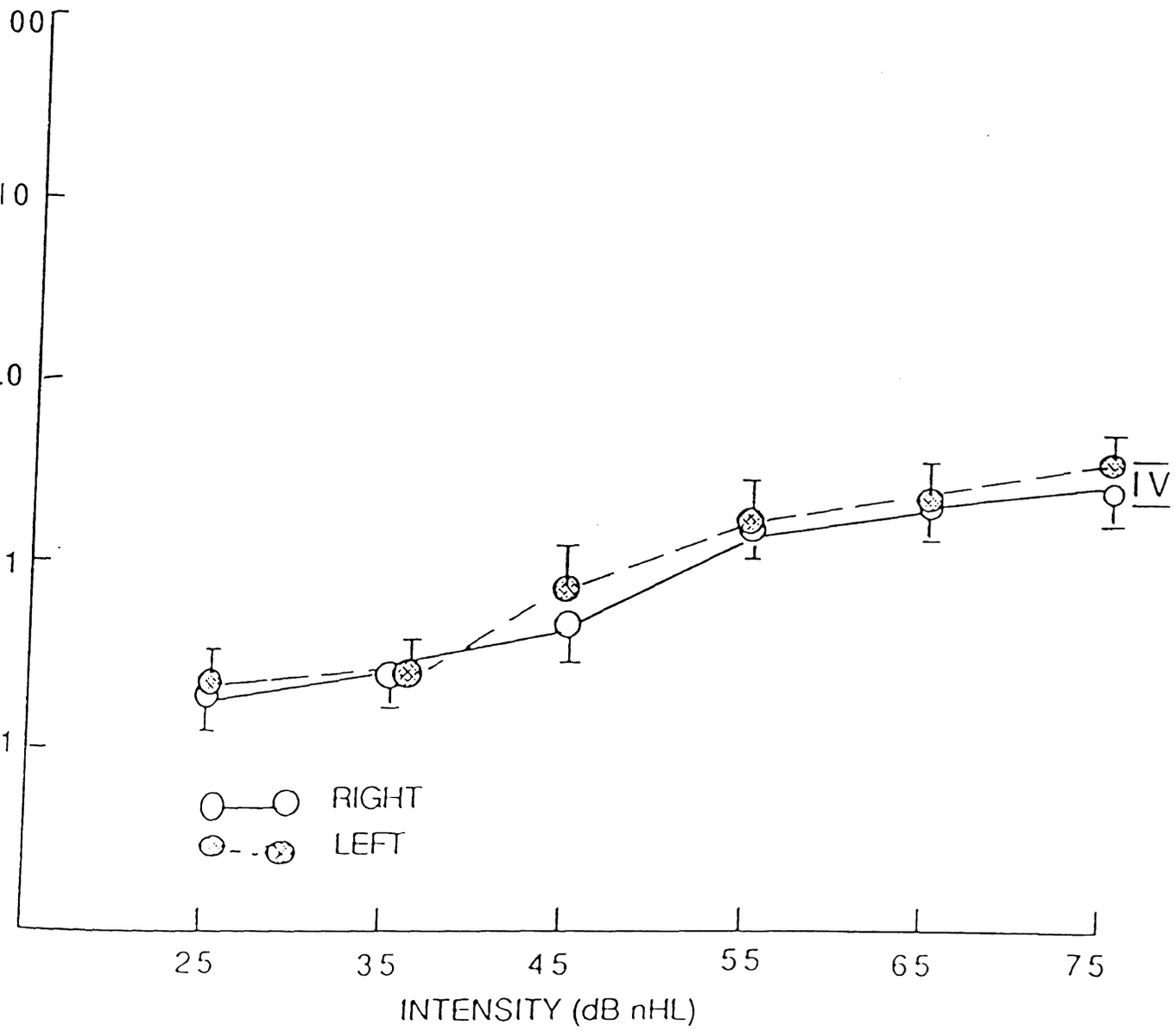

CREAM - AMPLITUDE
100
10
1.0
.1
.01
RIGHT
LEFT
25
35
45
55
65
75
INTENSITY (dB nHL)

Figure IV-17. Input-output amplitude functions for wave IV of the Cream (wh/wh, e/e).

AMPLITUDE (NV) x 100

CREAM - AMPLITUDE



right ear, and wave IV for the left ear. Genotype-intensity interaction was also minimal. We noted variability in the amplitude data, and coefficient of variation is above 50%. Duncan's (1955) test was used to determine differences in latency caused by varying intensity levels (Appendix T). The two-way ANOVA was computed to determine differences between ears across the various intensity levels, which revealed that there is no significant difference between the two ears (Appendix U)

Amplitude of the Hearing-Impaired

Inspection of the mean data (Appendix R) and the input -output functions in figures IV-18 through IV-25 depict gross diferences between normal and hearing-impaired genotypes. It can be seen that intensity-amplitude relationships are not described for the hearing-impaired genotypes for all waves at intensity levels below 45 dB nHL. The reason for this is that responses for the waves below 45 dB nHL are not identifiable. It is noted, however, that at intensities above 45 dB nHL and in cases where all ears are involved, amplitude did increase as a function of stimulus intensity. In other cases in which all ears are not involved, especially between 45 and 55 dB nHL, the pattern of amplitude intensity-relationship is quite different. This was noted in the left ear of the WBA at theintensity levels of 45 and 55 dB nHL for wave IV. The same

Figure IV-

Figure IV-18. Input-output amplitude functions for wave I of the Black-eyed white (Wh/wh, e/e).

AMPLITUDE (NV) X 100
100
10
1.0
.1
.01

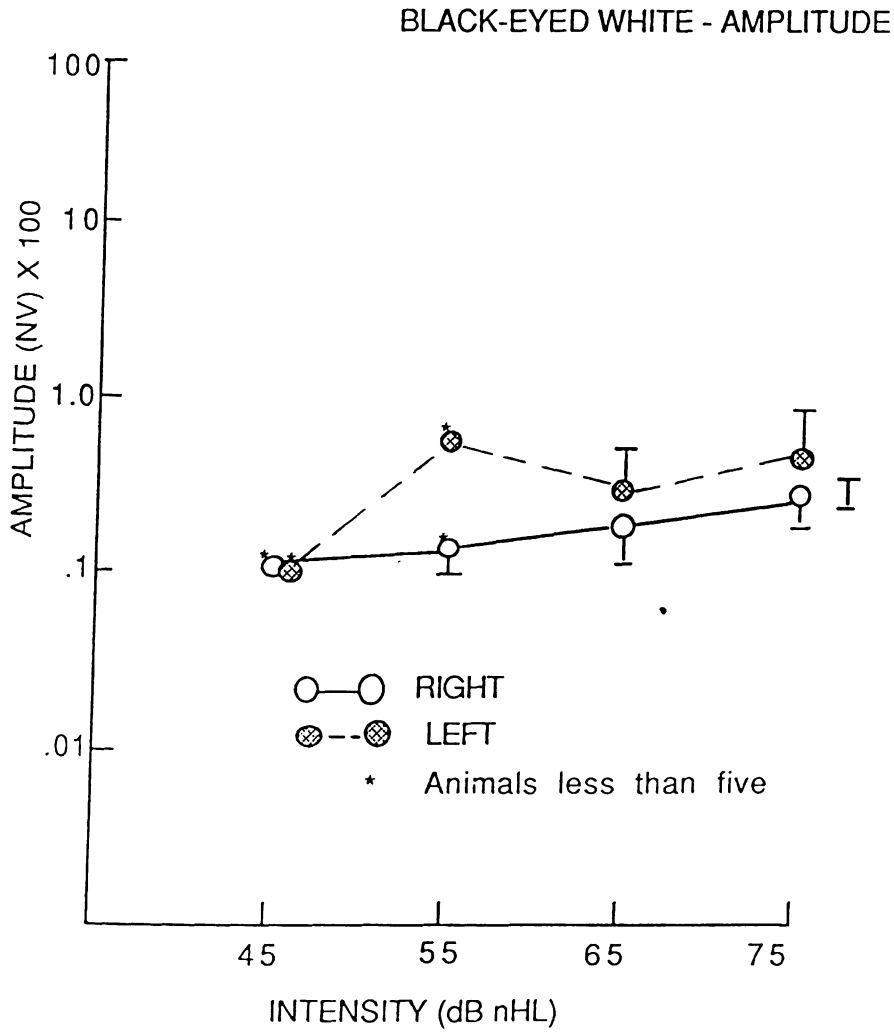

BLACK-EYED WHITE - AMPLITUDE
100
10
1.0
.1
.01
AMPLITUDE (NV) X 100
RIGHT
LEFT
* Animals less than five
45
55
65
75
INTENSITY (dB nHL)

Figure IV-1

Figure IV-19. Input-output amplitude functions for wave II of the Black-eyed white (Wh/wh, e/e).

AMPLITUDE (NV) X 100
100
10
1.0
.1
.01

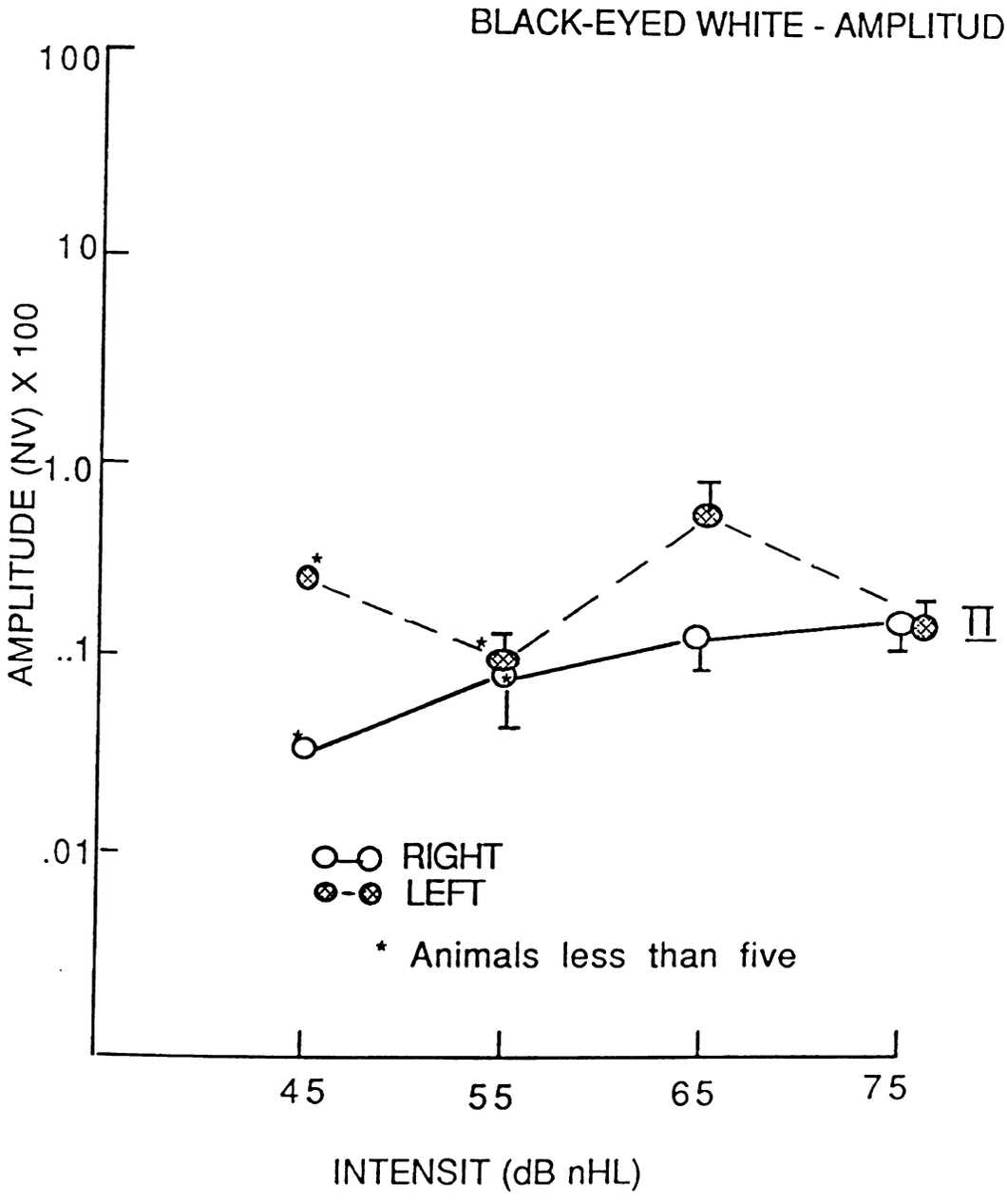

BLACK-EYED WHITE - AMPLITUDE
100
10
1.0
.1
.01
AMPLITUDE (NV) X 100
RIGHT
LEFT
* Animals less than five
45
55
65
75
INTENSIT (dB nHL)

Figure IV-1

Figure IV-20. Input-output amplitude functions for wave III of the Black-eyed white (Wh/wh, e/e).

100
10
1.0
.1
.01
AMPLITUDE (NV) X 100

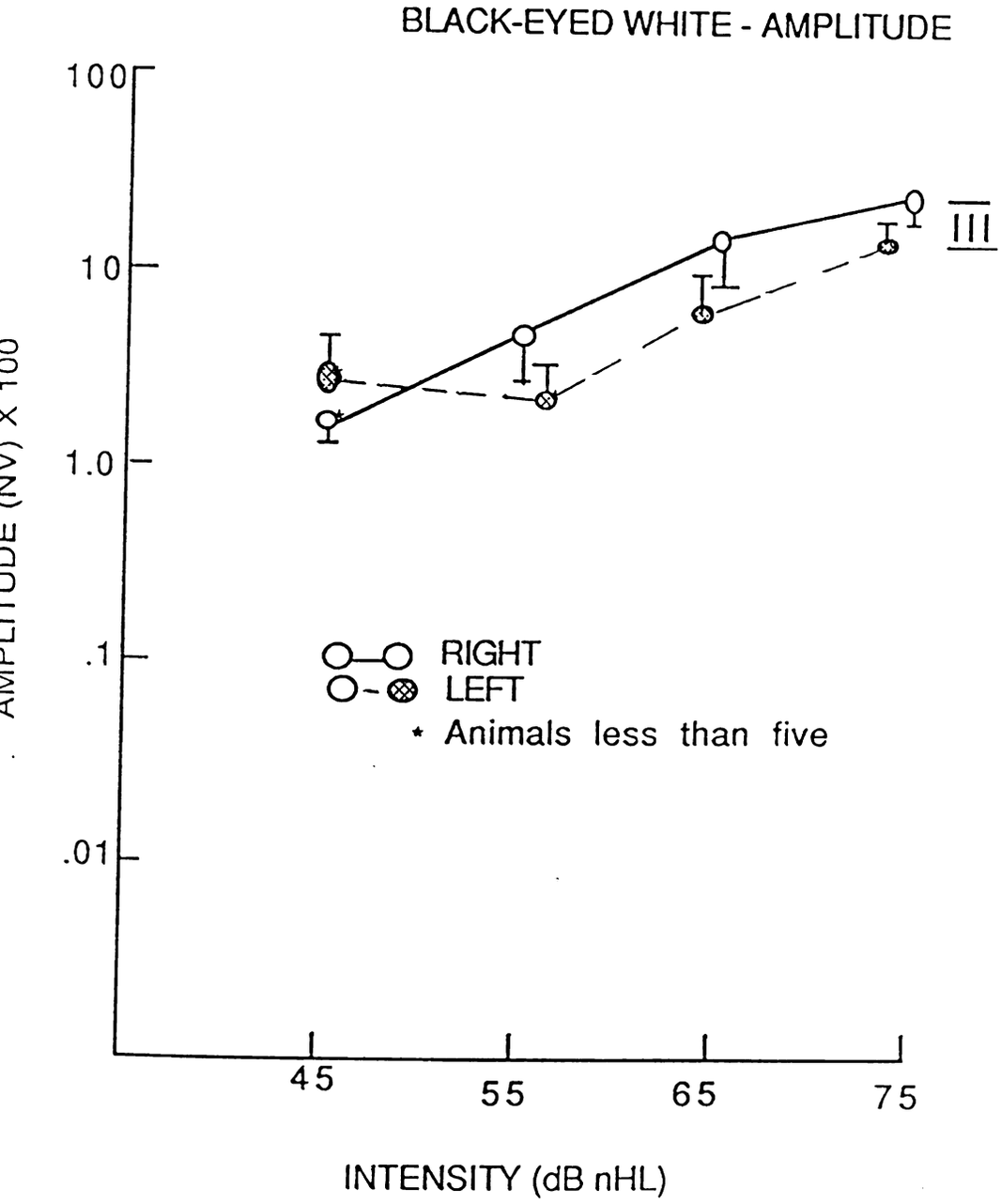

BLACK-EYED WHITE - AMPLITUDE
100
10
1.0
.1
.01
AMPLITUDE (NV) X 100
III
RIGHT
LEFT
* Animals less than five
45
55
65
75
INTENSITY (dB nHL)

Figure IV-

Figure IV-21. Input-output amplitude functions for wave IV of the Black-eyed white (Wh/wh, e/e).

100
10
1.0
.1
.01
AMPLITUDE (NV) X 100

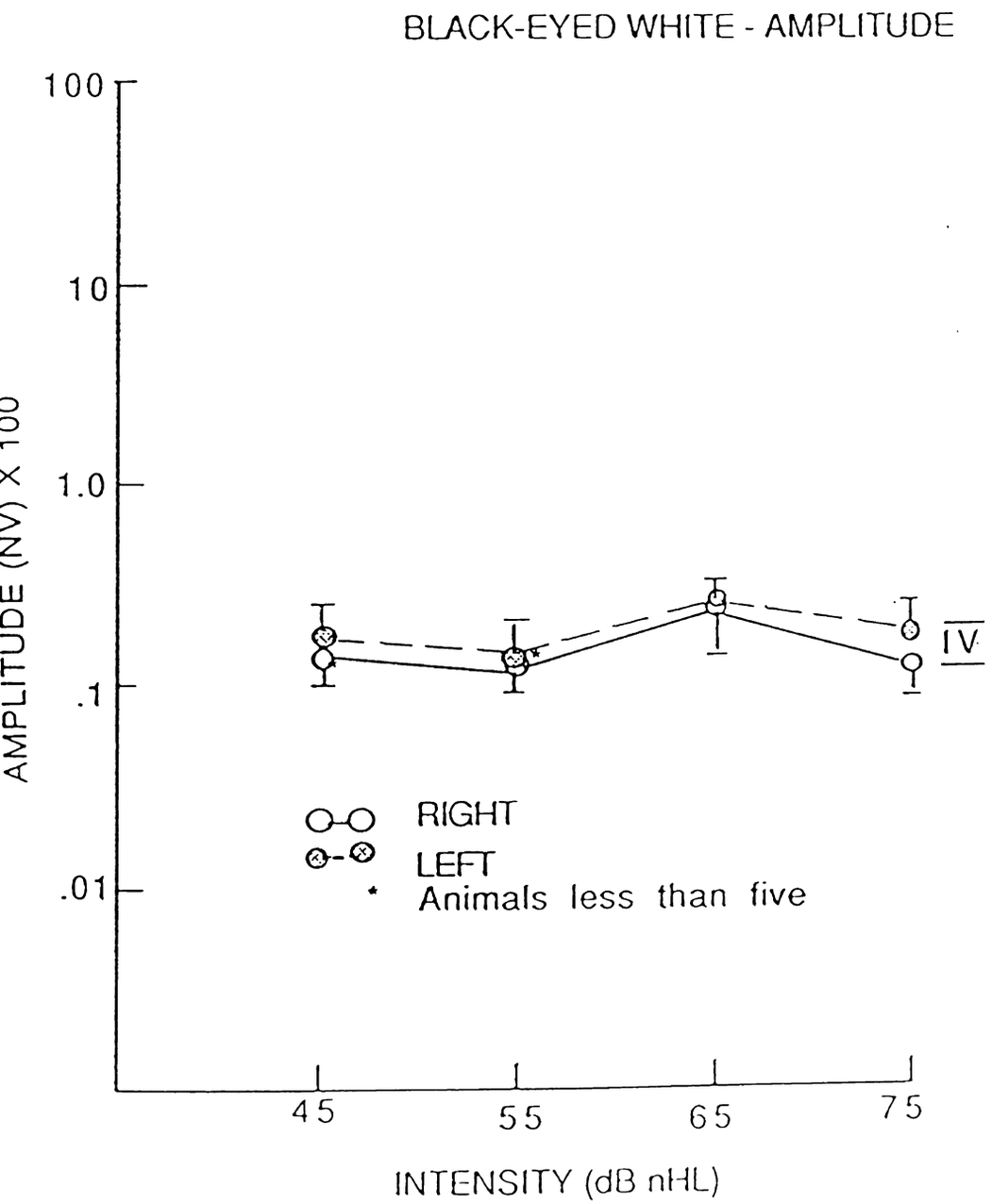
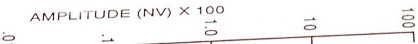

BLACK-EYED WHITE - AMPLITUDE
100
10
1.0
.1
.01
AMPLITUDE (NV) X 100
IV
RIGHT
LEFT
* Animals less than five
45
55
65
75
INTENSITY (dB nHL)

Figure I

Figure IV-22. Input-output amplitude functions for wave I of the White-belly Agouti (Wh/wh, E/e).


AMPLITUDE (NV) X 100
1.0
10
100

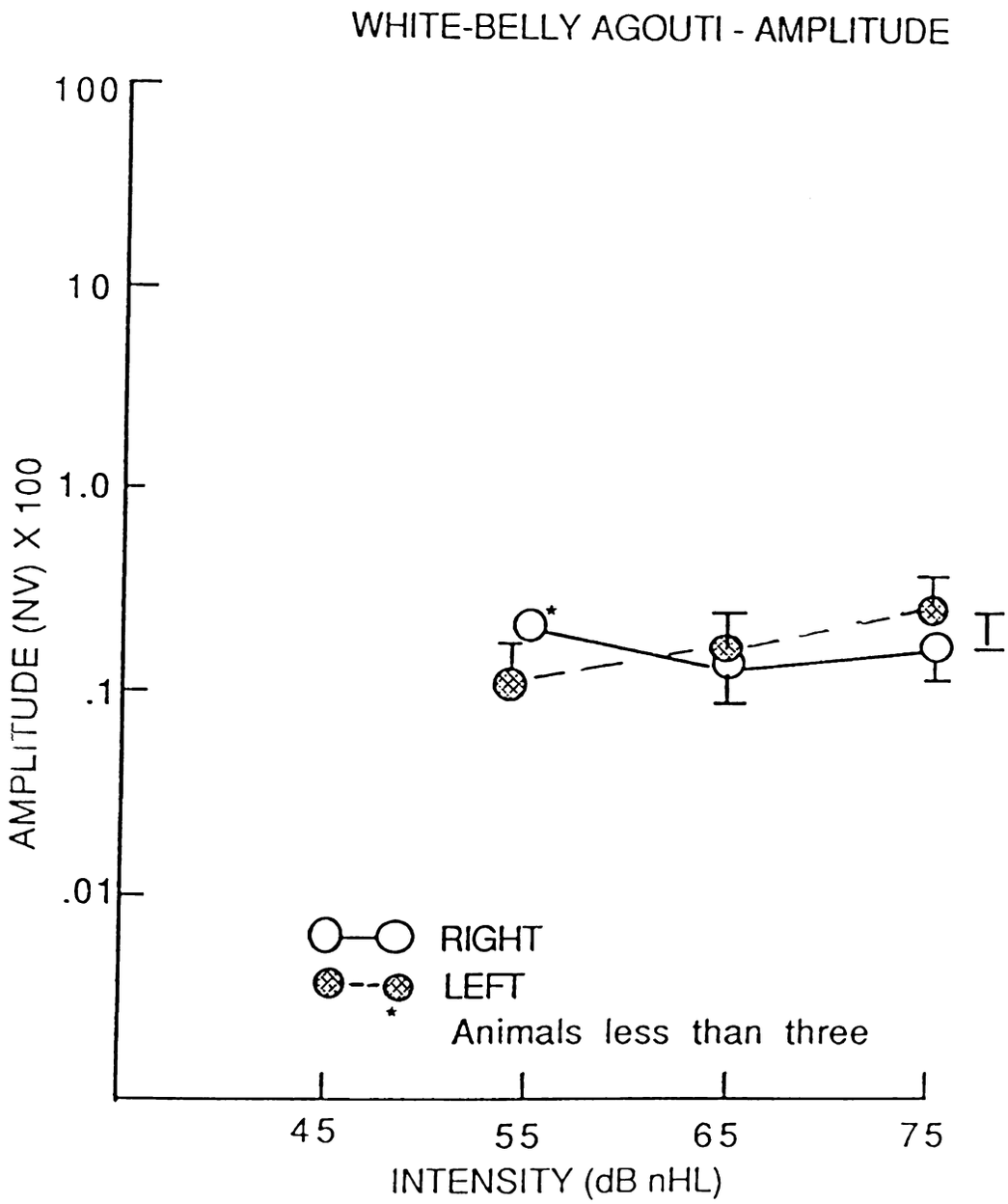

WHITE-BELLY AGOUTI - AMPLITUDE
100
10
1.0
.1
.01
AMPLITUDE (NV) X 100
RIGHT
LEFT
Animals less than three
45
55
65
75
INTENSITY (dB nHL)

Figure IV-

Figure IV-23. Input-output amplitude functions for wave II of the White-belly Agouti (Wh/wh, E/e).

100
10
1.0
.1
.01
AMPLITUDE (NV) X 100

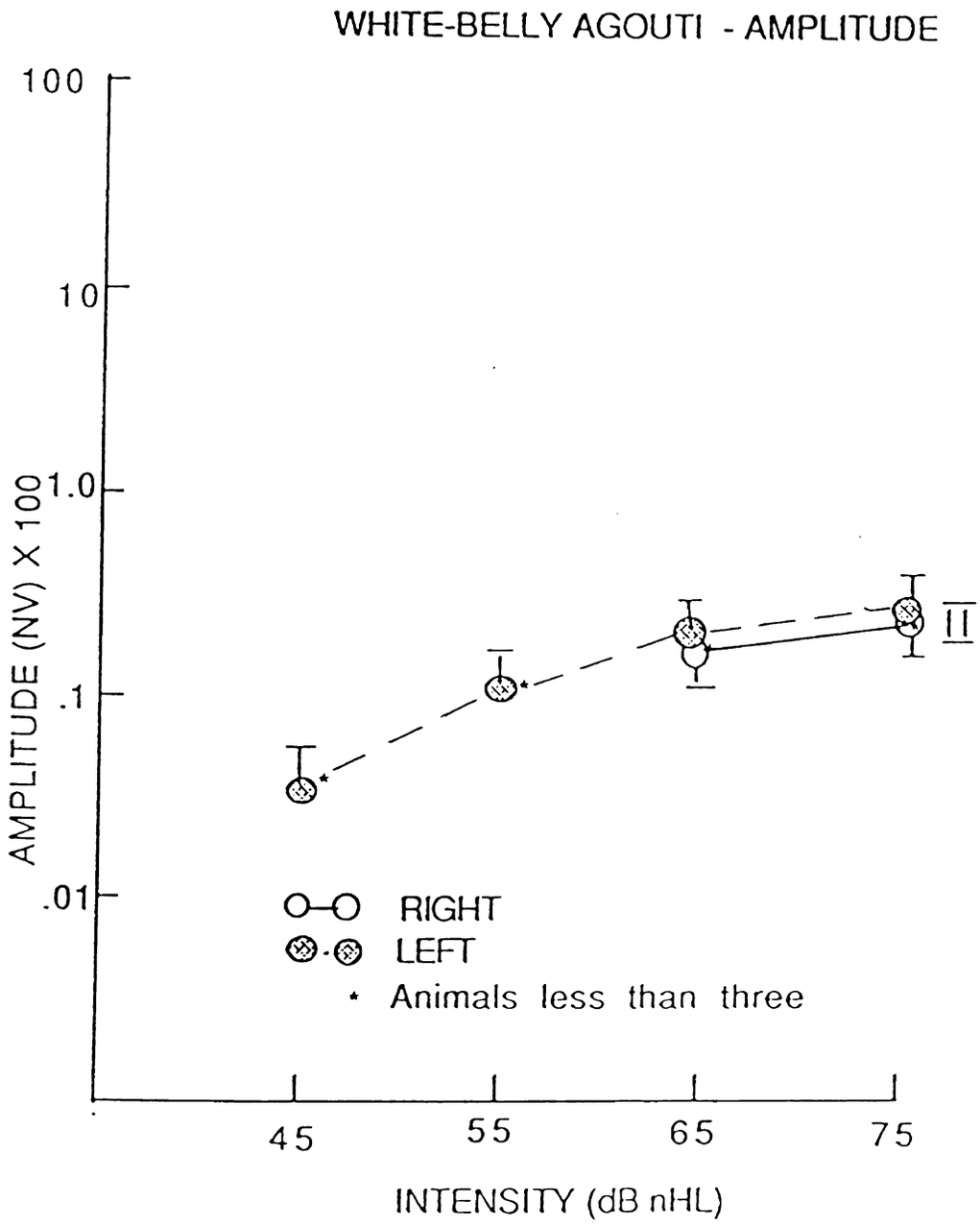

WHITE-BELLY AGOUTI - AMPLITUDE
100
10
1.0
.1
.01
AMPLITUDE (NV) X 100
II
RIGHT
LEFT
* Animals less than three
45
55
65
75
INTENSITY (dB nHL)

Figure IV-2

Figure IV-24. Input-output amplitude functions for wave III of the White-belly Agouti (Wh/wh, E/e).

AMPLITUDE (NV) X 100
100
10
1.0
.1
.01

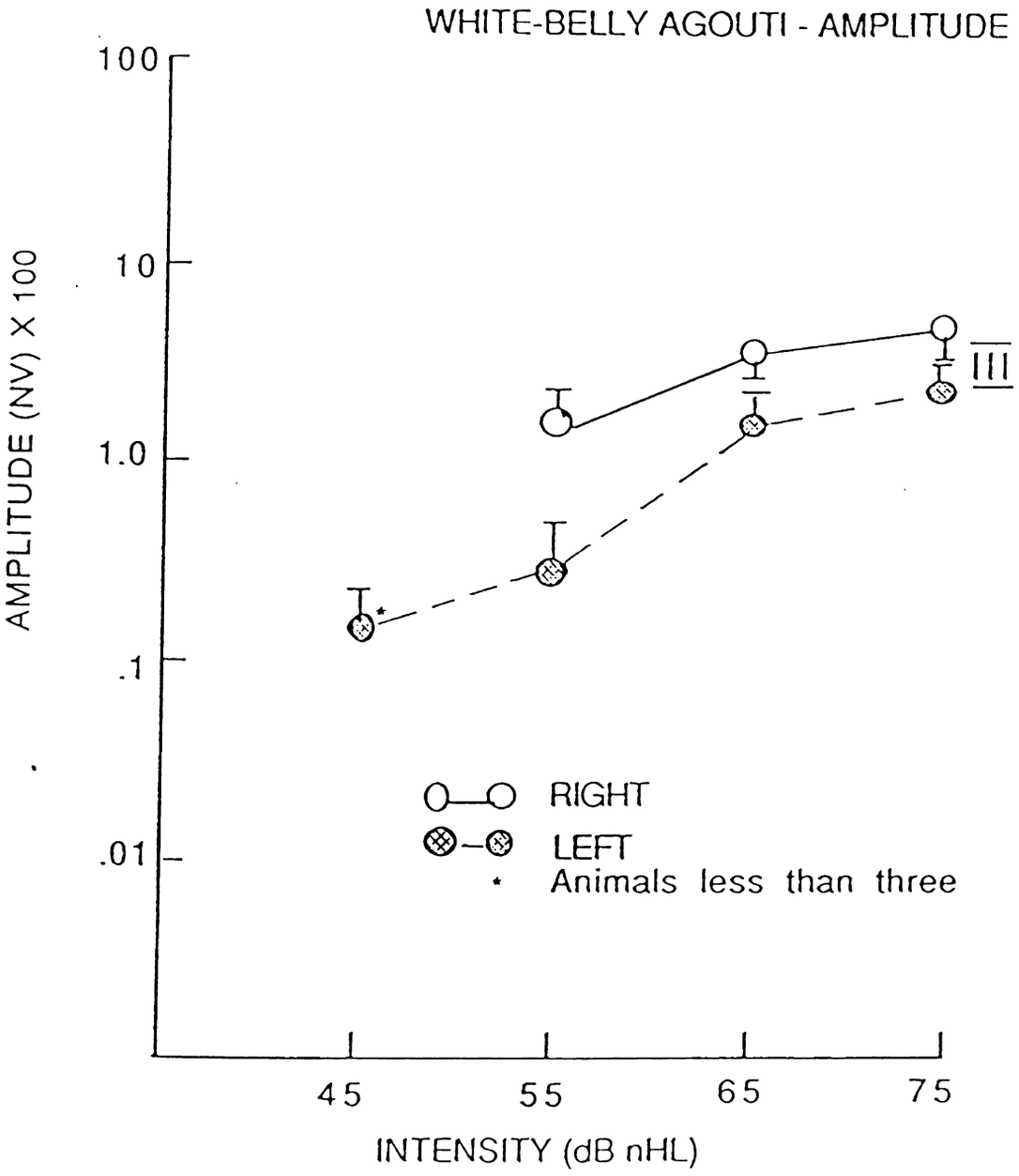
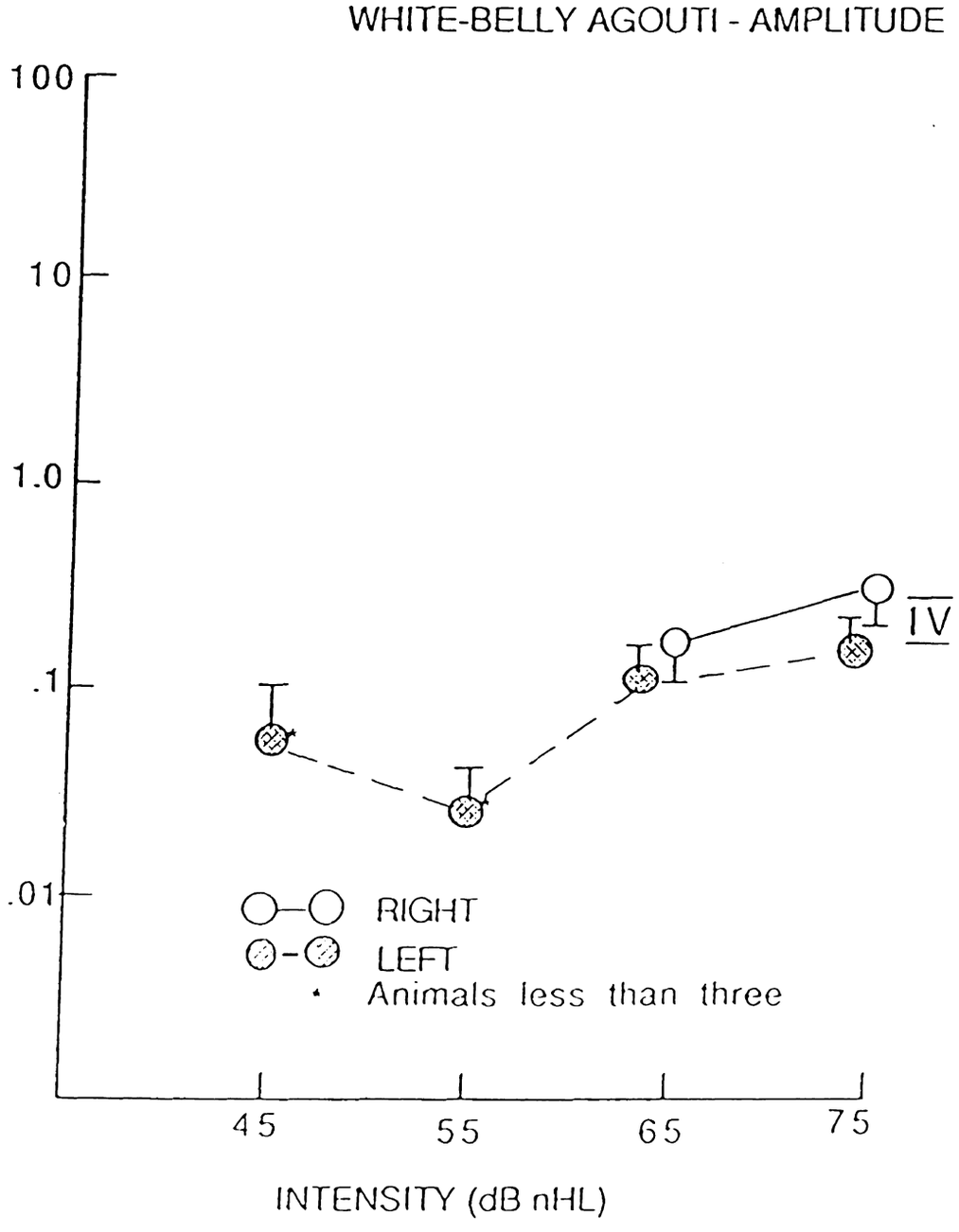

WHITE-BELLY AGOUTI - AMPLITUDE
100
10
1.0
.1
.01
AMPLITUDE (NV) X 100
III
RIGHT
LEFT
* Animals less than three
45
55
65
75
INTENSITY (dB nHL)

Figure IV-

Figure IV-25. Input-output amplitude functions for wave IV of the White-belly Agouti (Wh/wh, E/e).

100
10
1.0
.1
.01
AMPLITUDE (NV) X 100


WHITE-BELLY AGOUTI - AMPLITUDE
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IV
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Animals less than three
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INTENSITY (dB nHL)

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observations were noted in the BEW for all waves. We do note that amplitude actually decrease for the BEW for wave IV from 65-75 dB nHL. It would appear that the WBA showed a recruitment-like phenomenon, in that amplitude tend to increase abnormally as intensity of the stimulus is increased.

The two-way non-orthogonal ANOVA was computed to compare the mean amplitude values of the BEW and the WBA across the various intensity levels. It turns out that differences between the two genotypes with regard to amplitude were not statistically significant (Appendix V). We also compared inter-aural-amplitude differences for the two genotypes across the various intensity levels using as usual, a non-orthogonal ANOVA. Here again, no statistically significant differences were observed between the ears (Appendix W).

Furthermore, figures IV-26 through IV-33 display the input-output functions for waves I-IV. We see from these figures that, in general, as intensity is increased, amplitude is increased. It is noted that amplitude of WBA waves are greater than that of all the animals. Curiously, the BEW tends to decrease with increasing stimulus intensity. We also see that functions in these figures tend to intersect, depicting disordinal interaction. As

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such, superiority did not exist for any genotype across the various intensity levels. In view of this, a single statement cannot be made about the superiority of any genotype without reference to a particular intensity level. For example, figures IV-27 and IV-31 display a disordinal interaction for wave II for both right and left ear. Observe, however, that there is more interaction among genotypes in the right than in the left ear for this particular wave.

We do know that in a linear-linear plot, the correlation tells us how close the points generated by the fixed factor and the variable factor are to the straight line. This is determined by the equation:

$$Y = mx + b.$$

In a log plot, as shown in in the foregoing figures, the correlation tells us how the variable and the fixed factors are related in a log scale. As such the equation of the straight line is determined by the formula:

$$\text{Log } y = mx + \log b$$

where log y is represented by Y-axis, m is the slope, x represents the independent variable, and log b is the intercept.

Within this frame of reference, correlation coefficients were computed for the mean values of all genotypes. It

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turns out that all genotypes except WBA exhibit a strong positive correlation (Appendix X). The negative correlation value of the WBA is indicative of decreasing amplitude as a function of intensity.

In human studies the ratio of wave V to that of wave I is computed for amplitude values. In this study we found that wave III was the most robust and most stable. Therefore, the ratio of wave III to wave I was used. As it turned out, the relative amplitude ratio increased with increasing stimulus intensity. Observe that the ratio is greater than 1.0. It is noted also that amplitude ratio is not uniform for the hearing-impaired genotypes. This is because responses are not from all genotypes. As such we cannot make any confident statememnt about these effects (Appendix Y).

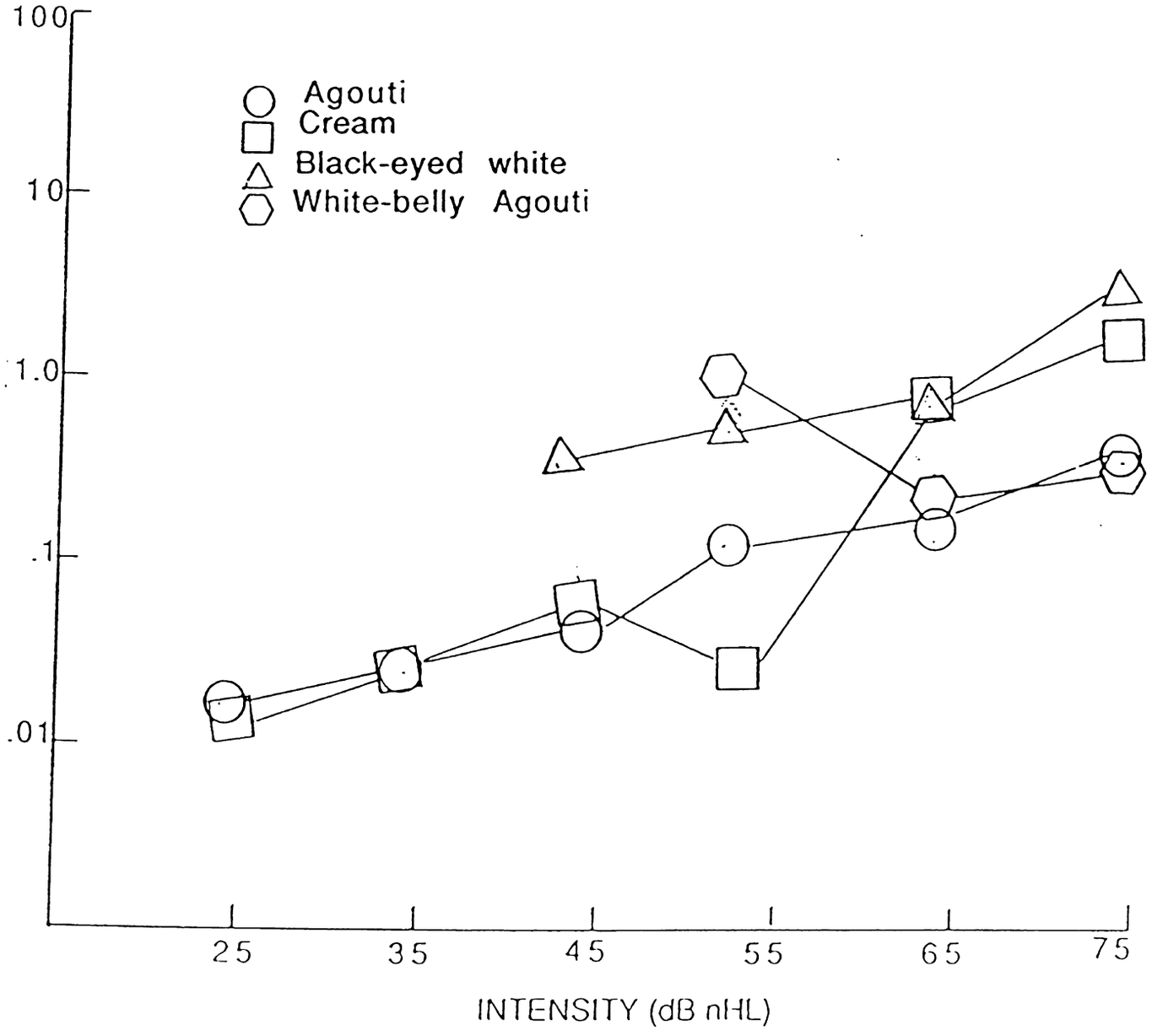
In order to determine whether there is a difference between the Wh-locus and the E-locus with respect to amplitude of the waves, the Chi-square test was performed. Inspection of the summary table (Appendix Z) ndicates that there is a significant difference between Wh-locus and E-locus for both ears with regard to amplitude for all waves. Again, it can be seen that there is an interaction between the two genes with regard to amplitude. It can be surmised that at intensity levels at which recordable responses are obtained,

Figure

Figure IV-26. Composite data points for the amplitude of wave I (R/E) as function of stimulus intensity and genotype.

100
10
1.0
.1
.01
AMPLITUDE (NV) X 100

RIGHT EAR: I

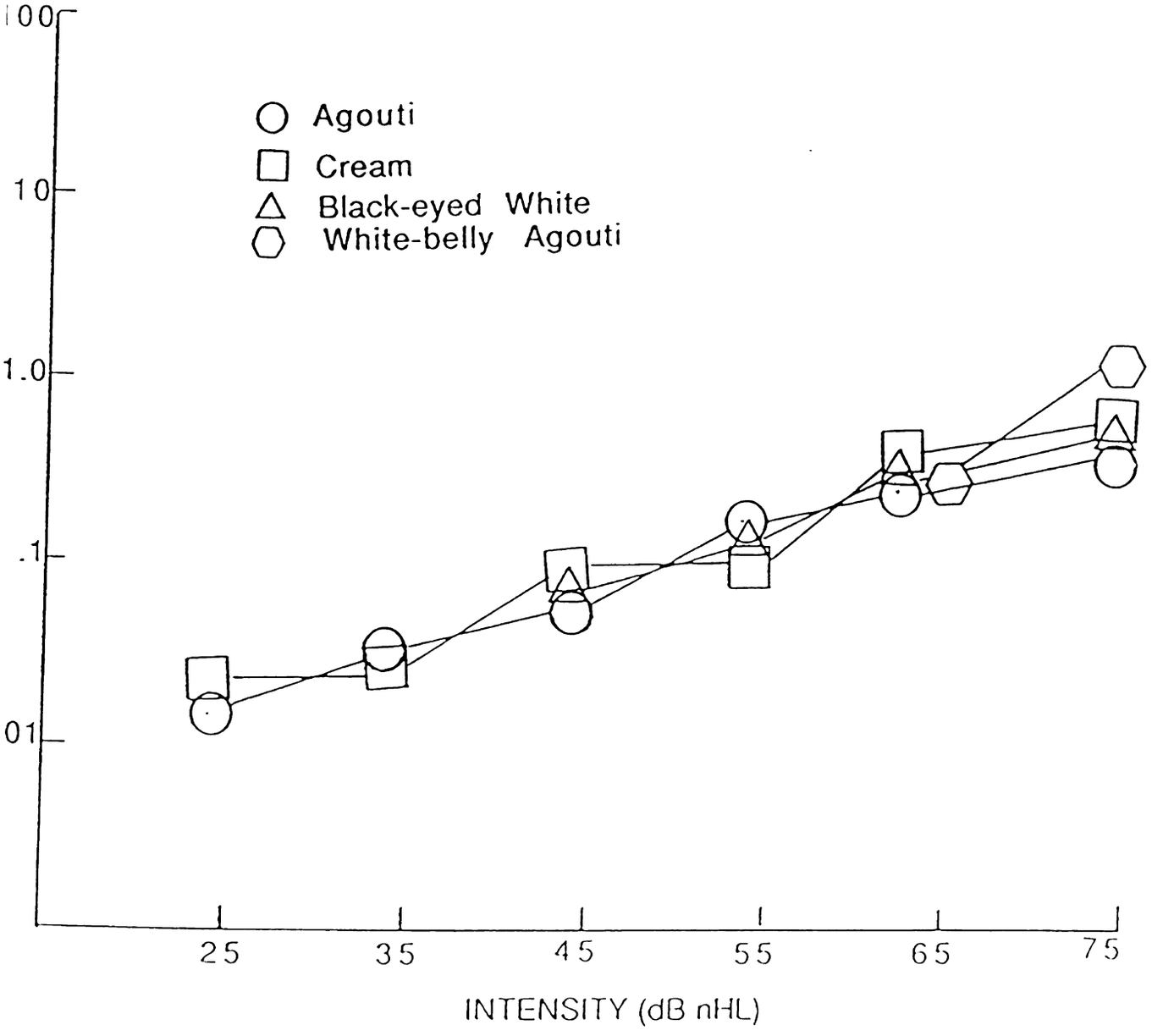

100
10
1.0
.1
.01
Agouti
Cream
Black-eyed white
White-belly Agouti
25
35
45
55
65
75
INTENSITY (dB nHL)

Figure

Figure IV-27. Composite data points for the amplitude of wave II (R/E) as function of stimulus intensity and genotype.

100
10
1.0
.1
.01
AMPLITUDE (NV) X 100

RIGHT EAR: WAVE II

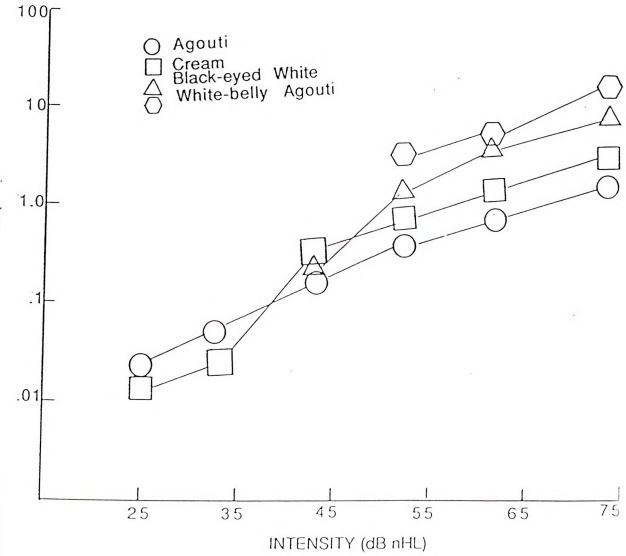


Figure

Figure IV-28. Composite data points for the amplitude of wave III (R/E) as function of stimulus intensity and genotype.

100
10
1.0
.1
.01
AMPLITUDE (NV) X 100

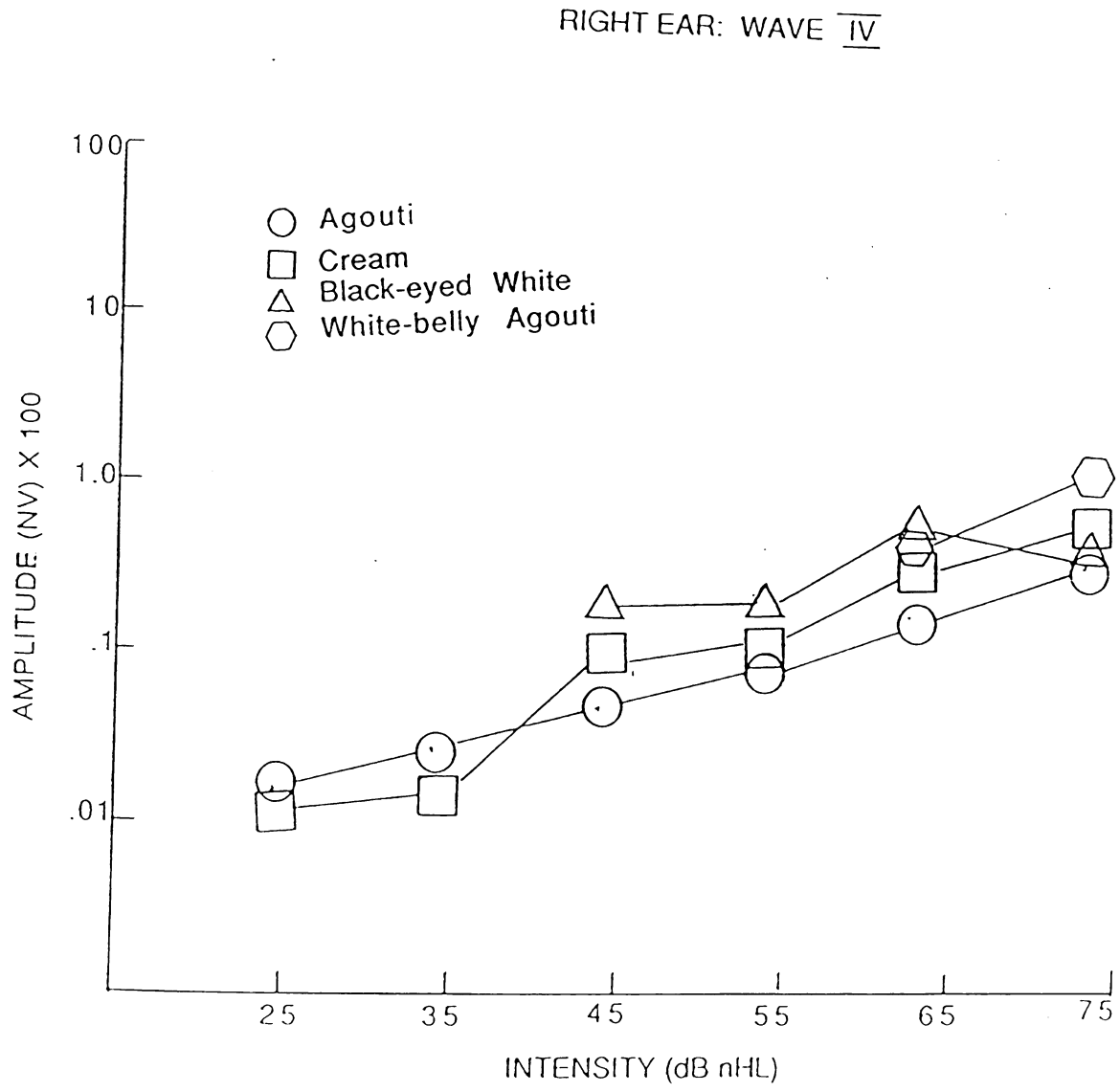
RIGHT EAR: WAVE III



Figure

Figure IV-29. Composite data points for the amplitude of wave IV (R/E) as a function of stimulus intensity and genotype.

100
10
1.0
.1
.01
AMPLITUDE (NV) X 100


RIGHT EAR: WAVE IV
100
10
1.0
.1
.01
AMPLITUDE (NV) X 100
Agouti
Cream
Black-eyed White
White-belly Agouti
25
35
45
55
65
75
INTENSITY (dB nHL)

Figure

Figure IV-30. Composite data points for the amplitude of wave I (L/E) as a function of stimulus intensity and genotype.

100
10
1.0
.1
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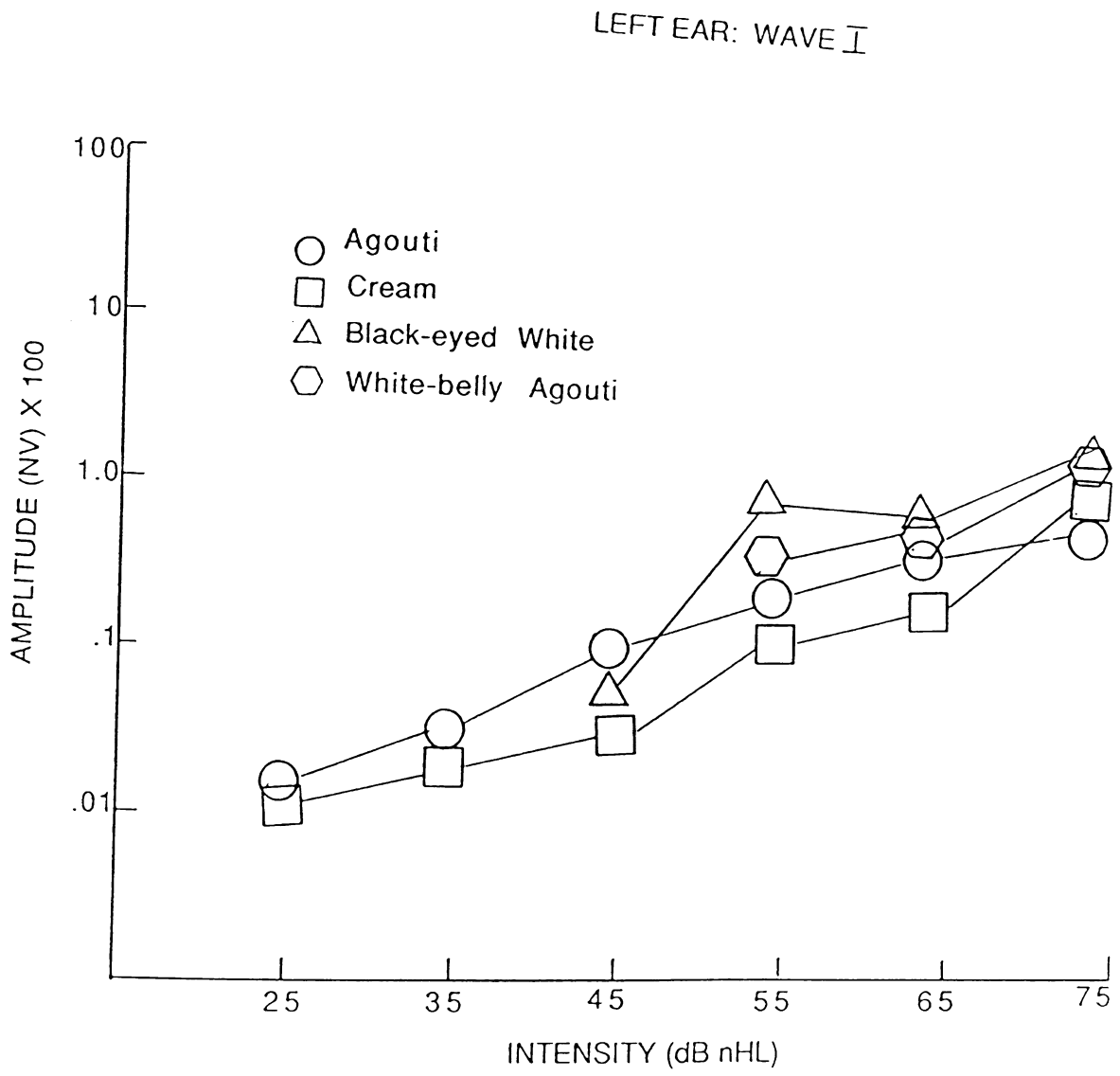
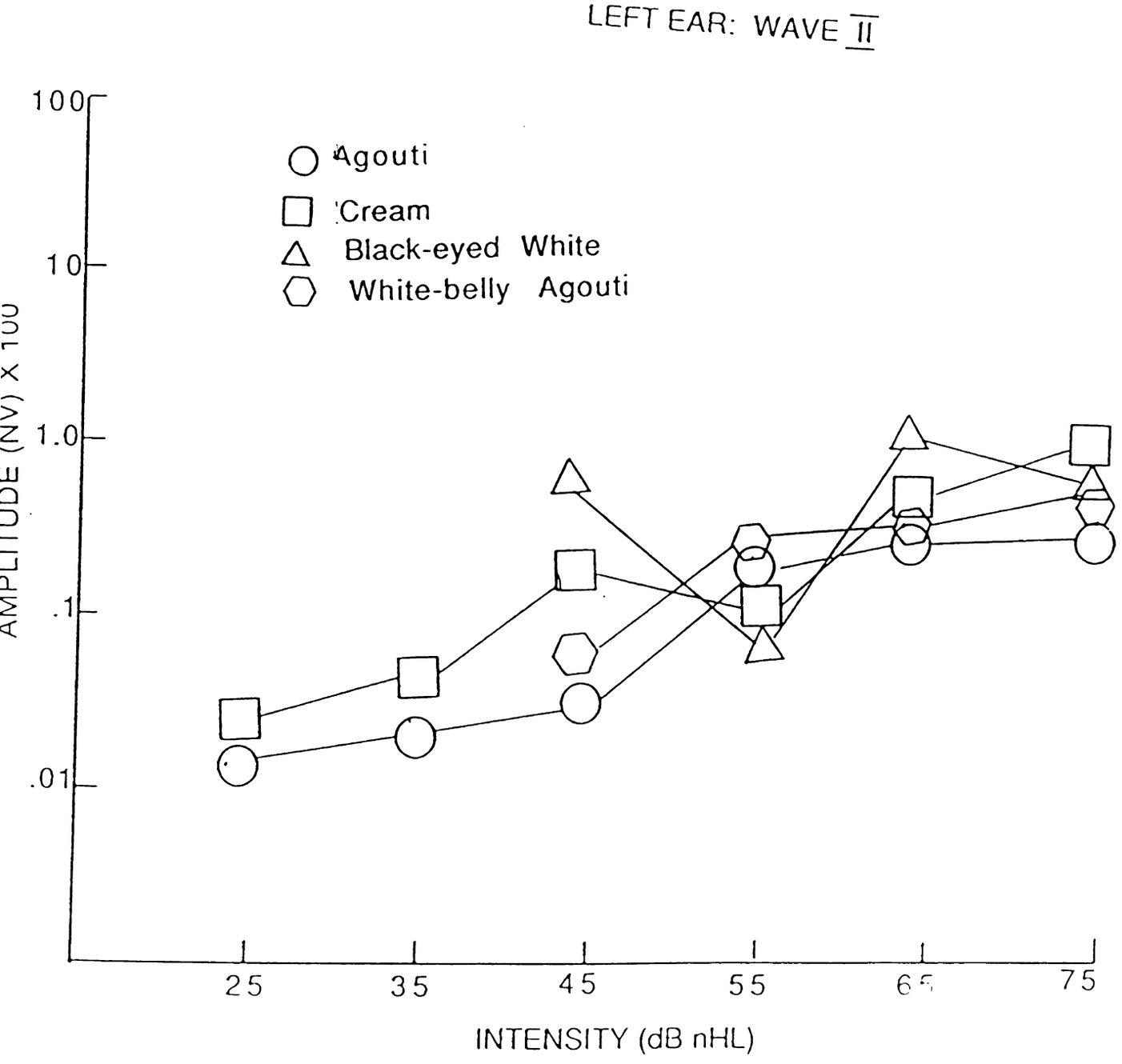
AMPLITUDE (NV) X 100


LEFT EAR: WAVE I
100
10
1.0
.1
.01
AMPLITUDE (NV) X 100
Agouti
Cream
Black-eyed White
White-belly Agouti
25
35
45
55
65
75
INTENSITY (dB nHL)

Figure

Figure IV-31. Composite data points for the amplitude of wave II (L/E) as a function of stimulus intensity and genotype.

100
10
1.0
.1
.01
AMPLITUDE (NV) X 100


LEFT EAR: WAVE II
100
10
1.0
.1
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AMPLITUDE (NV) X 100
Agouti
Cream
Black-eyed White
White-belly Agouti
25
35
45
55
75
INTENSITY (dB nHL)

Figure

Figure IV-32. Composite data points for the amplitude of wave III (L/E) as a function of stimulus intensity and genotype.

AMPLITUDE (μV) X 100
100
10
1.0
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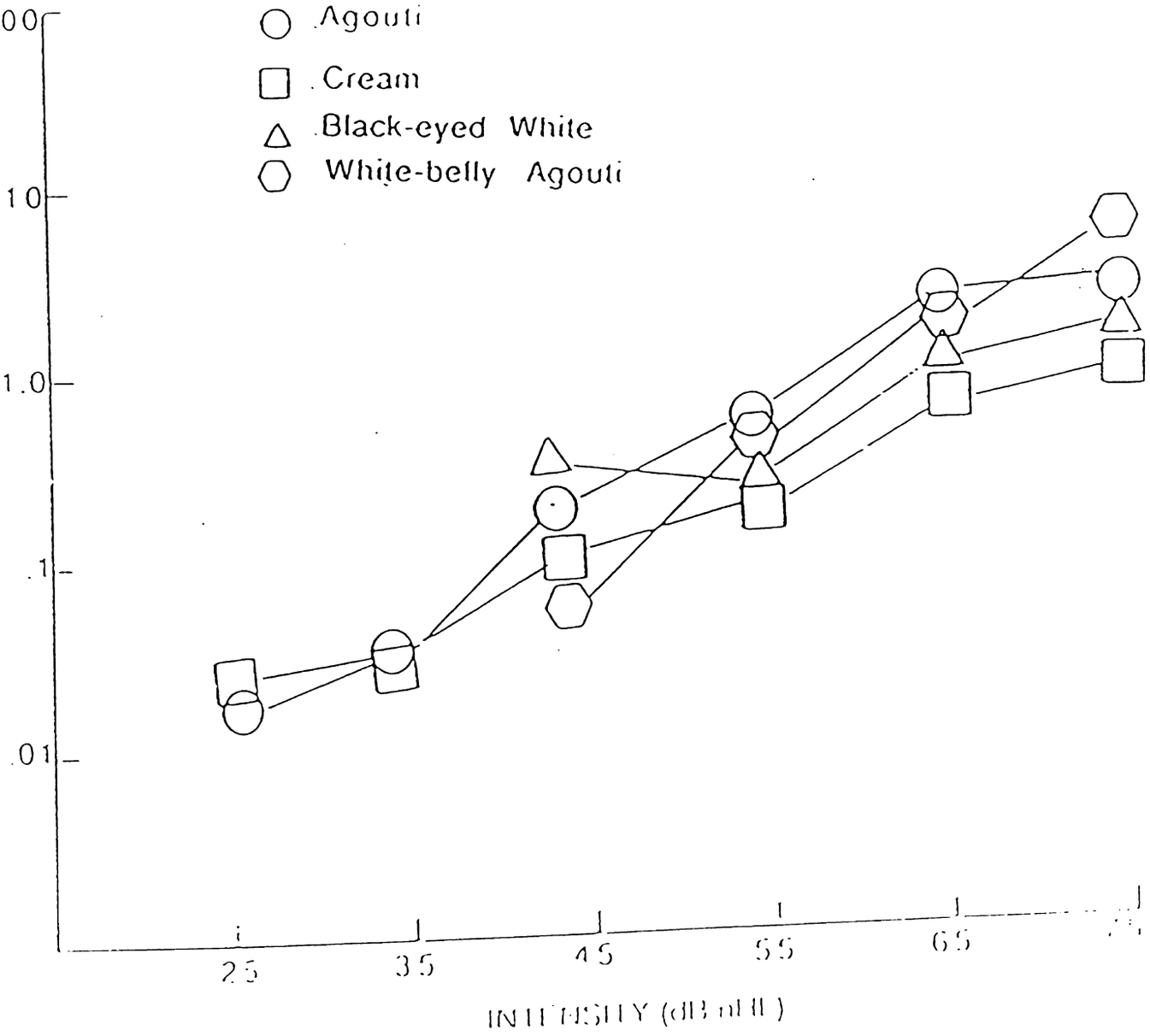
.01

LEFT EAR: WAVE III



Figur

Figure IV-33. Composite data points for the amplitude of wave IV (L/E) as a function of stimulus intensity and genotype.

AMPLITUDE (NV) × 100

100

10

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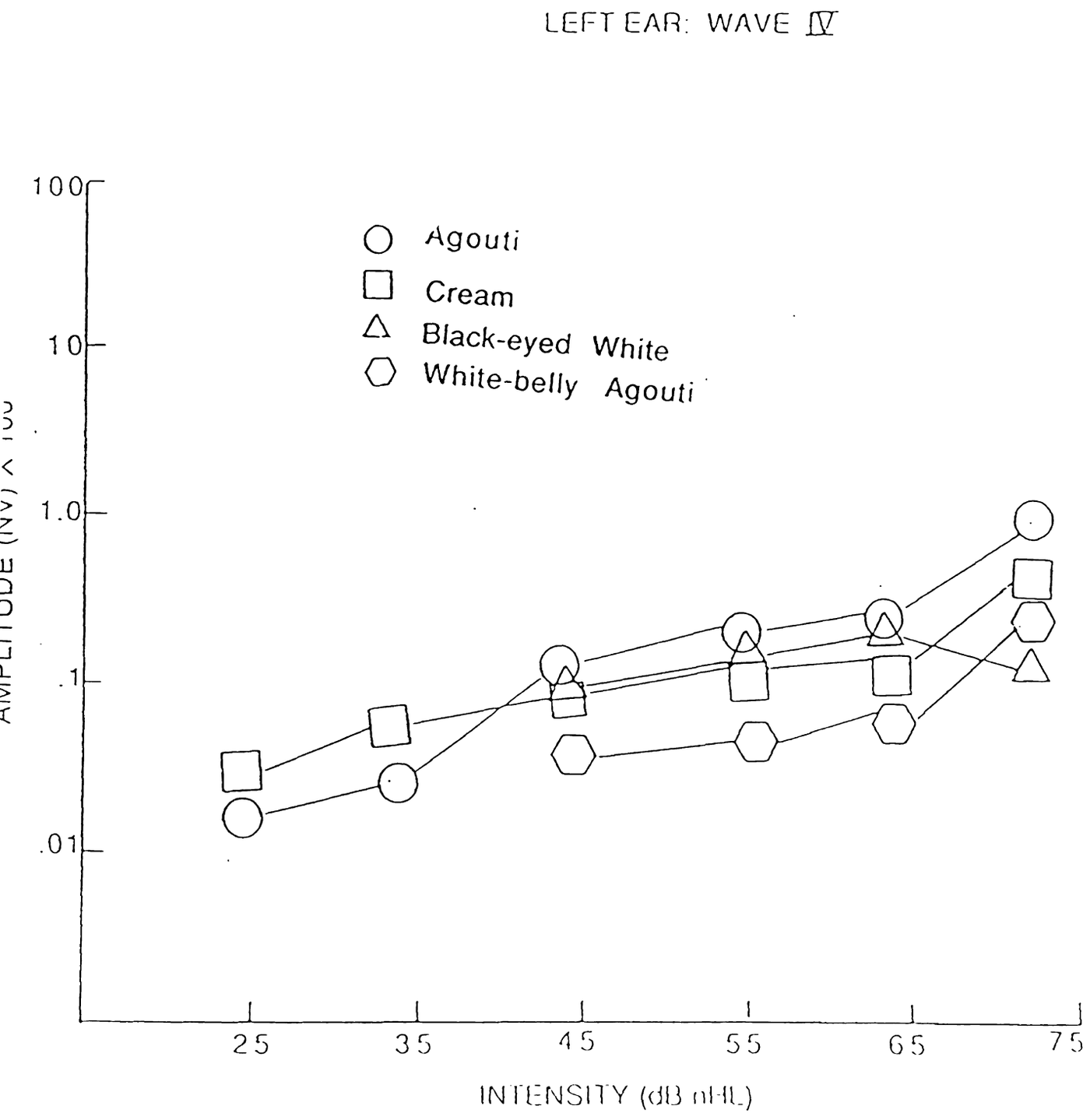
LEFT EAR: WAVE IV
100
10
1.0
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AMPLITUDE (IV) X 100
Agouti
Cream
Black-eyed White
White-belly Agouti
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the two genes interact in their effect on the BSER waves. The exception noted was wave I and III in the right ear and waves III and IV in the left ear in which the Chi-square results were not significant. For example the X^2 in this case is X^2 df = 1 = 2.57, P = .11 for wave I (R/E) and x^2df = 1.44 = .23 for wave III (L/E).

In summary, several studies have consistently explored the effects of the Wh-mutation on the Syrian hamster. Indeed, it has been demonstrated that the Wh-gene is a highly pleiotropic mutation causing numerous morphologic, physiologic and behavioural abnormalities on the Syrian hamster. The more obviours morphological effects of the gene as noted was to cause the homozygotes to be deaf, blind and white. Still another observation was that the Wh-gene in the Syrian hamster is homologous to the Waardenberg syndrome in humans, in that they both appear to affect the same developmental processes. It has been observed that deafness is one of the most serious defects of the Wh-gene (in the hamster), and the Waardenberg syndrome (in humans) of the individuals affected. We do know that BSER as a non-invasive measure has proved invaluable in monitoring the hearing capabilities of infants and in a variety of animals. There has been no investigations conducted to asess the capabilities of the hamsters in the AN/As-Wh strain. These fundamental observations formed the basis of this study.

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Our results have shown that, in general, wave-form morphology, thresholds, latencies and amplitudes are not similar across genotypes. The results revealed that responses obtained from two genotypes are similar to those obtained by other investigators (Schweitzer, 1987; Ahmadizadeh, et al 1987). Thus, the Agouti and the Cream have normal thresholds, and normal wave-form morphology. We do note that there is a direct relationship between latency and intensity and an inverse relationship between amplitude and intensity for both genotypes, a not too uncommon finding in humans and animals. The statistical results indicate significant differences between the Agouti and the Cream with regard to latency and amplitude. On the other hand, the BEW, the WBA and the Anophthalmia White (AW) presents a different picture. Thus, wave-form morphology, thresholds, latency and amplitudes were different compared to the Agouti and the Cream. In general, we found input-output latency and amplitude plots to be unlike those of the Agouti and the Cream. Fundamental to this finding is that the responses were not obtained for all the animals at all intensity levels. The ANOVA results indicate minimal and in some instances no significant difference between the BEW and WBA with regard to latency and amplitude. The study also revealed that there is no significant effect between Wh-locus and E-locus with regard to latency. Interestinly,

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the X^2 results showed significant differences between Wh-locus and E-locus.

Suffice it to say, that the finding of incomplete waveform morphology and the differential thresholds observed in these hamsters could pave the way for possible detection, differentiation and interpretation of a broad range of defects that may be due to the peripheral auditory dysfunction or brainstem level dysfunction caused by the Wh-mutation. The abberant responses in the Wh/Wh-- genotype even at the highest intensity level of 75 dB nHL and above did not show any definition of peaks compared to responses from normal-hearing hamsters. Thus, these results indicate the differential effect that the Wh-mutation has on developing hamsters auditory mechanism and brainstem. As such, genotypes can be classified as normal, moderate-severe and profound hearing loss.

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CHAPTER V

DISCUSSION

Non-invasive hearing testing has revealed that auditory brain-stem evoked potentials BSERs have considerable value in assessing auditory functioning. The amplitude and latency of the BSERs have been thoroughly investigated in the cat (Jewett, 1970, guinea pig (Dobie and Berlin, 1979b), rat (Church et al, 1984) and mouse (Henry and Lepkowsky, 1978). These studies have shown that as the intensity of the stimulus is increased, the amplitude of the BSER waves increases while latency decreases. In addition, studies have shown that BSERs could be obtained at stimulus intensity levels between 10 and 30 dB SL from normal and hearing-impaired subjects (Møller and Blegvad, 1976).

Studies of the evoked potential correlates of genetic progressive hearing loss in two genotypes of mice C57BL/6 and CBA/J have shown that hearing thresholds differed for the two genotypes. We know that at this point, there has not been any investigation conducted to determine the hearing capabilities of the five sets of genotypic hamsters

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available in the AN/As strain. This study was conducted to determine whether the threshold of hearing of the hamsters in the AN/As strain are normal. Secondly, it was the objective of this investigation to explore the differences in hearing thresholds, latencies and amplitudes as a function of genotype and at varying intensity levels.

We observed that waveform morphology, thresholds, latencies and amplitudes are not similar across genotypes. In general, it was noted that the waveform patterns of the Agouti and the Cream are similar to those of other rodents. In all, rodents that were studied exhibited a series of four or five waves. The waveform morphology, however, varied in different species. For example, the first two waves of the BSER obtained from the mouse are of relatively greater magnitude than the later waves. In the rat, the peak of the third wave is usually observed to fall on the downward slope of the second wave. In our study, we found that wave III is the most robust and stable of all the waves; wave II is often fused with wave III and is clear at higher intensity levels for most animals.

It was noticed, however, that the BEW, the WBA and the Anophthalmic white present different waveform morphology. It is noted that at high intensity levels of 65 and 75 dB nHL, waves I-IV can be seen in the BEW and the WBA. Below

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45 dB nHL, however, the waves become indistinguishable. In the case of the Anophthalmic white, waves I-IV were totally absent. Why should there be a difference in the waveform morphology and thresholds for the hamsters in the AN/As-Wh strain? We do not have direct evidence to answer this question, however, we know from the work of Jerger et al (1978) that a high frequency hearing loss can result in poor waveform morphology and even a flat BSER tracing. Furthermore, Selters and Brackmann (1979) reported that the absence of all BSER waves is characteristic of patients having acoustic neuromas. They found that approximately one-half of their acoustic neuroma subjects have no response for BSER. While there is no evidence that these hamsters posses tumors, on-going morphological analysis may reveal abnormalities of coclear and/or retrocochlear structures. In the same vein, Harker (1980) noted that 28% of his subjects did not exhibit BSER. Harker (1980) also observed that if hearing loss is severe enough, there may be an absent BSER without any retrocochlear involvement. Total absence of BSER waves is even more likely to occur in 8th nerve or low brain-stem lesions if there is a hearing loss. Thus, it is possible that the abberant waveform morphology observed in the Anophthalmic white may be due to the absence of tectorial membrane in this particular genotype as explained below. On the other hand, the incomplete waveform in the BEW and WBA can be attributed to either peripheral or

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retrocochlear pathology caused by the variable expression of the Wh-gene. This requires further investigation.

We know from the work of Asher (1988) that the Wh gene causes the degeneration of the tectorial membrane. This morphological abberation as noted by Asher (1988), is associated with the failure of the tectorial membrane to detach from the future inner sulcus cells of the organ of Corti. This finding is important since the tectorial membrane is involved in depolarization of hair cells as the membrane exerts a shearing action across the tips of the stereocilia. We also know from the work of Davis (1961) and Dallos (1975) that sound vibrations that are introduced to the scala vestibuli are conducted to the cochlear duct by the deformation of Ressiner's membrane. The endolymph is thereby disturbed, and thus, the vibrations continue. The basilar membrane is coupled to this movement, and therefore, it is similarly displaced, resulting in a pressure release at the round window membrane. Since the organ of Corti resides upon the basilar membrane, the vibrations to it are readily transmitted. As the tips of the hair cells are embedded in the tectorial membrane , when the basilar membrane is displaced upward, the hair cells are sheared in a complex manner. Part of this shearing action is facilitated by the basilar membrane and tectorial membrane

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Our notion is that the source of electrical charge is derived within the hair cells. It is known that the hair cells which contain an intracellular fluid are high in potassium and low in sodium. Several investigators (Davis, 1961; Dallos, 1975) accept the notion that when the cilium upon the hair cells are deformed, the electrical properties of the hair cell membrane alter, allowing electrical charge possibly related to the movement of potasium ions to enter from the endolymph. This possibly results in a positive on-going change of the intracellular potential. Within this frame of reference, we could say that it is the modulation of steady potassium flux which results in the generation of the cochlear microphonic (CM) and some of the components of the summating potentials (Dallos, 1975). The electrical changes in the hair cell causes it to release a chemical transmitter which diffuses through the clefts between the hair cell bodies and the afferent endings of the cochlear nerve. The transmitter substance, probably an amino acid, such as glutamate and/or aspartate (Pujol et al, 1980) causes a change in the local membrane permeability of the dendrite and results in the depolarization termed the generator potential. One can perhaps surmise that the hearing loss noted in the BEW, WBA, and the AW, together

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with the abberant waveform morphology in these animals, may be related to the tectorial membrane dysfunction, resulting in cochlear or retrocochlear pathology.

We also see that in some animals, the BSER wave I is preceded by a potential referred to as I'. Presently, there is a paucity of information on the origins of this particular wave. Recent studies by Moore et al (1988) suggested that the I' of the BSER and the positive wave of the compound action potential (CAP) recording indicates post synaptic afferent activity of the cochlear afferents. Hughes and Fino (1980) have shown however, that the I' is not part of the cochlear microphonic (CM) since it does not reverse in polarity with clicks of opposite polarity. There are suggestions that the I' may be related to excitatory post synaptic potential (EPSP) arising in the afferent terminals of the eighth nerve. Thus, it can be seen that the evidence regarding cochlear versus neural origin of the I' is still inconclusive. Additionally, there is no definite information on the origins of waves I-V in the hamster. MØller and Jannetta (1985) reported that the origins of the BSER waves are similar across small animals, such as cats, guinea pigs and rats. It is possible that waves I-IV may have the same origin as reported in the cat (Table 6) thus: wave I, auditory nerve; wave II, cochlear nucleus, wave III; superior olivary complex; wave IV,

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In another vein, it was observed that there were four and in some rare instances five major BSER peaks in the hamster as in other small rodents. In humans, seven major peaks are obsereved (Møller and Jannetta, 1985; Amedofu, 1985). A possible explanation for this difference may be found in the auditory nerve. Lang (1981) noted that the auditory nerve in man is much longer than it is in small animals usually used in auditory research. Thus, the auditory nerve in small animals such us cats, rats, and guinea pigs is 0.3 to 0.5 cm long, while it is about 2.5 cm long in man. Another difference between man and small experimental animals such as the cat is the smaller size of the auditory nuclei in man relative to the head size. Whether this difference is sufficient enough to cause significant differences in the BSERs recorded from these species is not clear. Further research is needed to determine this.

We also noted from the results of this study that genotypes can be classified as normal, moderate-to-severe, and profound hearing loss. Therefore, it is necessary to make comments about the possible pattern of inheritance among these hamsters with regard to hearing loss. We do know that there are three patterns of inheritance; dominant, recessive

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and X-linked. As already noted, the Wh gene was first described by Knapp and Polivanov (1958) as an autosomal recessive gene inherited independently of the albino C^{d} We also know from the work of Beher and Beher (1959) that the Wh mutation has a dominant inheritance in that the hetrozygotes may be distinguished from the homozygotes. On this premise they suggested that the gene acted as a partial dominant. Again, Robinson (1962, 1964) described the Wh gene as incompletly dominant and indicated that animals homozygous for the mutant showed a complete absence of coat and pigmentation, while aplasia of the eyes resulted in extreme anophthalmia. We noted that with regard to hearing of the hamsters in the AN/As-Wh strain, the Wh mutation is incompletely dominant. Our research has shown that animals homozygous for the mutant showed a complete lack of hearing, while the heterozygotes showed normal hearing, as well as varying degrees of hearing loss.

How do we know that it is the Wh gene that is causing the hearing loss? To answer this question, we need to note the purity of the strains. It is well known that the hamsters used in this study are from a single inbred strain with one subline. To be true, AN/As-Wh has been inbred brother and sister for 27 generations with at least one parent carrying the Wh gene. For this reason, all hamsters are 99.9 percent homozygous with respect to all genes not linked to Wh. With

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respect to genes within 10 map units of Wh, all hamsters are 89% homozygous. Thus, when making comparisons between hamsters with respect to the Wh gene, any difference found must be caused by the Wh gene, or a gene that is very closely linked. Additionally, the AN/As-E has been back crossed into the pure strain for seven generations. Therefore, with genes unlinked to E, including Wh, the substrain is 99.2 percent homozygous. With respect to genes 30 map units away from E, all animals are 92 percent homozygous. Thus, the comparisons we make in this study with respect to hearing are between hamsters which are nearly identical for all genes with the exception of the genes at the Wh locus and the E locus. As such, any hearing loss that is noted in any genotype must be attributed to the Wh and the E genes, or at least those genes closely linked to them.

We observed an inverse relationship between the intensity of the stimulus and latency for the BSERs in that it was seen to decrease with decreasing standard deviation as a function of stimulus intensity. The coefficient of variation between animals was found to be below 15%. This points to the fact that we can predict latency values quite accurately on the basis of the changing values of intensity. Significant differences were observed between the latency values of the Agouti and the Cream. Absolute latency values were found to

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be slightly later in the Agouti than the Cream. That is that, the Agouti appeared to be more normal than the Cream. The possible explanation of this finding is the alleleic composition of these two genotypes. The two genotypes are homozygous for the wh gene and heterozygous or homozygous for the e gene. The shorter latency values noted in the Cream is indicative of recruitment and may be due to the destruction of the outer hair cells in the cochlea.

It is also noted that for the BEW and the WBA, the latencies on the input-output curves are displaced to the right, and as a result, stimuli of high intensity is required to evoke a response. Why is it that latency of BSERs in the BEW and WBA shift to the right, and why is it that the slope of their responses is steeper than that of the Agouti and the Cream? A possible explanation can be found in the investigations of Hecox and Galambos (1978) and Yamada et al (1979). These investigators identified two types of abnormal latency-intensity functions, at least for wave V in humans. The first type of abnormal function is one in which wave V occurs within normal limits at high intensities. In this case, when intensity is increased, there is either a rapid increase in latency of wave V outside normal limits or an abrupt disappearance of the response. This abnormality, they noted, indicates cochlear hearing loss and is always associated with recruitment. The second type of abnormal

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latency-intensity function is one in which the entire function is skewed to the right on the abscissa, as indicated in our data. There are perhaps, three reasons to account for this observation. Firstly, a conductive hearing loss will displace the latency-intensity function by an amount approximately equivalent to the hearing loss. Secondly, a similar shift of the latency-intensity function may result from a steep high frequency hearing loss. In this case, the shift is not due to the decreased intensity of sound reaching the cochlea but is due to the travelling wave delay to reach a low-frequency response region of the basilar membrane. In a sense, the function is skewed upward rather than to the right. A third cause of this right-ward shift in latency-intensity function is the presence of retrococlear dysfunction that slows neural conduction between the ear and the neural generators of wave V. Therefore, it is reasonable to suggest that the shift in the latency-intensity function for waves I-IV in the BEW and WBA that is noted in our data is possibly due to either the destruction of the outer hair cells caused by the e mutation or, the alteration of the number of ganglionic cells by the Wh mutation.

We noted also that there was no significant difference between latency values for the BEW and the WBA. We can surmise why this should happen, in that the two genotypes

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are different. Thus, the following observation may suffice in that the most cogent speculation is that the two genotypes probably have the same type of hearing loss. The data of Selters and Brackmann (1979) suggests that latency is constant for hearing losses of less than 50 dB, but increases for greater losses. We know from the work of Don and Eggermont (1978) and Galambos and Hecox (1978) that hearing loss can be conceived in terms of a filtering action in the pattern of response arising from the various regions of the cochlea. Thus, a high frequency loss might be expected to produce latency delays compatible with the time taken by the cochlear travelling wavefront to traverse the basal region. A precipitous loss above 4000 Hz will not have marked effect on latency because contributions from such regions are masked by that of the 1000-4000 Hz region. In the case of a more gradually slopping hearing loss, the pattern may be difficult to interprete because of the interaction between the intensity-related change in stimulus excitation pattern and the hearing loss. The overall picture is that of extreme complexity, if the variety of degree and slope of hearing loss, and loss etiologies are taken into account.

We noted in our data that latency-intensity functions are not uniform, especially for intensities below 65 dB nHL and perhaps due to the differences in the degree of hearing loss

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among genotypes. It is possible that the BEW and WBA may have the same slope of hearing loss. As such, we should not expect a significant difference between the two genotypes with regard to latency. In practice, we should be cautious of using latency as an index of hearing loss. Kavanagh and Beardsley (1979) have shown that at high intensity levels, latency have little correlation with hearing loss. To be true, they found that some of their subjects have high frequency hearing loss but have normal latency values. Our data tend to suggest this possibility, but there is a need to obtain more frequency specific data.

Inpection of the input-output amplitude plots in figures IV-10 through IV-25 and the composite function in figures IV-26 through IV-33 reveal that a direct relationship exists between the amplitude of the ABR and the stimulus intensity (augmenting phenomenon). There were exceptions to this trend in that there were some animals in whom amplitude remained constant, and, in some cases, amplitude decreased as a function of stimulus intensity (reducing phenomenon). Curiously, too, the amplitude of the BEW as a group tend to decrease as a function of stimulus intensity. It may be that this increase or decrease in amplitude as a function of stimulus intensity might be due to the response characteristics of the inner hair cells. The work of Kiang et al (1965) revealed that while the actual threshold of the

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receptors is in doubt, there most certainly exist fibers in the auditory system that may respond only to tones of high intensity. Again, Pickles (1982) also reported that some nerve fibers did not saturate in a sharply defined manner, but went on increasing at high intensities. These fibers also tend to have high thresholds than others.

Can we term the augmenting/reducing phenomenon a normal phenomenon? The answer to this question is perhaps yes. Our data have shown that the Agouti and the Cream (normal genotypes) exhibit this phenomenon. This is not an uncommon finding, in that the same phenomena has been reported in somatosensory studies (Petrie, 1960), visual evoked potential studies (Buchsbaum and Silverman, 1968; Braden et al 1983) and brain stem evoked potential studies (Amedofu, 1985). Interestingly, this phenomenon was also observed in the hearing-impaired genotypes. On the other hand, we noted that the reducing phenomenon occured more in the hearing-impaired genotypes, especially within the BEW than the normal animals. This is also not surprising, in that Buchsbaum and Silverman (1968) reported that a larger number of their patients exhibit the reducing phenomenon. Again, Kavanagh and Beardsley (1979) showed that some of their subjects with sensori-neural hearing loss have abnormal input-output amplitude functions similar to that of reducers. It follows that the augmenting -reducing

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phenomenon must be considered as pertains to amplitude data.

We also determined the amplitude ratio of wave III to wave I in our study. Wave III was used because it is the most robust and stable of all waveform patterns in the Syrian hamster. In humans, this ratio is simply the amplitude of wave V compared to wave I; and a relative ratio of $\geq$ 1 is considered normal. This ratio is an important index in detecting hearing loss and retrocochlear lesions (Musiek, 1984). In our study, we found the waves III/I ratio to be > 1 for both normal and abnormal genotypes. Musiek and Gollegly (1985) noted that although amplitude ratio measurements are of value in detecting eighth nerve and brain-stem lesions, they must be used with caution. "Normal criteria for amplitude ratio", they pointed out, "may vary according to instrumentation used, intensity level, repetition rate, and a host of other varables". Hence, relative amplitude of >1 may not be a universal criterion.

The limitations inherent in this investigation relate mainly to stimulus parameters that of necessity were either controlled or held constant for the purpose of this study. For example, the utilization of male hamsters may limit our generalization to other subjects. Additionally, the use of clicks may limit our generalization to other types of stimuli. There is a suggestion (Selters and Brackmann,

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1977) that click stimuli at moderate or high intensities have their effective excitation maxima in the 1000-4000 Hz region of the cochlea. It is to be noted that the results of this study are within reason, in that BSER studies using derived narrow band frequencies (Don and Eggermont, 1978) suggest that contributions from high and lower frequency regions do affect BSER waveforms as a whole. Still another point in the use of clicks in this study is that the trend towards the use of more place-specific stimuli for otoneurologic investigations have met with little success (Laukli, 1983). Furthermore, at present there is little agreement about which alternatives to the click are most appropriate. Whatever the choice, a body of data concerning hearing loss effects using other kinds of stimuli remains to be developed (Stapells and Picton, 1981).

We also held repetition rate constant at 11.1/sec. There is evidence to suggest that a rate of 11.1/sec is within a reasonable margin of acceptability for obtaining BSERs. We know from the work of others (Moore, 1971; Row, 1980) that increasing the repetition rate also increases latency but decreases the magnitude of the BSER waves. Such an effect is more pronounced for repetition rates greater than 11.1/sec, although a latency increase and an amplitude decrease do not go undetected at rates below 11.1/sec.

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It is evident from the above findings that waveform morphology, thresholds, and latencies differ across genotypes. Sinificant differences were noted in latency and amplitude for the Agouti and the Cream. The results for the BEW and the WBA are different in that amplitude and latency values were not computed across all intensity levels due to absence of specific waves. Again, for these two genotypes, response thresholds vary between 45-75 dB nHL. As expected, we did not compute any numerical values for the Anophthalmic white because no recordable responses were obtained. Another interesting finding is that there appears to be no effect between the Wh-locus and the E-locus with regard to latency at the intensity level at which BSERs were obtained for all genotypes in the study. With regard to amplitude, statistically significant differences were noted between the Wh-locus and the E-locus. This finding may conote that latency and amplitude are the result of independent underlying physiologic processes.

Thus the results of this study have shown that genotypes differ in their BSERs at different intensity levels. Interestingly, we see from this variability, a corpus of hamsters who can be classified variously as normal (Agouti and Cream), moderate-to-severe (BEW and WBA) and profound (Anophthalmic white) hearing loss. This investigation has important implications for human studies, in that the

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CHAPTER VI

SUMMARY AND CONCLUSIONS

The gene, Anophthalmic white (Wh) in the Syrian hamster is a pleiotropic mutant causing deleterious effects on eye development, pigmentation and deafness (Robinson, 1962; Asher, 1968). Recent investigations of the cochlea (Asher, 1988) under light and electron microscopy have shown that the gene caused degeneration of the tectorial membrane which becomes apparent between 10 to 15 days of neonatal life. Other equally devastating effects caused by this gene include, infertility, growth retardation and metabolic rate. It would appear that the Wh mutation is homologous to the Waardenberg syndrome in humans in that both mutations apparently affect the same developmental processes (Waardenberg, 1951; Preus et al, 1983; Arias, 1984). We know that of all the defects caused by both mutations, deafness is the most pronounced (Asher, 1968; Wang et al, 1981). While deafness has been described in the hamsters, only behavioral observations were conducted. To our knowledge, no investigations have been conducted to determine the hearing of the various genotypes.

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The BSER can be used to effectively evaluate and monitor the hearing status of infants (Hecox and Galambos, 1974; Salamy and Mckean, (1976). In the same vein, the BSER has also been investigated in other animal species including the cat (Jewett and Romano, 1978), mouse (Henry and Lepkowsky, 1978) and gerbil, (Wolf and Ryan, 1985b). While the hamster has been chosen as a model for the study of the developing capabilities of the peripheral auditory apparatus (Relkin, et al, 1979; Stonek, 1977), apparently, very little is known about the Syrian hamster. A thorough review of the results of the literature revealed that there has not been any investigations conducted to systematically evaluate the hearing of various genotypes of hamsters. The present investigation was designed to determine the hearing capabilities of the Wh genotypes and phenotypes observed in the AN/As-Wh strain; and whether there are genotypes which have normal hearing as well as genotypes which can be classified as hearing-impaired. Twenty hamsters were used in the study to test the following null hypothesis:

(1) The genotype wh/wh, E/e (Agouti) has no effect on the morphology, threshold, latency and amplitude of waves I-IV of the auditory brain-stem response at varying intensity levels.

(2) The genotype wh/wh,e/e (Cream) has no effect on the morphology, thresholds, latency and amplitude

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(3) The genotype Wh/wh, e/e (BEW) has no effect on the morphology, thresholds, latency and amplitude of waves I-IV of the auditory brain-stem responses at varying intensity levels.

(4) The genotype Wh/wh,E/e (WBA) has no effect on the morphology, thresholds, latency and amplitude of waves I-IV of the auditory brain-stem response at varying intensity levels.

(5) The genotype Wh/Wh-- (Anophthalmic white) has no effect on the morphology, thresholds, latency and amplitude of waves I-IV of the auditory brain-stem response at varying intensity levels.

Animals were anesthetized with rompun (dose = 10 mg/kg) and sodium pentobarbitol (dose = 30 mg/kg). Supplemental dosage of rompun were administered as necessary to maintain a constant background EEG level. Clicks with a maximum spectra at 2000 Hz were presented to both ears of each animal at intensity levels of 25, 35, 45, 55, 65 and 75 dB nHL. The repetition rate was held constant at 11.1/sec with a computer analysis time of 10 ms and an average of 2048 responses. The band pass of the amplifier was 100 - 3 kHz (-3 dB). A dwell time of 10 uS was employed using 1000 data points and frequency conversion was 100 kHz. The

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A total of five genotypes were tested in a pilot study so as to eliminate independent and dependent variables having minimum influence on the resultant data. As expected, identifiable BSER responses were obtained for the Agouti and the Cream at 25-75 dB nHL. It turns out that elevated thresholds (45-75 dB nHL) were obtained for the BEW and WBA, while the Anophthalmic white showed complete obscure recordable responses. We observed also that while both the Agouti and the Cream have normal threshold, their amplitude and latency data indicate statistically significant differencecs. Therefore the null hypothesis that genotype wh/wh, E/e (Agouti) and the wh/wh, e/e (Cream) are not different is rejected for these genotypes for all waves. Threshold differences were observed between the BEW and the WBA.

We note also that even at intensity levels of 45-75 dB nHL, responses from individual animals varied considerably. Thus, for the only two intensity levels for which statistical analysis was possible, no significant difference was noted between the BEW and the WBA. This implies that at high intensity levels at which recordable responses were possible for these genotypes, differences between them were

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minimal. This does not mean that the two genotypes do not differ, since inspection of data revealed that all genotypes differ with respect to threshold, latency and amplitude, except for the Agouti and Cream for which thresholds were similar.

It was of interest to conduct a the Chi-square to determine the difference between Wh-locus and E-locus with regard to latency and amplitude for wave I-IV. No statistically significant difference was noted for latency. This means that at high intensity levels at which all animals can respond the Wh-locus and the E-locus have almost identical effects on latency. This finding is understandable, since animals used in the study are heterozygotes, and thus, there is a possibility that there may be an overlap in gene-locus effect. On the other hand, the Chi-square test of significance revealed that significant differences exist between Wh-locus and E-locus with regard to amplitude for waves I-IV at 75 dB nHL and their is interaction between them. Thus, it may be that latency and amplitude are the result of independent underlying physiologic processes. Thus, while absolute amplitudes reflect both the trade-off between the sizes and orientations of generators, their component neurons, the head volume, and mass of muscle, skin, bone and sinew; latency is dependent on the conduction time along the auditory pathway (Merzenich, et al 1983).

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For example, a large brain results in larger nerve-conduction pathways, resulting by itself, in slower conduction times.

We also see a direct relationship in the group data between amplitude of the BSER and stimulus intensity and an inverse relationship between latency and intensity for the Agouti and the Cream. The results of the statistical analysis revealed that differences exist between the various intensity levels and amplitude for waves I, II, III, and IV. Therefore the null hypothesis that intensity does not affect amplitude of the Agouti and the Cream is rejected. Secondly, the null hypothesis that the latency of the BSER does not decrease as function of stimulus intensity for these two genotypes is rejected. With regard to the BEW and the WBA, there was no significant difference for the latencies and amplitudes of waves I-IV as function of stimulus intensity. We observed that these genotypes have a tendency to be reducers more than augmenters, in that the amplitude of their BSER tend to decrease as function of stimulus intensity. We could not compute any statistical analysis for the Anophthalmia white since there were no recordable BSERs.

We noted from our results that, while consistent, a certain degree of variability exists, not only between genotypes but

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also within genotypes with respect to latency and amplitude. We also observed that thresholds vary between genotypes from 25-75 dB nHL. Based on these findings, we can describe the Wh-gene as incompletely dominant since animals homozygous for the mutant showed a complete absence of hearing while the heterozygous showed varying degrees of hearing capability. With these properties in mind, we see a cohort of genotypes whose hearing could be classified as normal (Agouti and Cream), moderate-to-severe (BEW and WBA) and profound hearing loss (Anophthalmic white). Within this context, this animal model of hearing loss may have important implications for individuals exhibiting manifestations of the Waardenberg syndrome, as well as other genetically-based hearing abnormalities.

Suggestions for Additional Research

In view of these results, the following recommendations are made as areas of additonal investigation:

(1) The use of other forms of stimuli with more frequency specificity such as short tone bursts and tone pips using a similar research design is recommended.

(2) A study similar to the present investigation should be conducted using different repetition rates so as to determine whether changes in thresholds, latency and amplitude values interact

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with genotype as a function of various repetition rates.

(3) It may be worthwhile to conduct an investigation of this nature on the mouse since the Mi^{or} in this species provides another suitable model of the Waardenberg syndrome

(4) Longitudinal studies using the various genotypes employed in this study should be conducted to investigate whether the variable expression of the Wh-mutation and the E-gene on these genotypes produce progressive hearing loss.

(5) A study that differenciates cochlear from retrocochlear pathology such as oto-acoustic emissions (Kemp, 1978) may be warranted in the various genotypes.

(6) Histological studies should be conducted on the Anophthalmic white, BEW and the WBA, so as to determine the site-of-lesion of their hearing loss. It follows that a histological examination should also be conducted on the Agouti so as to yield comparative normative data.

(7) Since there is a significant difference between the Agouti and the Cream with regard to latency, histological studies should be conducted on these two genotypes so as to determine whether the shorter latency values noted in the Cream are due

to the destruction of the outer hair cells or some other pathological condition in the ear.

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Appendix A. Definition of Terms

Acrosome. The anterior end of the head of a spermatozoon.

Agouti. A term in genetics used to indicate the natural wildcolor pattern of the hair of the hamster. The grizzled color of the fur often noticed results from the barring of each hair in several alternate dark and light bands.

Albinism. Congenital abnormal, non-pathological, partial or total absence of pigment in the skin, hair and eyes.

Alleles. A pair of genes, situated on the same site on a paired cromosome, containing specific inheritance characteristics. A pair of alleles is usually indicated by an alphabetical letter with a capital used for the dominant geneand a lower case letter for the recessive gene, e.g. wh/wh; (Cream) and Wh/Wh-- (Anophthalmia). An individual possessing a pair of identical alleles, either two dominant or two recessive genes is said to be "pure" to the characteristic controlled by the gene and is therefore homozygous for that gene, e.g., Wh/Wh--, The union of a dominant gene and its recessive allele produces heterozygous

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individual for that characteristic, e.g., Wh/wh, (Cream).

Anophthalmia. Absence of the eyes.

Aplasia. Incomplete or arrested development of the inner ear. It is a static condition.

Arginase. An enzyme existing primarily in the liver, but also in the testes and kidney which splits arginine into urea and ornithine.

Arginine. It is the chemical name of an amino acid produced by the digestion of proteins.

Autosomal. Any of the paired cromosomes other than the Sex-linked (X and Y) chromosomes.

Chromosomal aberrations. Anomalies due to the presence of an extra chromosome (e.g., trisomy D is characterised by absence of external auditory canal, absence of the middle ear, microphthalmia and cataracts). The problem may involve mutations such as translocation of a chromosome.

Canthorum. The angle at either end of the fissure between the eyelids.

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Citrulline. An acid that is formed from ornithine and is itself converted into ornithine in the urea cycle.

Dominant. Capable of expression when carried by only one of a pair of homologous chromosomes, e.g., dominant allele or trait.

Epistatic interaction. The interaction between genes at different loci of which one hereditary character is expressed, or is marked by the superimposition of another gene upon the other.

Enzyme. A complex protein (or organic catalyst produced by living cells) that is capable of inducing chemical change in other substances, without being chaged themselves.

Expressivity. The severity of a genetic abnormality in a particular individual.

Follicle. A small secretory sac or cavity.

Gamete. The mature male or female reproductive cell (i.e, a fertilized ovum).

Genotype. Basic hereditary combination of genes of an organism.

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Heterozygous. An individual in which the members of one or more pairs of genes are unalike.

Homozygous. An individual developing from similar gametes and thus possessing similar pairs of genes for any heredity characteristic.

Hypophysis. An undergrowth (the pituitary body).

Melanin. The pigment which gives color to the hair, skin or choroid of the eyes.

Melanocyte. Pigment forming cell, which gives rise to color of the eyes, skin and hair.

Microphthalmia. Abnormally small size of the eyes.

Mutation. A change in a gene potentially capable of being transmitted to an offspring.

Pelage. The hairy coat of animals.

Penetrance. The frequency with which a genetic abnormality is manifested among those who possess the gene or genes involved.

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Phenotype. The physical appearance or make up of an individual. In genetics, a group of individuals who resemble each other in appearance, may differ in genetic make up.

Pigment. Any organic coloring matter in the body.

Pleiotropic. Producing many effects in the phenotype.

Recessive. Incapable of expression unless (the responsible allele) is carried by both members of a pair of homologous chromosomes. e.g., a recessive trait.

Zygote. Cell produced by union of two gametes.

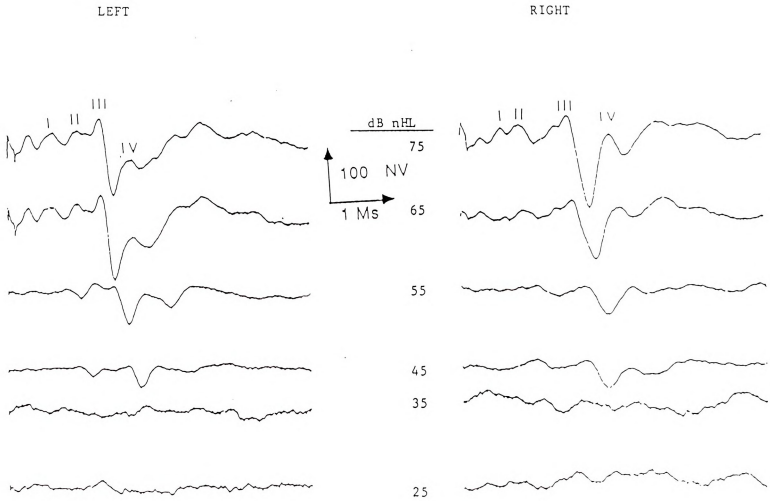
APPENDICES

Appen

Appendix B. Auditory brainstem evoked responses for twenty hamsters to clicks presented at varying intensity levels of 25, 35, 45, 55, 65 and 75 dB nHL.

Analog Traces for wh/wh, E/e (Agouti)

HM012 - wh/wh, E/e (AGOUTI)

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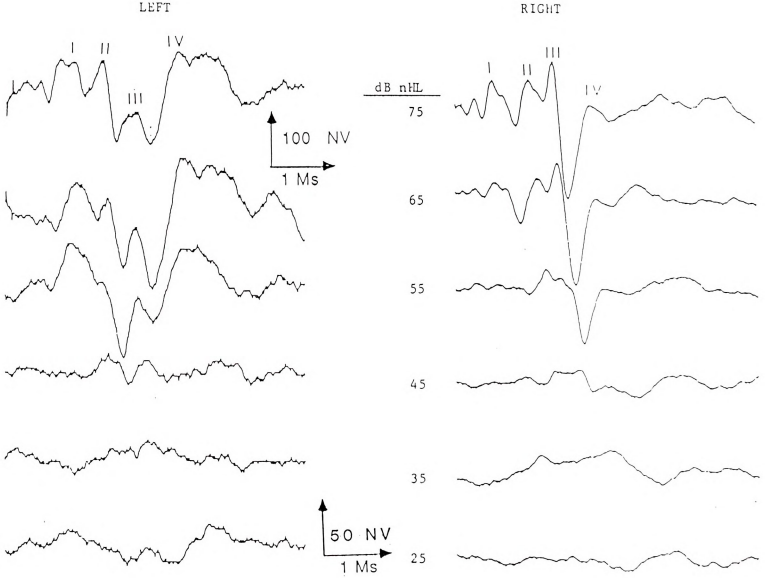
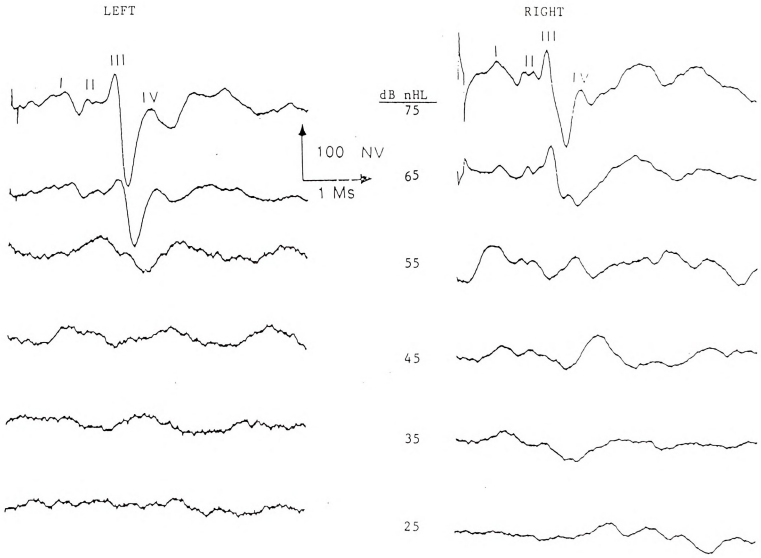
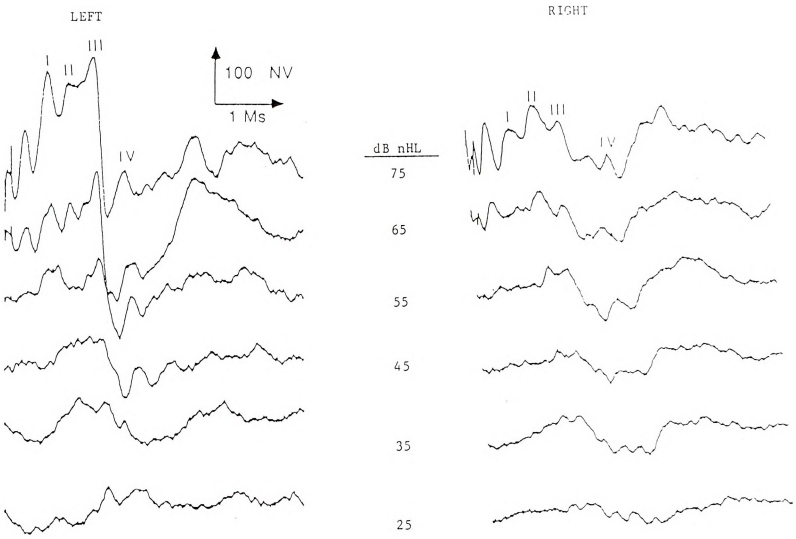
HM013 - wh/wh, E/e (AGOUTI)



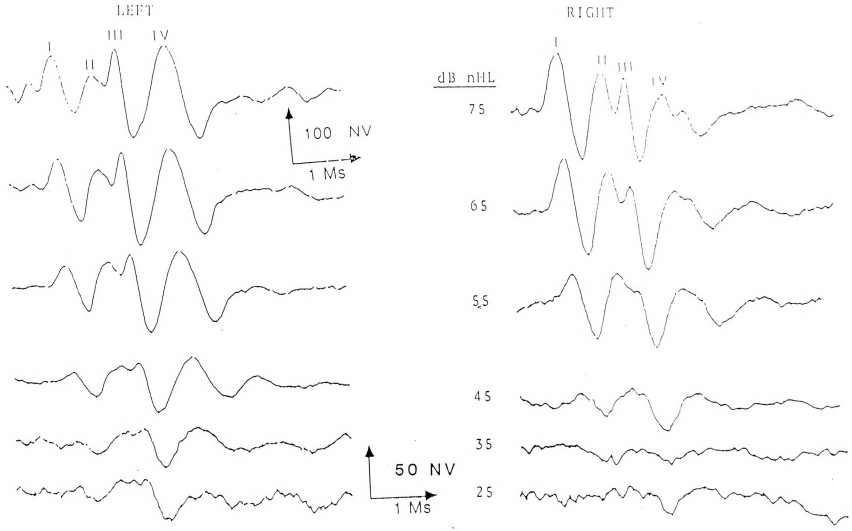
HM014 - wh/wh, E/e (AGOUTI)



HM016 - whh/wh, E/e (AGOUTI)

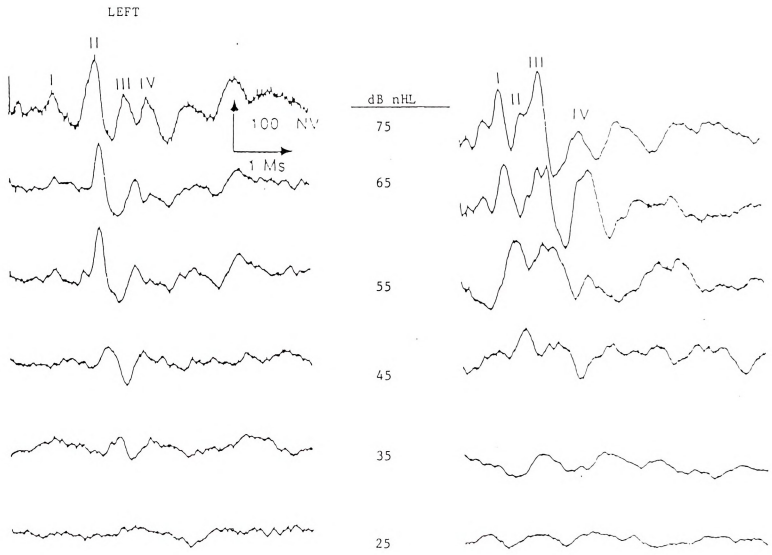


HM033 - wh/wh, E/e (AGOUTI)

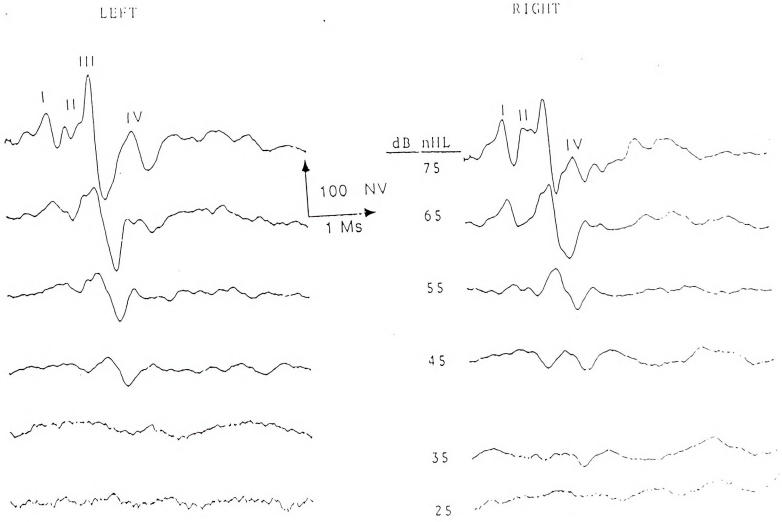


Analog Traces for Wh/wh, e/e (Cream)

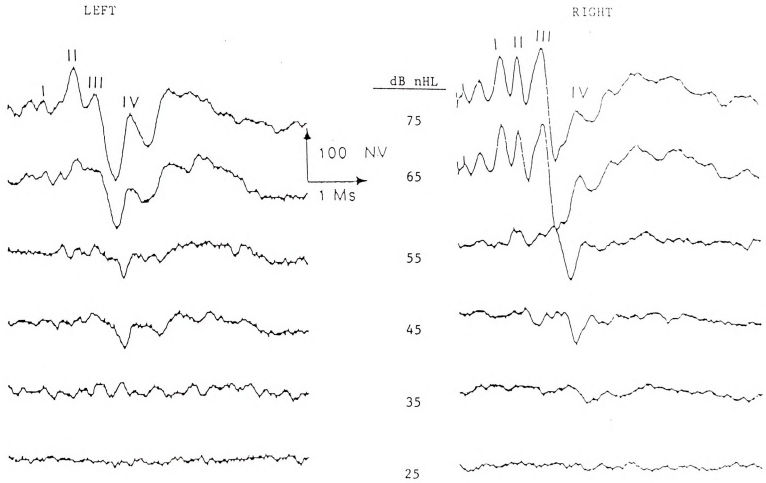
HM017 - wh/wh, e/e (CREAM)



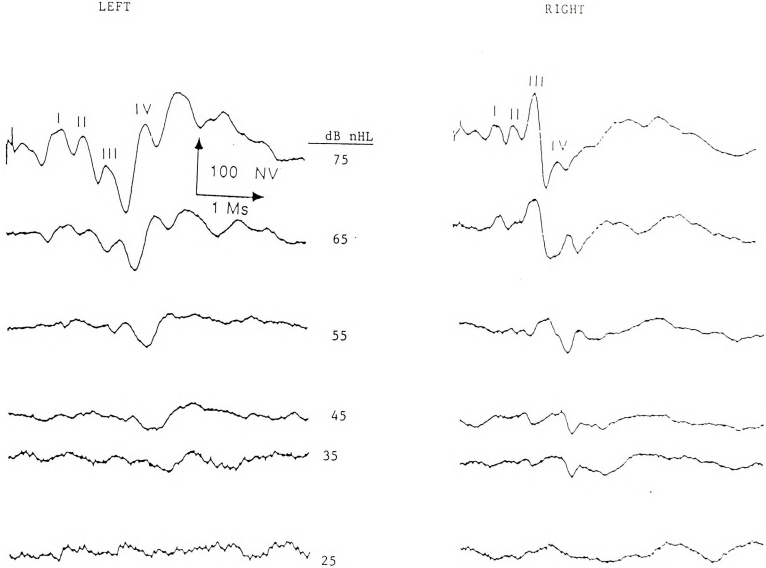
HM018 - wh/wh, e/e (CREAM)



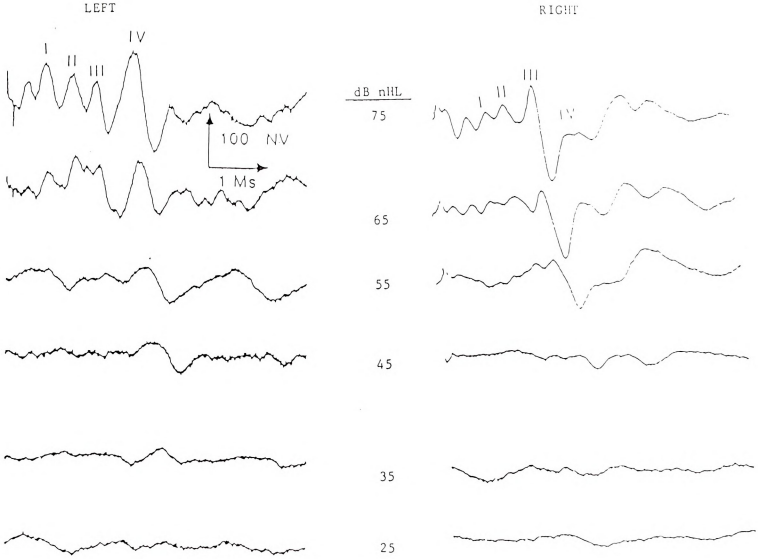
HM019 - wh/wh, e/e (CREAM)



HM020 - wh/wh, e/e (CREAM)

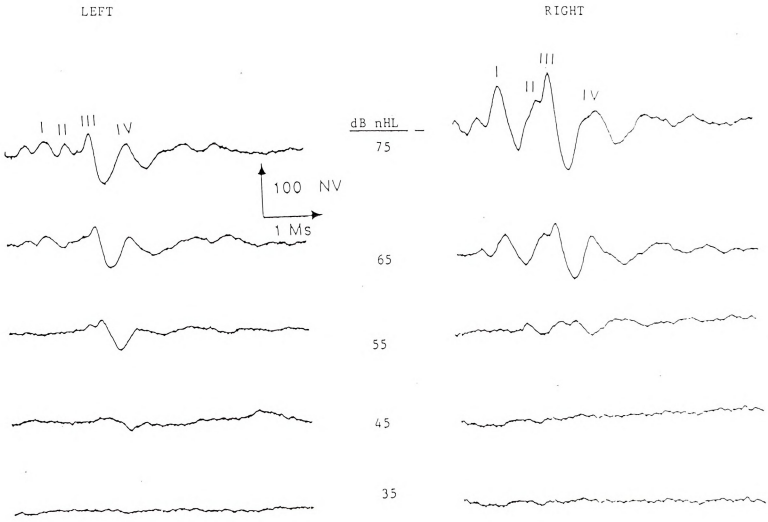


HM021- wh/wh, e/e (CREAM)

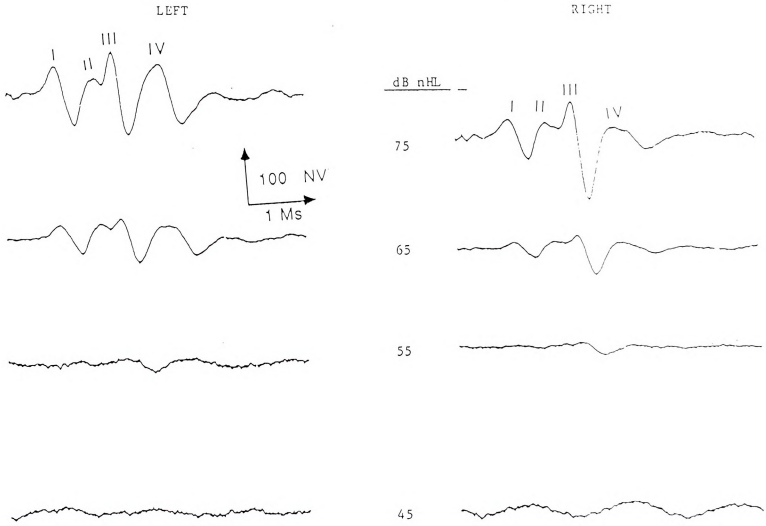


Analog Traces for Wh/Wh, e/e (Black-eyed White)

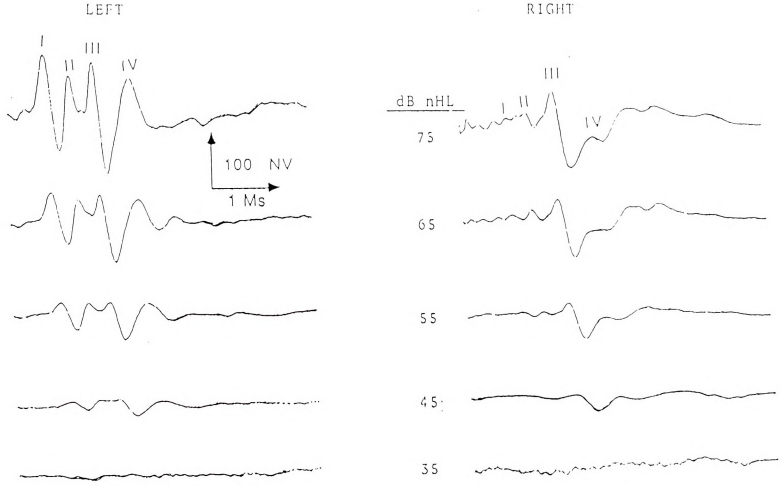
HM022 - Wh/wh, e/e (BLACK-EYED WHITE)



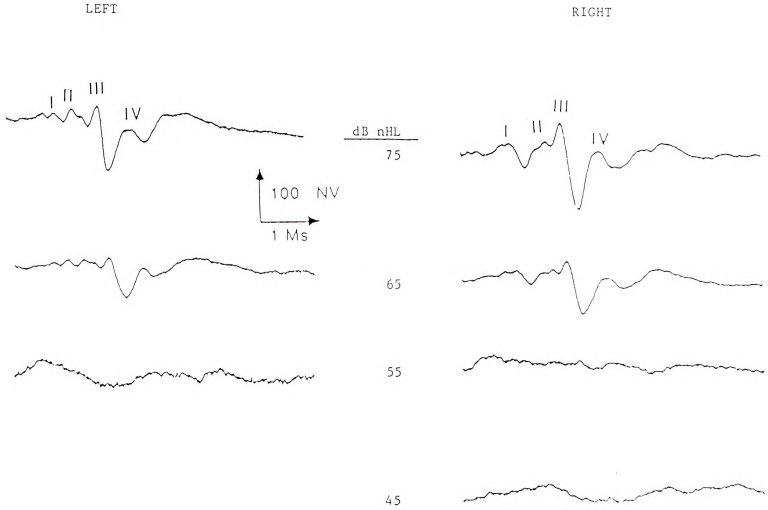
HM023 - Wh/wh, e/e (BLACK-EYED WHITE)



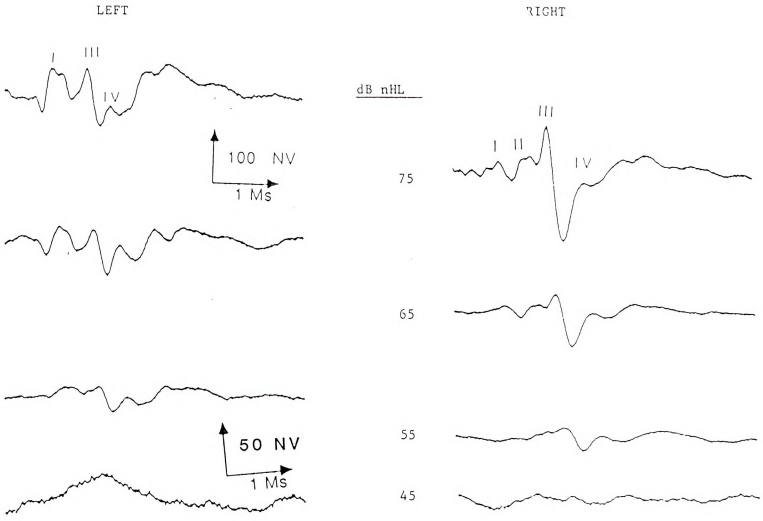
HM024 - Wh/wh, e/e (BLACK-EYED WHITE)



HM025 - Wh/wh, e/e (BLACK-EYED WHITE)

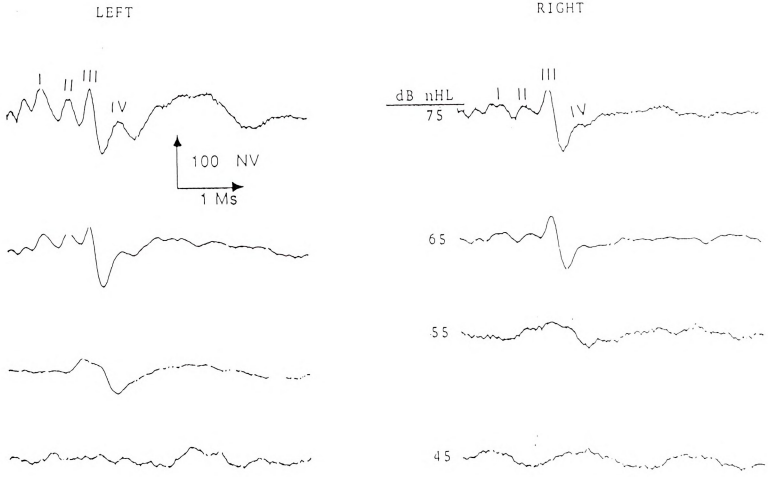


HM026 - Wh/wh, e/e (BLACK-EYED WHITE)

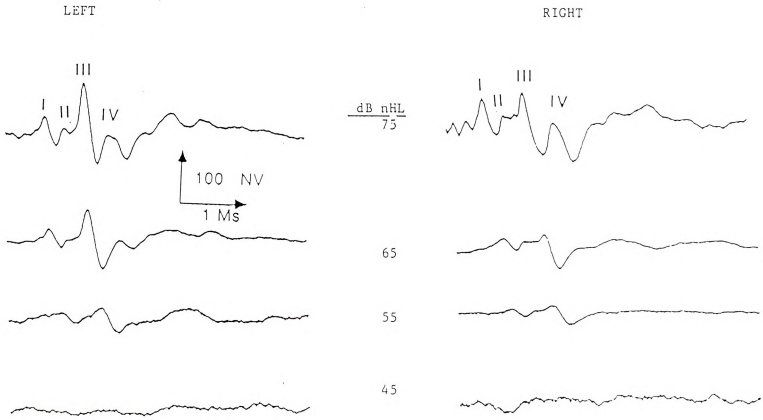


Analog Traces for Wh/Wh, E/e (White-belly Agouti)

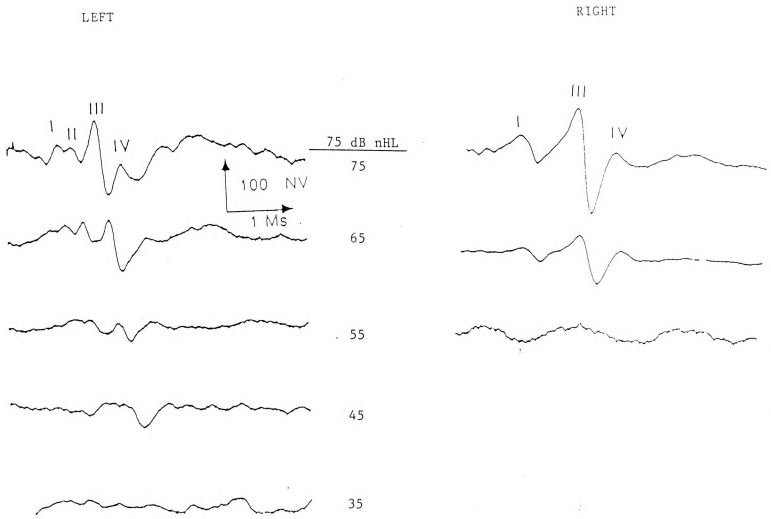
HM027 - Wh/wh, E/e (WHITE-BELLY AGOUTI)



HM028 - Wh/wh, E/e (WHITE-BELLY AGOUTI)

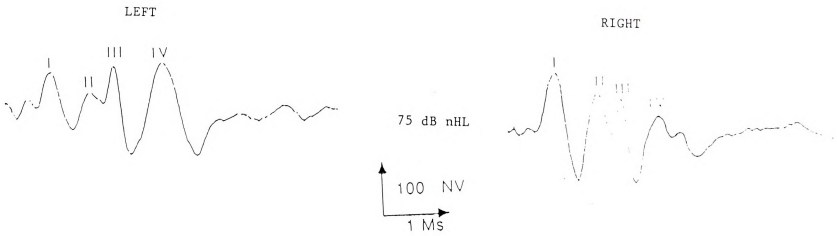


HM030 - Wh/wh, E/e (WHITE-BELLY AGOUTI)

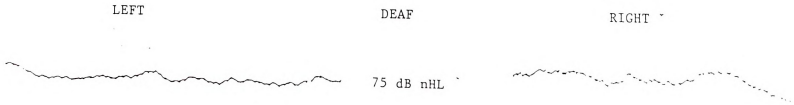


Analog Traces for Wh/Wh,-- (Anophthalmia White)

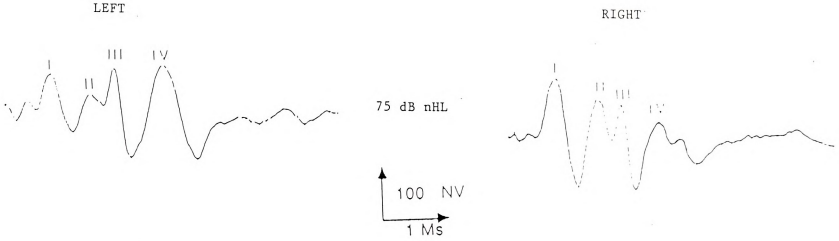
HM033 wh/wh, E/e (AGOUTI)



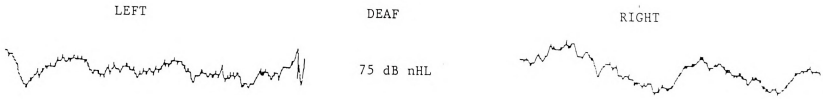
HM031 Wh/Wh,-- (ANOPHTHALMIA WHITE)



HM033 - wh/wh, E/e (AGOUTI)



HM032 - Wh/Wh,-- (ANOPHTHALMIA WHITE)



Appendix C. Latency and amplitudes for waves I-IV of Agouti.

Table 6. Latency of wave I (ms) from 25-75 dB nHL for Agouti(R/E)

Animal	25	35	45	55	65	75
HM012	2.80	2.02	1.98	1.94	1.50	1.45
HM013	2.20	2.10	2.04	1.94	1.68	1.50
HM014	2.10	1.82	1.76	1.56	1.46	1.40
HM016	2.28	2.20	2.08	1.90	1.60	1.52
HM033	1.96	1.90	1.88	1.74	1.60	1.50
TOTAL	9.90	9.98	9.74	9.08	7.84	7.36
X	1.90	1.80	1.79	1.72	1.56	1.48
SD	0.40	0.15	0.30	0.20	0.09	0.05

Table 7. Amplitude of wave I from 25-75 dB nHL for Agouti(R/E)

Animal	25	35	45	55	65	75
HM012	10	30	65	120	130	300
HM013	20	25	70	195	355	750
HM014	40	50	105	225	140	125
HM016	40	55	25	30	35	75
HM033	35	50	150	205	300	650
TOTAL	145	210	415	775	960	1920
X	29	42	83	155	192	380
SD	13.4	13.5	47	150	132	310

Table 8. Latency of wave I from 25-75 dB nHL for Agouti(L/E)

Animal	25	35	45	55	65	75
HM012	2.38	2.32	2.28	2.00	1.94	1.54
HM013	2.18	2.06	1.90	1.88	1.86	1.66
HM014	2.28	2.26	2.24	1.98	1.92	1.60
HM016	2.10	2.02	1.96	1.80	1.56	1.46
HM033	2.00	1.90	1.80	1.62	1.52	1.45
TOTAL	11	10.50	10.18	9.28	8.80	7.37
X	2.20	2.10	2.05	1.86	1.76	1.54
SD	0.16	0.20	0.20	0.20	0.20	0.10

Table 9. Amplitude of wave I from 25-75 dB nHL for Agouti(L/E)

Animal	25	35	45	55	65	75
HM012	35	40	115	140	145	170
HM013	20	30	25	50	50	60
HM014	30	45	45	120	190	225
HM016	15	20	45	90	235	350
HM033	35	100	255	575	585	595
TOTAL	135	235	485	975	1205	1400
X	27	47	97	195	241	280
SD	9	31	95	150	204	204

Table 10. Latency for wave II from 25-75 dB nHL for Agouti(R/E)

Animal	25	35	45	55	65	75
HM012	2.96	2.86	2.84	2.56	2.34	2.16
HM013	3.38	3.30	3.26	2.90	2.68	2.40
HM014	2.92	2.88	2.86	2.52	2.36	2.20
HM016	2.98	2.90	2.60	2.48	2.36	2.30
HM033	3.20	3.14	3.14	2.98	2.84	2.76
TOTAL	15.50	15.00	14.70	13.44	12.58	11.82
X	3.10	3.00	2.94	2.69	2.60	2.37
SD	0.20	0.19	0.30	0.20	0.20	0.25

Table 11. Amplitude for wave II from 25-75 dB nHL for Agouti (R/E)

Animal	25	35	45	55	65	75
HM012	10	40	25	15	15	20
HM013	5	20	45	125	155	200
HM014	20	30	55	210	115	70
HM016	10	50	45	85	200	225
HM033	20	35	45	105	115	220
TOTAL	65	185	215	540	640	735
X	13	35	43	108	118	147
SD	6.7	11	11	90	70	95.3

Table 12. Latency for wave II from 25-75 dB nHL for Agouti(L/E)

Animal	25	35	45	55	65	75
HM012	3.38	2.30	3.26	2.94	2.86	2.34
HM013	3.06	3.00	2.96	2.84	2.38	2.28
HM014	3.00	2.98	3.22	3.10	2.86	2.54
HM016	3.40	3.40	2.84	2.80	2.64	2.12
HM033	3.30	3.20	2.98	2.88	2.66	2.52
TOTAL	16.0	14.8	14.70	14.56	13.40	11.80
X	3.2	2.97	2.90	2.90	2.68	2.36
SD	0.19	0.40	0.20	0.15	0.20	0.20

Table 13. Amplitude for wave II from 25-75 dB nHL for Agouti (L/E)

Animal	25	35	45	55	65	75
HM012	10	30	48	66	88	65
HM013	10	20	25	50	150	220
HM014	20	30	30	25	45	65
HM016	15	25	25	200	135	48
HM033	10	15	75	185	200	110
TOTAL	65	120	203	526	618	508
X	13	24	40.6	105	124	108
SD	5	6.5	72	85	60	70

Table 14. Latency for wave III from 25-75 dB nHL for Agouti(R/E)

Animal	25	35	45	55	64	75
HM012	4.68	4.60	4.56	4.04	3.70	3.54
HM013	4.10	4.02	4.00	3.52	3.40	3.28
HM014	4.78	4.76	4.60	3.80	3.12	3.00
HM016	3.48	3.26	3.18	3.08	3.14	3.10
HM033	3.84	3.80	3.74	3.62	3.50	3.42
TOTAL	21	21.5	20.02	18.06	16.86	16.34
X	4.201	4.10	4.01	3.62	3.40	3.27
SD	0.55	0.60	0.60	0.36	.30	0.23

Table 15. Amplitude for wave III from 25-75 dB nHL for Agouti (R/E)

Animal	25	35	45	55	65	75
HM012	10	20	70	415	955	1520
HM013	70	100	300	820	950	1565
HM014	20	80	250	255	700	1305
HM016	95	130	175	280	285	330
HM033	40	100	250	350	505	520
TOTAL	235	330	995	2120	3395	5240
X	47	86	199	424	679	1048
SD	36	41	87	220	290	581

Table 16. Latency for wave III from 25-75 dB nHL for Agouti (L/E)

Animal	25	35	45	55	65	75
HM012	4.35	4.30	4.20	3.80	3.46	3.06
HM013	4.04	4.00	3.96	3.42	3.36	3.22
HM014	4.13	4.10	4.08	3.74	3.66	3.44
HM016	3.48	3.36	3.28	3.16	3.08	2.98
HM033	3.90	3.86	3.76	3.54	3.38	3.26
TABLE	21	1.92	18.86	17.66	16.94	15.96
X	4.20	3.84	3.72	3.53	3.39	3.20
SD	0.22	0.41	0.40	0.30	0.21	0.20

Table 17. Amplitude for wave III from 25-75 dB nHL for Agouti(L/E)

Animal	25	35	45	55	65	75
HM012	70	55	350	625	125	1295
HM013	30	50	130	145	330	475
HM014	10	45	25	305	800	850
HM016	20	100	480	540	2005	2250
HM033	50	140	550	1850	2000	1005
TOTAL	180	390	1535	3465	6388	5875
X	36	78	307	693	1278	1175
SD	24	41	225	450	738	670

Table 18. Latency for wave IV from 25-75 dB nHL for Agouti (R/E)

Animal	25	35	45	55	65	75
HM012	5.82	5.70	5.60	5.40	5.20	4.90
HM013	4.96	4.95	4.90	4.78	4.56	4.42
HM014	5.36	5.34	5.30	5.20	5.12	4.12
HM016	5.00	4.90	4.86	4.66	4.34	4.04
HM033	5.20	5.0	4.99	4.94	4.74	4.34
TOTAL	27.00	26.00	25.65	24.98	23.96	21.84
X	5.30	5.20	5.13	5.00	4.80	4.37
SD	0.34	0.33	0.30	0.30	0.40	0.35

Table 19. Amplitude for wave IV from 25-75 dB nHL for Agouti(R/E)

Animal	25	35	45	55	65	75
HM012	25	30	15	110	150	350
HM013	30	45	65	25	65	120
HM014	35	35	55	150	160	225
HM016	20	25	65	80	95	100
HM033	30	40	25	70	90	105
TOTAL	140	175	225	435	560	900
X	28	35	45	87	112	180
SD	6	8	24	75	41	108

Table 20. Latency for wave IV from 25-75 dB nHL for Agouti(L/E)

Animal	25	35	45	55	65	75
HM012	5.50	5.48	5.02	4.86	4.50	4.24
HM013	4.88	4.82	4.76	4.64	4.38	4.30
HM014	5.40	5.30	5.20	5.10	4.94	4.60
HM016	4.90	4.70	4.52	4.26	4.18	4.00
HM033	5.16	5.10	4.98	4.89	4.84	4.76
TOTAL	26.00	25.40	24.48	23.75	22.84	21.90
X	5.20	5.10	4.89	4.75	4.57	4.38
SD	0.30	0.33	0.30	0.31	0.32	0.40

Table 21. Amplitude for wave IV from 25-75 dB nHL for Agouti(L/E)

Animal	25	35	45	55	65	75
HM012	20	30	65	85	120	140
HM013	10	20	70	85	265	200
HM014	10	30	15	140	180	750
HM016	15	20	145	185	124	185
HM033	45	50	450	750	1200	1350
TOTAL	100	150	745	1245	1889	2650
X	20	30	149	249	378	525
SD	15	12	175	250	463	524

Appendix D. Latency and amplitude values for waves I-IV of Cream.

Table 22. Latency for wave I from 25-75 nHL for Cream (R/E)

Animal	25	35	45	55	65	75
HM017	2.38	2.30	2.02	1.80	1.56	1.42
HM018	2.04	2.02	2.00	1.62	1.52	1.40
HM019	1.98	1.90	1.86	1.84	1.56	1.50
HM020	1.90	1.84	1.82	1.72	1.62	1.46
HM021	2.20	2.00	2.00	1.98	1.96	1.90
TOTAL	10.00	9.80	9.70	8.96	8.22	7.68
X	2.00	1.98	1.94	1.79	1.65	1.54
SD	0.45	0.18	0.09	0.14	0.18	0.20

Table 23. Amplitude for wave I from 25-75 dB nHL for Creme (R/E)

Animal	25	35	45	55	65	75
HM017	45	100	195	200	450	250
HM018	20	45	100	105	495	900
HM019	20	30	100	110	345	350
HM020	10	20	25	25	200	215
HM021	15	15	25	25	150	235
TOTAL	110	190	445	465	1640	1950
X	21	42	89	93	328	390
SD	15	34	70	73	152	290

Table 24. Latency for wave I from 25-75 dB nHL for Cream (L/E)

Animal	25	35	45	55	65	75
HM017	2.02	1.98	1.96	1.80	1.66	1.50
HM018	2.30	2.04	1.98	1.86	1.62	1.45
HM019	2.00	1.96	1.94	1.86	1.76	1.40
HM020	2.36	2.32	2.30	1.96	1.86	1.65
HM021	2.20	2.04	2.02	1.56	1.54	1.46
TOTAL	10.90	9.50	9.40	9.06	8.44	7.46
X	2.18	1.90	1.98	1.81	1.70	1.50
SD	0.17	0.40	0.15	0.15	0.13	0.15

Table 25. Amplitude for wave I from 25-75 dB nHL for Cream (L/E)

Animal	25	35	45	55	65	75
HM017	50	60	20	30	50	200
HM018	10	70	130	155	300	550
HM019	45	15	25	35	45	75
HM020	15	20	25	155	125	365
HM021	20	30	75	210	95	255
TOTAL	140	200	275	585	615	1445
X	28	40	55	117	123	289
SD	18	24	48	81	204	180

Table 26. Latency for wave II from 25-75 dB nHL for Cream (R/E)

Animal	25	35	45	55	65	75
HM017	2.94	2.88	2.64	2.60	2.58	2.14
HM018	2.80	2.64	2.36	2.40	2.54	2.32
HM019	3.00	2.50	2.28	2.08	2.06	2.02
HM020	2.70	2.32	2.30	2.16	2.06	2.00
HM021	3.18	3.16	3.12	3.10	2.92	2.58
TOTAL	14.50	13.50	12.70	12.34	12.16	11.06
X	2.92	2.70	2.54	2.47	2.43	2.20
SD	0.19	0.35	0.35	0.40	0.37	0.24

Table 27. Amplitude for wave II from 25-75 dB nHL for Cream (R/E)

Animal	25	35	45	55	65	75
HM017	20	30	45	75	90	30
HM018	25	90	150	155	95	120
HM019	20	15	25	25	300	425
HM020	10	20	35	35	50	195
HM021	15	15	60	95	280	325
TOTAL	90	170	310	385	815	1095
X	18	34	62	77	165	219
SD	6	31	51	52	118	158

Table 28. Latency for wave II from 25-75 dB nHL for Cream (L/E)

Animal	25	35	45	55	65	75
HM017	3.00	2.90	2.86	2.60	2.05	2.00
HM018	3.34	3.00	2.92	2.66	2.58	2.04
HM019	2.70	2.62	2.58	2.44	2.34	2.20
HM020	2.80	2.98	2.86	2.70	2.68	2.24
HM021	2.90	2.70	2.68	2.38	2.28	2.24
TOTAL	14.70	14.10	13.90	12.78	11.93	11.10
X	2.94	2.82	2.78	2.50	2.39	2.21
SD	0.23	0.16	0.14	0.20	0.30	0.20

Table 29. Amplitude for wave II from 25-75 dB nHL for Cream (L/E)

Animal	25	35	45	55	65	75
HM017	25	40	185	25	65	275
HM018	30	60	400	40	145	225
HM019	10	30	55	155	95	210
HM020	45	50	200	225	320	550
HM021	20	45	115	45	110	250
TOTAL	130	225	955	490	735	1510
X	26	45	191	98	147	302
SD	13	11	130	88	101	141

Table 30. Latency for wave III from 25-35 dB nHL for Cream (R/E)

Animal	25	35	45	55	65	75
HM017	3.92	3.72	3.18	3.08	2.92	2.64
HM018	3.80	3.30	3.28	3.02	2.86	2.74
HM019	3.90	3.60	3.50	3.18	2.94	2.86
HM020	3.40	3.30	3.28	3.00	2.80	2.68
HM021	4.40	4.38	4.38	3.89	3.68	3.54
TOTAL	19.5	18.30	17.62	16.17	15.20	14.46
X	3.9	3.66	3.53	3.24	3.04	2.89
SD	0.35	0.45	0.49	0.37	0.36	0.37

Table 31. Amplitude for wave III from 25-75 dB nHL for Cream (R/E)

Animal	25	35	45	55	65	75
HM017	35	45	225	415	700	1025
HM018	30	100	335	750	865	1500
HM019	45	50	225	420	840	950
HM020	30	45	225	465	800	2500
HM021	15	90	255	820	1500	1700
TOTAL	155	330	1265	2870	4705	7675
X	31	66	253	574	941	1535
SD	11	27	48	195	319	625

Table 32. Latency for wave III from 25-75 dB nHL for Cream (L/E)

Animal	25	35	45	55	65	75
HM017	3.70	3.68	3.50	3.24	3.16	3.00
HM018	3.66	3.44	3.34	3.06	2.96	2.94
HM019	3.90	3.90	3.72	3.03	3.02	2.96
HM020	4.10	4.08	4.06	4.02	3.80	3.44
HM021	4.04	4.00	3.68	3.48	3.12	3.00
TOTAL	19.40	19.10	18.40	16.83	16.06	13.34
X	3.88	3.82	3.68	3.67	3.22	3.06
SD	0.18	0.30	0.30	0.40	0.34	0.21

Table 33. Amplitude for wave III from 25-75 dB nHL for Cream (L/E)

Animal	25	35	45	55	65	75
HM017	150	80	185	215	480	490
HM018	55	100	400	825	1600	2460
HM019	25	30	55	155	285	450
HM020	45	80	200	330	450	575
HM021	30	45	115	250	335	390
TOTAL	205	330	955	1775	3150	4365
X	41	67	191	355	630	873
SD	13	29	130	270	548	890

Table 34. Latency for wave IV from 25-75 dB nHL FOR Cream (R/E)

Animal	25	35	45	55	65	75
HM017	5.20	4.88	4.84	4.26	4.20	4.00
HM018	5.30	4.68	4.46	4.16	4.00	3.84
HM019	4.92	4.80	4.42	4.20	4.10	4.10
HM020	5.32	5.20	4.20	4.02	3.92	3.46
HM021	5.48	5.28	5.25	5.08	4.86	4.56
TOTAL	26.00	24.60	23.17	21.72	21.08	19.96
X	5.24	4.97	4.64	4.34	4.22	3.99
SD	0.22	0.26	0.40	0.42	0.37	0.40

Table 35. Amplitude for wave IV from 25-75 dB nHL for Cream (R/E)

Animal	25	35	45	55	65	75
HM017	30	35	95	110	550	560
HM018	45	50	80	95	125	465
HM019	30	45	65	185	195	200
HM020	10	20	75	110	225	225
HM021	15	15	40	40	185	110
TOTAL	130	165	355	540	1275	1560
X	26	33	71	108	255	312
SD	14	6	21	52	169	191

Table 36. Latency for Wave IV from 25-75 dB nHL for Cream (L/E)

Animal	25	35	45	55	65	75
HM017	4.60	4.58	4.54	4.42	4.12	3.82
HM018	4.96	4.92	4.84	4.26	4.22	4.18
HM019	5.20	5.00	4.58	4.42	4.22	4.12
HM020	5.40	5.20	5.00	4.40	4.94	4.68
HM021	5.48	5.40	5.16	4.72	4.48	4.24
TOTAL	26.00	25.10	24.92	23.02	21.98	21.04
X	5.20	5.02	4.98	4.61	4.40	4.20
SD	0.35	0.30	0.32	0.40	0.34	0.31

Table 37. Amplitude for wave IV from 25-75 dB nHl for Cream (L/E)

Animal	25	35	45	55	65	75
HM017	30	45	48	75	150	300
HM018	25	55	150	165	230	150
HM019	10	30	45	50	75	175
HM020	25	25	55	75	230	300
HM021	35	60	150	135	300	400
TOTAL	140	215	448	500	985	1925
X	25	43	90	100	197	385
SD	9.4	15	55	48	77	220

Appendix E. Latency and amplitude values for waves I-IV of the Black-eyed white.

Table 38. Latency for wave I from 25-75 for Black-eyed white (R/E)

Animal	25	35	45	55	65	75
HM022	NR	NR	2.04	2.20	1.74	1.60
HM023	NR	NR	NR	NR	2.04	1.94
HM024	NR	NR	NR	1.80	1.64	1.58
HM025	NR	NR	NR	NR	1.94	1.72
HM026	NR	NR	NR	NR	1.68	1.58
TOTAL	NR	NR	2.04	4.06	9.04	8.42
X	NR	NR	0.44	0.82	1.81	1.70
SD	NR	NR	I.0	1.5	1.50	0.20

Table 39. Amplitude for wave I from 25-75 for Black-eyed white(R/E)

Animal	25	35	45	55	65	75
HM022	NR	NR	100	120	300	575
HM023	NR	NR	NR	NR	400	750
HM024	NR	NR	NR	220	150	55
HM025	NR	NR	NR	NR	255	420
HM026	NR	NR	NR	NR	490	500
TOTAL	NR	NR	100	340	1595	2300
X	NR	NR	20	68	319	460
SD	NR	NR	44	71	131	257

Table 40. Latency for wave I from 25-75 for Black-eyed white (L/E)

Animal	25	35	45	55	65	75
HM022	NR	NR	NR	NR	2.12	2.02
HM023	NR	NR	NR	NR	1.80	1.62
HM024	NR	NR	1.96	1.66	1.50	1.40
HM025	NR	NR	NR	NR	1.78	1.54
HM026	NR	NR	NR		1.86	1.60
TOTAL	NR	NR	1.96	1.66	9.06	8.24
X	NR	NR	0.40	0.33	1.81	1.65
SD	NR	NR	0.90	0.70	0.22	0.20

Table 41. Amplitude for wave I from 25-75 for Black-eyed white (L/E)

Animal	25	35	45	55	65	75
HM022	NR	NR	NR	NR	150	200
HM023	NR	NR	NR	NR	500	950
HM024	NR	NR	80	750	1050	1300
HM025	NR	NR	NR	NR	35	365
HM026	NR	NR	NR	NR	120	120
TOTAL	NR	NR	80	750	1855	2935
X	NR	NR	11	150	371	587
SD	NR	NR	36	335	419	514

Table 42. Latency for wave II from 25-75 dB nHL for Black-eyed (R/E)

Animal	25	35	45	55	65	75
HM022	NR	NR	3.20	3.08	2.94	2.80
HM023	NR	NR	NR	0	3.08	2.90
HM024	NR	NR	3.56	2.52	2.30	2.22
HM025	NR	NR	NR	0	3.10	2.82
HM026	NR	NR	NR	2.76	2.62	2.50
TOTAL	NR	NR	6.76	8.36	14.04	13.24
X	NR	NR	0.64	1.70	2.81	2.65
SD	NR	NR	1.90	1.5	0.34	0.28

Table 43. Amplitude for wave II from 25-75 dB nHL for Black-eyed (R/E)

Animal	25	35	45	55	65	75
HM022	NR	NR	50	140	35	35
HM023	NR	NR	NR	NR	55	110
HM024	NR	NR	NR	120	375	400
HM025	NR	NR	NR	NR	75	75
HM026	NR	NR	NR	25	125	185
TOTAL	NR		50	285	665	805
X			10	57	133	161
SD			22	62	134	145

Table 44. Latency for wave II from 25-75 dB nHL for Black-eyed (L/E)

Animal	25	35	45	55	65	75
HM022	NR	NR	NR	2.72	2.54	2.40
HM023	NR	NR	NR	NR	3.14	2.92
HM024	NR	NR	2.74	2.50	2.28	2.02
HM025	NR	NR	NR	NR	2.32	2.18
HM026	NR	NR	NR	3.16	3.00	2.80
TOTAL	-	-	2.74	8.36	13.28	12.34
X	-	-	0.60	1.68	2.66	2.06
SD	-	-	1.20	1.5	0.39	0.38

Table 45. Amplitude for wave II from 25-75 dB nHL for Black-eyed (L/E)

Animal	25	35	45	55	65	75
HM022	NR	NR	NR	25	525	20
HM023	NR	NR	NR	NR	855	110
HM024	NR	NR	400	220	1500	495
HM025	NR	NR	NR	NR	195	100
HM026	NR	NR	NR	35	720	385
TOTAL	-	-	400	280	3795	1110
X	-	-	80	56	759	222
SD	-	-	179	110	483	206

Table 46. Latency for wave III from 25-75 dB nHL for Black-eyed (R/E)

Animal	25	35	45	55	65	75
HM022	NR	NR	4.02	3.60	3.25	3.24
HM023	NR	NR	NR	3.10	4.00	3.88
HM024	NR	NR	3.56	3.36	3.18	3.10
HM025	NR	NR	NR	3.19	3.08	3.00
HM026	NR	NR	3.82	3.66	3.40	3.16
TOTAL	-	-	11.40	16.91	17.34	16.16
X	-	-	2.28	3.39	3.47	3.35
SD	-	-	2.0	0.25	0.33	0.34

Table 47. Amplitude for wave III from 25-75 dB nHL for Black-eyed (R/E)

Animal	25	35	45	55	65	75
HM022	NR	NR	155	350	550	800
HM023	NR	NR	NR	25	1300	1900
HM024	NR	NR	30	1750	1900	2000
HM025	NR	NR	NR	100	505	545
HM026	NR	NR	300	1050	2500	2750
TOTAL	-	-	685	3275	6755	7995
X	-	-	137	655	1351	1599
SD	-	-	87	719	863	912

Table 48. Latency for wave III from 25-75 dB nHL for Black-eyed (L/E)

Animal	25	35	45	55	65	75
HM022	NR	NR	NR	3.12	2.96	2.80
HM023	NR	NR	NR	4.00	3.80	3.54
HM024	NR	NR	3.50	3.24	3.02	2.88
HM025	NR	NR	NR	NR	3.12	3.04
HM026	NR	NR	NR	4.10	3.88	3.58
TOTAL	-	-	3.50	14.66	16.18	15.84
X	-	-	0.70	2.94	3.36	3.17
SD	-	-	1.60	1.60	0.44	0.36

Table 49. Amplitude for wave III from 25-75 dB nHL for Black-eyed (L/E)

Animal	25	35	45	55	65	75
HM022	NR	NR	NR	400	525	560
HM023	NR	NR	NR	75	855	1700
HM024	NR	NR	400	1005	1500	1495
HM025	NR	NR	NR	NR	195	500
HM026	NR	NR	NR	390	720	800
TOTAL	-	-	400	1870	3795	5055
X	-	-	80	374	759	101
SD	-	-	179	389	483	623

Table 50. Latency for wave IV from 25-75 nHL for Black-eyed (R/E)

Animal	25	35	45	55	65	75
HM022	NR	NR	5.20	4.96	4.50	4.40
HM023	NR	NR	NR	4.40	4.26	4.08
HM024	NR	NR	NR	4.58	4.52	4.42
HM025	NR	NR	NR	NR	4.84	4.54
HM026	NR	NR	5.00	4.82	4.56	4.36
TOTAL	-	-	10.20	18.76	23.68	22.80
X	-	-	2.04	3.75	4.74	4.56
SD	-	-	2.7	2.1	0.33	0.30

Table 51. Amplitude for wave IV from 25-75 dB nHL for Black-eyed (R/E)

Animal	25	35	45	55	65	75
HM022	NR	NR	50	140	150	320
HM023	NR	NR	NR	200	500	400
HM024	NR	NR	NR	95	1050	155
HM025	NR	NR	NR	NR	35	300
HM026	NR	NR	200	220	120	75
TOTAL	-	-	250	655	1855	1250
X	-	-	50	131	371	250
SD	-	-	87	88	419	132

Table 52. Latency for wave IV from 25-75 dB nHL for Blak-eyed (L/E)

Animal	25	35	45	55	65	75
HMO22	NR	NR	4.54	4.34	4.10	4.06
HMO23	NR	NR	NR	5.40	5.12	5.10
HMO24	NR	NR	4.86	4.38	4.28	4.12
HMO25	NR	NR	NR	NR	4.32	4.10
HMO26	-	-	NR	5.28	4.96	4.64
TOTAL	-	-	9.40	14.10	23.06	22.04
X	-	-	1.88	2.82	4.62	4.41
SD	-	-	2.50	2.50	0.54	0.45

Table 53. Amplitude for wave IV from 25-75 dB nHL for Black-eyed (L/E)

Animal	25	35	45	55	65	75
HMO22	NR	NR	25	65	200	250
HMO23	NR	NR	NR	NR	510	75
HMO24	NR	NR	165	400	650	550
HMO25	NR	NR	NR	NR	200	360
HMO26	NR	NR	NR	125	135	145
TOTAL	-	-	190	590	1695	1380
X	-	-	38	118	339	276
SD	-	-	71	179	227	182

Appendix F. Latency and amplitude for waves I-IV for the White-belly agouti.

Table 54. Latency for Wave I from 25-75 dB nHL for White-belly (R/E)

Animal	25	35	45	55	65	75
HH027	NR	NR	NR	NR	1.38	1.30
HM028	NR	NR	NR	1.66	1.54	1.40
HM030	NR	NR	NR	NR	2.04	1.82
TOTAL	-	-	-	1.66	5.96	4.82
X	-	-	-	0.33	1.99	1.20
SD	-	-	-	0.95	0.34	0.30

Table 55. Amplitude for wave I from 25-75 dB nHL for White-belly (R/E)

Animal	25	35	45	55	65	75
HM027	NR	NR	NR	NR	450	600
HM028	NR	NR	NR	360	425	500
HM030	NR	NR	NR	NR	500	550
TOTAL	-	-	-	360	1275	1600
X	-	-	-	72	255	330
SD	-	-	-	208	75	50

Table 56. Latency for wave I from 25-75 dB nHL for White-belly (L/E)

Animal	25	35	45	55	65	75
HM027	NR	NR	NR	1.70	1.58	1.52
HM028	NR	NR	NR	1.66	1.52	1.40
HM030	NR	NR	NR	NR	1.90	1.70
TOTAL	-	-	-	3.36	4.98	4.62
X	-	-	-	1.12	1.66	1.54
SD	-	-	-	0.02	0.20	0.12

Table 57. Amplitude for wave I from 25-75 dB nHL for White-belly (L/E)

Animal	25	35	45	55	65	75
HM027	NR	NR	NR	185	155	250
HM028	NR	NR	NR	360	500	750
HM030	NR	NR	NR	NR	120	125
TOTAL	-	-	-	545	755	1125
X	-	-	-	273	258	375
SD	-	-	-	124	210	330

Table 58. Latency for wave II from 25-75 dB nHL for White-belly (R/E)

Animal	25	35	45	55	65	75
HM027	NR	NR	NR	NR	2.14	2.12
HM028	NR	NR	NR	NR	2.08	2.02
HM030	NR	NR	NR	NR	NR	NR
TOTAL	-	-	-	-	4.22	4.14
X	-	-	-	-	1.40	1.38
SD	-	-	-	-	0.32	0.30

Table 59. Amplitude for wave II from 25-75 dB nHL for White-belly (R/E)

Animal	25	35	45	55	65	75
HM027	NR	NR	NR	NR	250	500
HM028	NR	NR	NR	NR	25	25
HM030	NR	NR	NR	NR	NR	NR
TOTAL	-	-	-	-	275	525
X	-	-	-	-	91	175
SD	-	-	-	-	55	105

Table 60. Latency for wave II from 25-75 dB nHL for white-belly (L/E)

Animal	25	35	45	55	65	75
HM027	NR	NR	NR	NR	2.48	2.34
HM028	NR	NR	NR	NR	2.12	2.02
HM030	-	NR	2.90	220	2.52	2.40
TOTAL	-	-	2.90	220	7.12	6.76
X	-	-	0.97	0.73	1.43	1.40
SD	-	-	1.60	1.50	0.20	0.20

Table 61. Amplitude for wave II from 25-75 dB nHL white-belly (L/E)

Animal	25	35	45	55	65	75
HM027	NR	NR	NR	NR	75	125
HM028	NR	NR	NR	NR	45	125
HM030	NR	NR	50	110	250	200
TOTAL	-	-	50	110	370	450
X	-	-	17	37	123	150
SD	-	-	29	64	110	43

Table 62. Latency for wave III from 25-75 dB nHL white-belly (R/E)

Animal	25	35	45	55	65	75
HM027	NR	NR	NR	3.0	2.92	2.85
HM028	NR	NR	NR	3.0	2.84	2.72
HM030	NR	NR	NR	NR	3.92	3.64
TOTAL	-	-	-	6.0	9.66	9.21
X	-	-	-	2.0	3.22	3.07
SD	-	-	-	1.7	.61	0.50

Table 63. Amplitude for wave III from 25-75 dB nHL for white-belly (R/E)

Animal	25	35	45	55	65	75
HM027	NR	NR	NR	1005	1100	1300
HM028	NR	NR	NR	1500	1800	2000
HM030	NR	NR	NR	NR	1500	2000
TOTAL	-	-	-	2505	4400	5300
X	-	-	-	835	1252	1650
SD	-	-	-	764	351	494

Table 64. Latency for wave III from 25-75 dB nHL for white-belly (L/E)

Animal	25	35	45	55	65	75
HM027	NR	NR	NR	3.14	3.06	3.02
HM028	NR	NR	NR	2.94	2.74	2.62
HM030	NR	NR	3.72	3.62	2.36	2.93
TOTAL	-	-	3.72	9.70	8.16	8.90
X	-	-	1.24	3.23	2.72	2.96
SD	-	-	2.20	0.35	0.35	0.20

Table 65. Amplitude for wave III from 25-75 dB nHL for white-belly (L/E)

Animal	25	35	45	55	65	75
HM027	NR	NR	NR	950	1050	1500
HM028	NR	NR	NR	1200	1550	1900
HM030	NR	NR	180	200	580	1000
TOTAL	-	-	180	2350	3180	4400
X	-	-	60	483	1060	1466
SD	-	-	104	625	485	451

Table 66. Latency for wave IV from 25-75 dB nHL for white-belly (R/E)

Animal	25	35	45	55	65	75
HM027	NR	NR	NR	NR	4.00	3.98
HM028	NR	NR	NR	NR	3.82	3.62
HM030	NR	NR	NR	NR	5.26	5.02
TOTAL	-	-	-	-	13.08	2.62
X	-	-	-	-	4.36	4.21
SD	-	-	-	-	0.80	0.71

Table 67. Amplitude for wave IV from 25-75 dB nHL for white-belly (R/E)

Animal	25	35	45	55	65	75
HM027	NR	NR	NR	NR	95	340
HM028	NR	NR	NR	NR	500	750
HM030	NR	NR	NR	NR	300	380
TABLE	-	-	-	-	895	1470
X	-	-	-	-	298	490
SD	-	-	-	-	203	226

Table 68. Latency for wave IV from 25-75 dB nHL for white-belly (L/E)

Animal	25	35	45	55	65	75
HM027	NR	NR	NR	NR	4.08	4.92
HM028	NR	NR	NR	NR	3.94	3.64
HM030	NR	NR	5.10	4.80	4.58	3.84
TABLE	-	-	5.10	4.80	12.60	12.40
X	-	-	1.70	1.60	4.20	4.13
SD	-	-	2.90	2.80	3.40	0.70

Table 69. Amplitude for wave IV from 25-75 dB nHL for white-belly (L/E)

Animal	25	35	45	55	65	75
HM027	NR	NR	NR	NR	140	200
HM028	NR	NR	NR	NR	220	505
HM030	NR	NR	75	45	45	210
TABLE	-	-	75	45	405	915
X	-	-	25	15	135	305
SD	-	-	43	26	88	173

Appendix G. Mean latency data of waves I-IV for all genotypes.

Table 70. Mean Latency for wave I from 25-75 dB nHL for all animals(R/E)

Genotypes	25	35	45	55	65	75	(N)
Agouti	1.90	1.80	1.79	1.72	1.56	1.48	5
Cream	2.00	1.98	1.94	1.79	1.65	1.54	5
BEW	*	*	2.04+	2.00+	1.81	1.70	5
WBA	*	*	*	1.66+	1.99	1.20	3
TOTAL	3.90	3.78	6.13	7.20	7.10	5.95	18
MEAN	1.95	1.89	2.10	1.75	1.75	1.48	
# OF ANIMALS	10	10	11	13	18	18	

Table 71. Mean Latency for wave II from 25-75 dB nHL for all animals (R/E)

Genotypes	25	35	45	55	65	75	N
Agouti	3.10	3.00	2.94	2.69	2.60	2.37	5
Cream	2.90	2.70	2.54	2.47	2.43	2.20	5
BEW	*	*	3.88+	2.78+	2.81	2.65	5
WBA	*	*	*	*	2.11	2.07	3
TOTAL	6.02	5.70	9.36	7.94	9.95	9.29	18
MEAN	3.01	2.85	3.12	2.70	2.49	2.30	
# OF ANIMALS	10	10	12	13	18	18	

* No response from any animal
\+ Response from reduced number of animals

Table 72. Mean Latency for wave III from 25-75 dB nHL for all animals (R/E)

Genotype	25	35	45	55	65	75	(N)
Agouti	4.20	4.10	4.00	3.62	3.40	3.27	5
Cream	3.90	3.66	3.53	3.24	3.04	2.89	5
BEW	*	*	3.80+	3.39	3.47	3.35	5
WBA	*	*	*	3.00+	3.22	3.07	3
TOTAL	8.10	7.76	11.33	13.25	13.13	12.58	18
MEAN	4.50	3.88	3.80	3.30	3.28	3.16	
# OF ANIMALS	10	10	13	17	18	18	

Table 73. Mean Latency for wave IV from 25-75 dB nHL for all animals (R/E)

Genotypes	25	35	45	55	65	75	(N)
Agouti	5.30	5.20	5.13	5.00	4.80	4.30	5
Cream	5.20	4.97	4.64	4.34	4.22	3.99	5
BEW	*	*	5.10+	4.65+	4.74	4.56	5
WBA	*	*	*	*	4.36	4.21	3
TOTAL	10.50	10.17	14.87	13.99	18.12	17.06	18
MEAN	5.10	5.08	4.95	4.69	4.53	4.27	
# OF ANIMALS	10	10	12	14	18	18	

* No response from any animal
\+ Response from reduced number of animals.

Table 74. Mean Latency for wave I from 25-75 dB nHL for all animals (L/E)

Genotypes	25	35	45	55	65	75	(N)
Agouti	2.22	2.10	2.05	1.86	1.76	1.54	5
Cream	2.18	1.90	1.88	1.81	1.70	1.50	5
BEW	*	*	1.96+	1.66+	1.81	1.65	5
WBA	*	*	*	1.68+	1.66	1.54	3
TOTAL	5.40	4.00	5.89	7.01	6.93	1.56	18
# OF ANIMALS	10	10	14	16	18	18	

Table 75. Mean Latency for wave II fro 25-75 dB nHL for all animals (L/E)

Genotypes	25	35	45	55	65	75	(N)
Agouti	3.20	2.97	2.90	2.90	2.68	2.36	5
Cream	2.94	2.82	2.78	2.50	2.39	2.21	5
BEW	*	*	2.74+	2.78+	2.66	2.06	5
WBA	*	*	2.90+	2.20+	1.43	1.40	3
TOTAL	6.14	5.79	11.32	10.38	9.16	8.03	18
MEAN	3.07	2.89	2.83	2.60	2.29	2.00	
# OF ANIMALS	10	10	13	15	18	18	

* No response from any animal
+ Response from reduced number of animals

Table 76. Mean Latency for wave III from 25-75 dB nHL for all animals (L/E)

Genotype	25	35	45	55	65	75	(N)
Agouti	4.20	3.84	3.72	3.53	3.39	3.20	5
Cream	3.28	3.62	3.68	3.67	3.22	3.06	5
BEW	*	*	3.50+	3.88+	3.36	3.17	5
WBA	*	*	3.72+	3.23+	2.72	2.72	3
TOTAL	4.08	7.66	14.62	14.31	12.69	12.15	18
MEAN	2.04	3.83	3.66	3.57	3.17	3.04	
# OF ANIMALS	10	10	12	14	18	18	

Table 77. Mean Latency for wave IV from 25-75 for animals (L/E)

Genotype	25	35	45	55	65	5	(N)
Agouti	5.20	5.10	4.89	4.75	4.57	4.38	5
Cream	5.20	5.02	4.98	4.61	4.40	4.20	5
BEW	*	*	4.70+	4.85+	4.62	4.41	5
WBA	*	*	5.10+	4.80+	4.20	4.13	3
TOTAL	10.40	10.12	19.67	19.01	17.79	17.09	18
MEAN	5.20	5.06	4.90	4.69	4.45	4.27	
# OF ANIMALS	10	10	13	15	18	18	

* No response from any animal
+ Response from reduced number of animals

Appendix H1. Differences between Agouti and Cream with respect to wave latency as given by F-value.

Table 78. Differences between Agouti and Cream with respect to wave latency as given by F-value.

Parameter	Difference	I	II	III	IV
R/E	Genotype	0.32	7.16*	8.32*	6.49*
Latency	Intensity	12.26**	6.84**	4.85**	5.54**
	Interaction	0.52	0.42	0.08	0.95
Parameter	Difference	I	II	III	IV
L/E	Genotype	0.967	19.06*	3.46	6.52*
Latency	Intensity	4.26**	17.47**	10.69**	5.56**
	Interaction	0.65	0.264	0.04	0.96

* Indicates significance at P = .05 with F1,40 = 4.08
** Indicates significance at P = .05 with F5,40 = 2.45

Appendix H2. Summary of two-way ANOVA for waves I-IV for genotype (Agouti and Cream) and intensity for right and left ears.

Table 79. Summary of genotype (Agouti and Cream) x intensity x ANOVA X ABR latency for the Right Ear

Wave	Source	SS	DF	MS	F
I	Between Genotypes	0.01	1	0.01	0.33
	Between intensities	1.90	5	0.38	12.26**
	Genotype-intensity-Interaction	0.08	5	0.016	0.516
	Error	1.22	40	0.031	
II	Between Genotypes	0.68	1	0.68	7.16*
	Between Intensities	3.27	5	0.65	6.84**
	Genotype-intensity Interaction	0.20	5	0.04	0.42
	Error	3.79	40	0.095	
III	Between Genotypes	2.00	1	1.99	8.32*
	Between Intensities	5.79	5	1.16	4.85**
	Genotype-intensity Interaction	0.09	5	0.018	0.08
	Error	9.58	40	0.239	
IV	Between Genotypes	1.22	1	1.22	6.42*
	Between Intensities	5.19	5	1.04	5.46**
	Genotype-Intensity Interaction	0.89	5	0.18	0.94
	Error	7,49	40	0.19	

* Indicates significance at P = .05 with $F_{1,40} = 4.08$
** Indicates significance at P = .05 with $F_{5,40} = 2.45$

Table 80. Summary of genotype (Agouti and Cream) x intensity x ABR latency for the left ear

Wave		SS	DF	MS	F
I	Between Genotypes	0.03	1	0.03	0.967
	Between Intensities	0.66	5	0.132	4.26**
	Genotype-Intensity-Interaction	0.01	5	0.002	0.065
	Error	1.22	40	0.031	
II	Between Genotypes	1.01	1	1.01	19.06*
	Between Intensities	4.63	5	0.926	17.47**
	Genotype-intensity-Interaction	0.07	5	0.014	0.264
	Error	2.11	40	0.053	
III	Between Genotypes	0.36	1	0.36	3.46
	Between Intensities	5.56	5	1.112	10.69**
	Genotype-intensity-Interaction	0.02	5	0.004	0.04
	Error	4.14	40	0.104	
IV	Between Genotypes	1.22	1	1.22	6.52**
	Between Intensities	5.19	5	1.04	5.56**
	Genotype-intensity Interaction	0.89	5	0.18	0.96
	Error	7.49	40	0.19	

* Indicates significance at P = .05 with $F_{1,40} = 4.08$
** Indicates significance at P = .05 with $F_{5,40} = 2.45$

Appendix I. Duncan's test to determine differences between latencies caused by various intensity levels for waves I-IV for right and left ears. Significant studentized ranges are (2) 2.86, (3) 3.01, (4) 3.10, (5) 3.17, (6) 3.22 Means underscored by a line are considered equal.

Duncan's test for latency of wave I (R/E).

Rp at P = .05 with 40 degree of freedom (No. of means given).
Rp (2) 0.22 (3) 0.23, (4) 0.238, (5) 0.24, (6) 0.247

	A=75	B=65	C=55	D=45	E=35	F=25
*Means (in ms) pooled for Agouti & Cream	1.51	1.61	1.76	1.86	1.89	1.95

* No genotype-intensity interaction.

Duncan's test for latency of wave II (R/E)

Rp at P = .05 with 40 degrees of freedom (No. of means given).
Rp (2) 0.36, (3) 0.38, (4) 0.39, (5) 0.40, (6) 0.41

	A=75	B=65	C=55	D=45	E=35	F=25
* Means (in ms) pooled for Agouti & Cream	2.3	2.51	2.58	2.74	2.85	3.00

* No genotype-intensity interaction

Duncan's test for latency of wave III (R/E)

Rp at P = .05 with 40 degrees of freedom (No. of means given).
RP (2) 0.58, (3) 0.61, (4) 0.63, (5) 0.64, (6) 0.66.

	A=75	B=65	C=55	D=45	E=35	F=25
* Means (in ms) pooled for Agouti & Cream	3.08	3.22	3.43	3.80	3.88	4.05

* No genotype-intensity interaction

Duncan's test for latency of wave IV (R/E)

Rp at P = .05 with 40 degrees of freedom (No. of means given).
Rp (2) 0.56, (3) 0.57, (4) 0.59, (5) 0.60, (6) 0.61.

	A=75	B=65	C=55	D=45	E=35	F=25
* Means (in ms) pooled for Agouti & Cream	4.15	4.51	4.67	4.89	5.08	5.25

* No genotype-intensity interaction

Duncan's test for latency of wave I (L/E)

Rp at P = .05 with 40 degrees of freedom (No. of means given).
Rp (2) 0.48, (3) 0.52, (4) 0.54, (5) 0.55, (6) 0.58

	A=75	B=65	C=55	D=45	E=35	F=25
* Means (in ms) pooled for Agouti & Cream	1.52	1.73	1.84	1.97	2.00	2.20

* No genotype-intensity interaction

Duncan's test for latency of eave II (L/E)

Rp at P = .05 with 40 degrees of freedom (No of means given).
Rp (2) 0.26, (3) 0.27, (4) 0.28, (5) 0.285, (6) 0.29

	A=75	B=65	C=55	D=45	E=35	F=25
* Means (in ms) pooled for Agouti & Cream	2.29	2.54	2.70	2.84	2.89	3.07

* No genotype-intensity interaction

Duncan's test for latency of wave III (L/E).

Rp at P = .05 with 40 degrees of freedom (No. of means given).
RP (2) 0.37, (3) 0.39, (4) 0.40, (5) 0.41, (6) 0.42

	A=75	B=65	C=55	D=45	E=35	F=25
* Means (in ms) pooled for Agouti & Cream	3.11	3.31	3.60	3.70	3.73	3.74

* No genotype-intensity interaction

Duncan's test for latency of wave IV (L/E)

Rp at P = .05 with 40 degrees of freedom (No. of means given).
Rp (2) 0.54, (3) 0.57, (4) 0.58, (5) 0.60, (6) 0.61.

	A=75	B=65	C=55	D=45	E=35	F=25
* Means (in ms) pooled for Agouti & Cream	4.29	4.49	4.68	4.94	5.06	5.20

* No genotype-intensity interaction

Appendix J. Mean inter-aural latency differencies (ILDs) for ABR waves I-IV all normal genotypes (Agouti and Cream).

Table 81. Mean ILDs for all genotypes for waves I-IV for Agouti and Cream

Genotype	I	II	III	IV
Agouti	0.06	0.01	0.07	0.08
Cream	0.04	0.01	0.17	0.21

Appendix K. Summary of two-way ANOVA for differences between ears for waves I-IV across the various intensity levels Agouti and Cream genotypes.

Table 82. Summary of Ear x intensity x latency x ANOVA for BSER waves I-IV of Agouti

Wave		SS	DF	MS	F
I	Between Ears	0.13	1	0.13	3.33
	Between Intensities	2.90	5	0.58	14.87**
	Ear-intensity-Interaction	0.21	5	0.042	1.08
	Error	1.57	40	0.03	
II	Between Ears	0.00	1	0.00	0.00
	Between Intensities	5.68	5	1.14	5.59
	Ear-intensity-Interaction	1.66	5	0.33	1.62
	Error	8.16	40	0.204	
III	Between Ears	0.02	1	0.02	0.67
	Between Intensities	1.39	5	0.28	9.03**
	Ear-intensity-Interaction	0.08	5	0.16	0.52
	Error	1.22	40	0.03	
IV	Between Ears	0.47	1	0.47	0.61
	Between Intensities	2.61	5	0.52	0.675**
	Ear-intensity-Interaction	2.14	5	0.43	5.58**
	Error	3.06	40	0.077	

* Indicates significance at P = .05 with $F_{1, 40} = 4.08$

** Indicates significance at P = .05 with $F_{5, 40} = 2.45$

Table 83. Summary of Ear x intensity xx Latency x ANOVA of BSER waves I-IV of the Cream

Wave		SS	DF	MS	F
I	Between Ears	0.03	1	0.03	0.0.43
	Beween Intensities	2.28	5	0.46	6 6.57**
	Ear-intensity-interaction	0.07	5	0.014	0 0.20
	Error	2.94	40	0.07	
II	Between Ears	0.87	1	0.87	108.7*
	Between Intensities	0.01	5	0.002	0.25
	Error	0.04	5	0.008	
III	Between Ears	0.00	1	0.00	0.00
	Between Intensities	0.45	5	0.09	22.50**
	Error	0.02	5	0.004	
IV	Between Ears	3.62	1	3.62	40.22*
	Between Intensities	5.94	5	1.19	13.22**
	Ear-intensity-Interaction	5.45	5	1.09	15.33**
	Error	3.69	40	0.09	

* Indicates significance at P = .05 with $F_{1,40} = 4.08$
** Indicates significance at P = .05 with $F_{5,40} = 2.25$

Appendix L. Summary of two-way ANOVA for latencies of waves I-IV for genotype (BEW and WBA) and intensity for right and left ears.

Table 84. Summary of genotype (BEW and WBA) X intensity x ANOVA of BSER latency of waves I-IV for right ear

Wave		SS	DF	MS	F	P	S/NS
I	Between Genotypes (UC)+	0.11	1	0.11			
	Btween Intensities(UC)+	0.08	1	0.08			
	Between Genotypes (COR)*	0.09	1	0.09	2.28	0.16	NS
	Bwteen Intensities (COR)*	0.05	1	0.05	1.34	0.27	NS
	Interaction	0.00	1	0.00	0.00		
II	Between Genotypes (UC)*	10.00	1	8.69			
	Between Intensities (UC)*	3.00	1	3.12			
	Between Genotypes(COR)+	8.00	1	7.83	17.14	0.01	S
	Between Intensities(COR)+	1	1	1.27	2.77	0.11	NS
	Interaction			1.14	2.50	0.13	NS
III	Between Genotypes (UC)*	0.14	1	0.14			
	Between Intensities(UC)*	0.07	1	0.07			
	Between Genotypes(CORR)+	0.14	1	0.14	0.63		NS
	Between Intensities(COR)+	0.07	1	0.07	0.32		NS
	Interaction	0.00	1	0.00	0.00		NS
IV	Between Genotypes(UC)*	0.32	1	0.32			
	Between Intensities(UC)*	0.28	1	0.28			
	Between Genotypes (COR)+	0.38	1	0.38	2.67	0.13	NS
	Between Intensities(COR)+	0.34	1	0.34	2.37	0.15	NS
	Interaction	0.00	1	0.12	0.82		

\+ Uncorrected
* Corrected
S Indicates significance at P = .05 level
NS Indicates non-significance at P = .05 level

Table 85. Summary of genotype (BEW and WBA) x intensity x ANOVA of the BSER latency for waves I-IV of the left ear.

Wave		SS	DF	MS	F	P	S/NS
I	Between Genotypes (UC)*	1.00	1	1.00			
	Between Intensities(UC)*	0.48	1	0.48			
	Between Genotypes (COR)+	0.44	1	0.44	2.46	0.14	NS
	Between Intensities(COR)+	0.41	1	0.41	2.31	0.15	NS
	Interaction	0.11	1	0.11	0.64		NS
II	Between Genotypes (UC)*	0.22	1	0.22			
	Between Intensities(UC)*	0.10	1	0.10			
	Between Genotypes COR)+	0.25	1	0.25	2.06	0.18	NS
	Between Intensities(COR)+	0.13	1	0.13	1.09	0.32	NS
	Interaction	0.00	1	0.00	0.00		NS
III	Between Genotypes (UC)*	3.00	1	3.13			
	Between Intensities UC)*	0.33	1	0.33			
	Between Genotypes (COR)+	3.00	1	3.00	4.41	0.36	S
	Between Intensities(COR)+	0.21	1	0.21	0.37		NS
	Interaction	1.03	1	1.03	1.85	0.20	NS
IV	Between Genotypes (UC)*	0.27	1	0.27			
	Between Intensities(UC)*	0.26	1	0.26			
	Between Genotypes (COR)+	0.25	1	0.25	1.04	0.33	NS
	Between Genotypes (COR)+	0.24	1	0.24	1.02	0.34	NS
	Interaction	0.09	1	0.09	0.38		NS

* Uncorrected
\+ Corrected.
S Indicates significance at P = .05 level
NS Indicates non-significance at P = .05 level

Appendix M. Inter-aural latency difference for BEW and WBA for waves I-IV.

Table 86. Inter-aural latency difference for BEW and WBA.

Genotype	I	II	III	IV
BEW	-0.05	0.59	0.28	0.15
WBA	0.34	1.40	0.35	0.11

Appendix N. Summary of ANOVA for latency differences between ears for waves I-IV for BEW and WBA.

Table 87. Summary of Ear x intensity x Latency x ANOVA of BSER waves I-IV for the BEW.

Wave		SS	DF	MS	F
I	Between Ears	0.04	1	0.04	1.33
	Between Intensities	0.03	1	0.03	1.00
	Ear-intensity-Interaction	0.00	1	0.00	0.00
	Error	0.50	15	0.03	
II	Between Ears	1.00	1	1.00	2.33
	Between intensities	1.04	1	1.04	2.42
	Ear-intensity-Interaction	0.44	1	0.44	1.03
	Error	6.40	15	0.43	
III	Between ears	0.33	1	0.33	0.43
	Between intensities	0.16	1	0.15	0.21
	Ear-intensity-interaction	0.67	1	0.67	0.86
	Error	11.67	15	0.78	
IV	Between ears	0.05	1	0.05	0.08
	Between intensities	2.24	1	2.24	3.93
	Ear-intensity-Interaction	0.01	1	0.01	0.018
	Error	8.52	15	0.57	

* Indicates significance at P = .05 with F1,15 = 4.54

Table 88 Summary of Ear x intensity x latency x ANOVA of BSER waves I-IV of the WBA

Wave		SS	DF	MS	F
I	Between ears	0.00	1	0.00	0.00
	Between intensities	0.05	1	0.05	0.77
	Ear-intensity-Interaction	0.00	1	0.00	0.00
	Error	0.52	8	0.065	
II	Between ears (UC)+	0.10	1	0.10	
	Between intensities(UC)+	0.01	1	0.01	
	Between ears (CORR)++	0.10	1	0.10	3.52
	Between intensities(ORR)++	0.02	1	0.02	0.75
	Ear-intensity-Interaction	0.02	1	0.02	0.56
III	Between ears	0.39	1	0.39	2.05
	Between intensities	0.00	1	0.00	0.00
	Ear-intensity-Interaction	0.06	1	0.06	0.315
	Error	1.55	8	0.19	
IV	Between ears	0.04	1	0.04	0.093
	Between intensities	0.04	1	0.04	0.093
	Ear-intensity-Interaction	0.01	1	0.006	0.002
	Error	3.46	8	0.43	

\+ Uncorrected
++ Corrected
$F_{1,8} = 5.32$ at $P = .05$

Appendix O. Linear regression latency values for waves I-IV for both ears of all genotypes.

Table 89. Correlation latency values for waves I-IV of both ears of all genotypes.

Genotype	I	II	III	IV
AG	-0.95	-0.95	-0.99	-0.98
CR	-0.97	-0.99	-0.97	-0.98
BEW	-0.88	-0.96	0.42	-0.94
WBA	0.66	-0.95	-0.90	-0.94

Table 90. Intercept of latency values for waves I-IV for all genotypes.

Genotype	I	II	III	IV
AG	2.89	3.50	4.70	5.80
CR	1.20	3.36	4.50	5.90
BEW	4.6	5.18	3.00	5.30
WBA	1.41	6.00	5.10	7.26

Table 91. Slopes of latency values for waves I-IV for all genotypes

Genotype	I	II	III	IV
AG	-0.003	-0.002	-0.002	-0.002
CR	-0.002	-0.002	-0.002	-0.002
BEW	-0.006	0.004	0.001	-0.001
WBA	-0.002	-0.007	0.003	-0.003

Appendix P. Individual and mean thresholds for waves I-IV for all genotypes.

Table 92. Individual thresholds for wave I for all genotypes

Genotype	Ear	25	35	45	55	65	75
Agouti	R	x					
	L	x					
Cream	R	x					
	L	x					
BEW	R			x	x	xxx	
	L			x	x	xxx	
WBA	R				x	xx	
	L				xx	x	

Table 93. Individual thresholds for wave II for all genotypes

Genotype	Ear	25	35	45	55	65	75
Agouti	R	x					
	L	x					
Cream	R	x					
	L	x					
BEW	R			xx	x	xx	
	L			x	xx	xx	
WBA	R			x	xx		
	L			x	xx		

Table 94. Individual thresholds for wave III for all genotypes

Genotype	Ear	25	35	45	55	65	75
Agouti	R	x					
	L	x					
Cream	R	x					
	L	x					
BEW	R			xxx	xx		
	L			x	xxx	x	
WBA	R				xx	x	
	L			x	xx		

Table 95. Individual thresholds for wave IV for all genotypes

Genotype	Ear	25	35	45	55	65	75
Agouti	R	x					
	L	x					
Cream	R	x					
	L	x					
BEW	R			xx	xx	x	
	L			xx	x	xx	
WBA	R				xxx		
	L			x	xx		

Table 96. Mean thresholds for waves I-IV for all genotypes (R/E) in dB nHL

Genotype	I	II	III	IV
Agouti	25	25	25	25
Cream	25	25	25	25
BEW	60	55	49	53
WBA	62	65	59	55

Table 97. Mean thresholds for waves I-IV for all genotypes (L/E) in dB nHL

Genotype	I	II	III	IV
Agouti	25	25	25	25
Cream	25	25	25	25
BEW	63	57	55	55
WBA	59	56	52	55

Appendix Q. Differences between Wh-locus and E-locus with regard to wave latency as given by Chi-square.

Table 98. Differences between Wh-locus and E-locus with regard to latency as given by Chi-square.

Right Ear Parameter	X2	P	Left Ear Parameter	X2	P
Wave I	0.208	0.65	Wave I	0.61	0.44
Wave II	0.53	0.45	Wave II	0.26	0.61
Wave III	0.624	0.80	Wave III	9.11	0.76
Wave IV	0.334	0.56	Wave IV	7.08	0.79

Appendix R . Mean amplitude values for waves I-IV for all genotypes.

Table 99. Mean amplitude (NV) for wave I from 25-75 dB nHL for all animals (R/E)

Genotype	25	35	45	55	65	75	(N)
Agouti	29	42	83	155	192	380	5
Cream	21	42	89	93	328	390	5
BEW	*	*	100+	170+	319	460	5
WBA	*	*	*	360+	257+	330	3
TOTAL	50	84	272	778	1096	1560	18
MEAN OF MEANS	25	42	91	195	274	390	
# OF ANIMALS	10	10	11	13	18	18	

Table 100. Mean amplitude (NV) for wave II from 25-75 dB nHL for all animals (R/E)

Genotype	25	35	45	55	65	75	(N)
Agouti	36	35	43	108	118	147	5
Cream	18	34	62	77	165	219	5
BEW	*	*	50+	95+	133	161	5
WBA	*	*	*	*	137	265	3
TOTAL	54	69	155	280	553	792	18
MEAN OF MEANS	27	35	52	93.3	138	198	
# OF ANIMALS	10	10	11	13	17	17	

* Indicates no response from any animal
+ Indicates response from reduced number of animals

Table 101. Mean amplitude (NV) for wave III from 25-75 dB nHL for all animals (R/E)

Genotype	25	35	45	5	65	75	(N)
Agouti	47	86	199	424	679	1048	5
Cream	31	66	253	474	941	1535	5
BEW	*	*	228+	655	1351	1599	5
WBA	*	*	*	1253	1252	1650	3
TOTAL	78	152	680	2906	4223	5832	18
MEAN OF MEANS	39	76	227	727	1056	1458	
# OF ANIMALS	10	10	12	17	18	18	

Table 102. Mean amplitude (NV) for wave IV from 25-75 dB nHL for all animals (R/E)

Genotype	25	35	45	55	65	75	(N)
Agouti	28	45	45	87	112	180	5
Cream	26	71	71	108	255	312	5
BEW	*	*	125+	164+	371	250	5
WBA	*	*	*	*	298	490	3
TOTAL	54	116	241	359	1036	1232	18
MEAN OF MEANS	27	58	80	120	259	308	
# OF ANIMALS	10	10	12	14	18	18	

* Indicates no reponse from animals
+ Indicates response from reduced number of animals

Table 103. Mean amplitude (NV) for wave I from 25-75 dB nHL for all animals (L/E)

Genotype	25	35	45	55	65	75	(N)
Agouti	27	47	97	195	241	108	5
Cream	40	40	55	117	123	302	5
BEW	*	*	80+	750+	371	222	5
WBA	*	*	*	273+	258	150	3
TOTAL	67	87	232	1335	993	1531	18
MEAN OF MEANS	34	44	77	334	248	383	
# OF ANIMALS	10	10	11	13	18	18	

Table 104. Mean amplitude (NV) for wave II from 25-75 dB nHL for all animals (L/E)

Genotype	25	35	45	55	65	75	(N)
Agouti	13	24	41	105	124	108	5
Cream	26	45	191	98	147	302	5
BEW	*	*	400+	94+	759	222	5
WBA	*	*	50+	110	123	150	3
TOTAL	39	6	682	407	1153	890	18
MEAN OF MEANS	19	3	171	102	288	223	
# OF ANIMALS	10	1	12	14	18	18	

* Indicates no response from animals
+ Indicates reesponse from reduced number of animals

Table 105. Mean amplitude for wave III from 25-75 dB nHL for all animals (L/E)

Genotype	25	35	45	55	65	75	(N)
Agouti	25	30	145	249	378	525	5
Cream	20	43	90	100	197	387	5
BEW	*	*	400+	468+	759	1011	5
WBA	*	*	75	75	135	305	3
TOTAL	45	73	710	892	1469	2228	18
MEAN OF MEANS	23	37	178	223	367	557	
# OF ANIMALS	10	10	12	14	18	18	

Table 106. Mean amplitude (NV) for wave IV from 25-75 dB nHL for all animals (L/E)

Genotype	25	35	45	55	65	75	(N)
Agouti	20	30	145	249	378	525	5
Cream	25	43	90	100	194	387	5
BEW	*	*	95+	197+	339	276	5
WBA	*	*	75+	75+	134	305	3
TOTAL	45	73	405	621	1045	1493	18
MEAN OF MEANS	23	37	101	155	261	373	
# OF ANIMALS	10	10	13	14	18	18	

* Indicates no response
+ Indicates response from less animals

Appendix S1. Differences between Agouti and Cream with regard to wave amplitude as given by F-values.

Table 107. Difference between Agouti and Cream with regard to wave amplitude as given by F-values

Parameter	Difference	I	II	III	IV
Right Ear Amplitude	Genotype	0.12	0.14	4.30*	4.13*
	Intensity	8.32**	6.37**	3.70*	9.95**
	Interaction	0.45*	0.453	0.96	1.08

Parameter	Difference	I	II	III	IV
Left Ear Amplitude	Genotype	33.28*	9.49*	50.95*	4.01
	Intensity	50.07**	6.48**	2.45**	9.95**
	Interaction	12.99**	2.63**	1.24	1.06

* Indicates significance at P = .05 with $F_{1,40} = 4.08$
** Indicates significance at P = .05 with $F_{5,40} = 2.45$

Appendix S2. Summary of two-way ANOVA for amplitudes of waves I-IV for genotype (Agouti and Cream) and intensity for both ears.

Table 108. Summary of genotype (Agouti and Cream) x intensity x ANOVA for ABR amplitudes for right ear.

Wave	Source	SS	DF	MS	F
I	Between genotypes	2870.42	1	2870.41	0.12
	Between intensities	991768.75	5	198353.75	8.32**
	Genotype-intensity-interaction	53442.08	5	10688.42	0.45
	Error	953150.00	40	238288.8	
II	Between genotypes	836.27	1	836.27	0.14
	Between intensities	188181.33	5	37636.27	6.37**
	Genotype-intensity-interaction	13362.33	5	2672.47	0.453
	Error	236204.80	40	5905.12	
III	Between genotypes	438250.42	1	438250.42	4.30*
	Between intensities	1883222.08	5	376644.42	3.70**
	Genotype-intensity interaction	487782.08	5	97556.42	0.96
	Error	4075300.00	40	101882.50	
IV	Between genotypes	34843.75	1	34843.75	4.13*
	Between intensities	420228.75	5	84045.75	9.95**
	Genotype-intensity-interaction	44818.75	5	8963.75	1.08
	Error	337550.00	40	8438.75	

* Indicates significance at P = .05 with $F_{1,40} = 4.08$
** Indicates significance at P = .05 with $F_{5,40} = 2.45$

Table 109. Summary of genotype (Agouti and Cream) x intensity x ANOVA of ABR amplitudes for waves I-IV of the left ear.

Wave	Source	SS	DF	MS	F
I	Between genotypes	47943.52	1	47943.52	7.328*
	Between intensities	165796.86	5	33159.37	5.070**
	Genotype-intensity-interaction	42508.75	5	8501.75	1.29**
	Error	261691.60	40	6542.4	
II	Between genotypes	67000.42	1	67000.42	9.49*
	Between intensities	226199.88	5	45239.98	6.48**
	Genotype-intensity-interaction	92973.88	5	18594.77	2.63**
	Error	282388.40	40	7054.71	
III	Between genotypes	3255080.44	1	3255080.44	50.95*
	Between intensities	781894.56	5	156378.91	2.45**
	Genotype-intensity-interaction	395409.73	5	79081.95	1.24
	Error	2555144.40	40	63878.6	
IV	Between genotypes	33843.75	1	33843.75	4.01
	Between intensities	420228.75	5	84045.75	9.95
	Genotype-intensity-interaction	44818.75	5	8963.75	1.06
	Error	337550.00	40	8438.75	

* Indicates significance at P = .05 with $F_{1,40} = 4.08$
** Indicates significance at P = .05 with $F_{5,40} = 2.45$

Appendix T. Duncan's test to determine the differencies in latencies caused by varying intensity levels for waves I-IV. Significant Studentized ranges are (2) 2.86, (3) 3.01, (4) 3.10, (5) 3.17, (6) 3.22. Means underscored by a line are considered equal.

Duncan's test for amplitude of wave I (R/E)

Rp at P = .05 40 with degrees of freedom (No. of means given).
Rp: (2) 197, (3) 208, (4) 214, (5) 218, (6) 222

	A=25	B=35	C=45	D=55	E=65	F=75
* Means (in ms) pooled for Agouti & Cream	25	42	86	124	260	385

* No genotype-intensity interaction

Duncan's test for amplitude of wave II (R/E)

Rp at P = .05 with 40 degrees of freedom (No. of means given)
RP: (2) 98, (3) 102, (4) 105, (5) 108, (6) 109.

	A=25	B=35	C=45	D=55	E=65	F=75
* Means (in ms) pooled for Agouti & Cream	27	35	53	93	142	183

* No genotype-intensity interaction

Duncan's test for amplitude for wave III (R/E).

RP at P = .05 with 40 degrees of freedom (No. of means given).
Rp: (2) 408, (3) 429, (4) 442, (5) 452, (6) 459.

	A=25	B=35	C=45	D=55	E=65	F=75
* Means (in ms) pooled for Agouti & Cream	39	76	226	499	810	1292

* No genotype-intensity interaction

Duncan's test for amplitude for wave IV (R/E).

Rp at P = .05 with 40 degrees of freedom (No. of means given).
RP: (2) 117.5, (3) 123.4, (4) 127, (5) 129.9, (6) 132

	A=25	B=35	C=45	D=55	E=65	F=75
* Means (in ms) pooled for Agouti & Cream	27	58	58	98	184	246

* No genotype-intensity interaction

Duncan's test for amplitude of wave I (L/E).

Rp at P = .05 with 40 degrees of freedom (No. of means given).
Rp: (2) 102, (3) 108, (4) 112, (5) 114, (6) 116.

	A=25	B=35	C=45	D=55	E=65	F=75
* Means (in ms) pooled for Agouti & Cream	34	44	76	156	182	285

* No genotype-intensity interaction

Duncan's test for amplitude of wave II (L/E)

Rp at P = .05 with 40 degrees of freedom (No. of means given).
Rp: (2) 107, (3) 112.8, (4) 116, (5) 118.8, (6) 120.

	A=25	B=35	C=55	D=45	E=65	F=75
* Means (in ms) pooled for Agouti & Cream	19	35	102	116	136	205

* No genotype-intensity interaction

Duncan's test for amplitude of wave III (L/E).

Rp at P = .05 with 40 degrees of freedom (No. of means given).
Rp: (2) 323, (3) 340, (4) 350, (5) 358, (6) 363.8.

	A=35	B=25	C=45	D=55	E=65	F=75
* Means (in ms) Pooled for Agouti & Cream	37	39	118	175	288	456

* No genotype-intensity interaction

Duncan's test for amplitude of wave IV (L/E).

Rp at P = .05 with 40 degrees of freedom (No. of means given)
Rp: (2) 117, (3) 123.6, (4) 127, (5) 129.9, (6) 132.

	A=25	B=35	C=45	D=55	E=65	F=75
* Means (in ms) pooled for Agouti & Cream	23	37	118	175	286	456

* No genotype-ntensity interaction

Appendix U. Summary of two-way ANOVA showing differences between ears for in waves I-IV amplitude across various intensity levels for the Cream and Agouti.

Table 110. Summary of Ear x intensity x amplitude x ANOVA of BSER waves I-IV for the Agouti.

Wave	Source	SS	DF	MS	F
I	Between ears	4083	1	4083	0.17
	Between intensities	418913	5	83782	3.43**
	Ear-intensity-interaction	26033	5	5206	0.22
	Error	977650	40	24441	
II	Between ears	400	1	400	0.64
	Between intensities	45493	5	9098	1.45
	Ear-intensity-interaction	13442	5	2688	0.43
	Error	250381	40	6260	
III	Between ears	117483	1	117483	0.577
	Between intensities	7163757	5	1432751	7.05**
	Ear-intensity-interaction	845183	5	169036	0.83
	Error	8133790	40	203344	
IV	Between ears	129828	1	129828	2.70
	Between intensities	583984	5	116796	2.43
	Ear-intensity-interaction	282366	5	56473	1.18
	Error	1919262	40	4798	

* Indicates significance at P = .05 with $F_{1,40} = 4.08$
** Indicates significance at P = .05 with $F_{5,40} = 2.45$

Table 111. Summary of Ear x intensity x amplitude x ANOVA of BSERS waves I-IV of the Cream.

Wave	Source	SS	DF	MS	F
I	Between ears	226	1	226	0.873
	Between intensities	19569	5	3913.80	15.13**
	Ear-intensity-interaction	8631	5	1726.20	6.67**
	Error	10346	40	258.70	
II	Between ears	56733	1	56733	6.20*
	Between intensities	201558	5	40311	4.40**
	Ear-intensity-interaction	131448	5	26289	2.87**
	Error	365890	40	9147.30	
III	Between ears	4155	1	4155	2.99
	Between intensities	94491	5	18898	13.61**
	Ear-intensity-interaction	52142	5	10428.40	7.51**
	Error	5529	40	1388.20	
IV	Between ears	4896	1	4896.07	0.52
	Between intensities	500682	5	100136.47	10.53**
	Ear-intensity-interaction	29856	5	5971.27	0.63
	Error	380103	40	95026.60	

* Indicates significance at P = .05 with F1,40 = 4.08
** Indicates significance at P = .05 with F5,40 = 2.45

Appendix V. Summary of two-way ANOVA for genotype (BEW and WBA) and intensity for waves I-IV of both ears.

Table 112. Summary of genotype (BEW and WBA) x intensity x ANOVA of ABR amplitudes of waves I-IV of right ear.

Wave	Source	SS	DF	MS	F	P	S/NS
I	Between genotypes	49307	1	49306.64	1.73	0.21	NS
	Between intensities	60025	1	60024.99	2.11	0.17	NS
	Genotype-intensity-interaction	2282	1	2281.67	0.08		NS
II	Between genotypes	28995	1	28994.81	1.52	0.23	NS
	Between intensities	300	1	300.17	0.02		NS
	Genotype-intensity-interaction	2400	1	2400.19	0.13		NS
III	Between genotypes	261255	1	261255	0.47		NS
	Between intensities	44018	1	44017	0.08		NS
	Genotype-intensity-interaction	445887	1	448557	0.80		NS
IV	Between genotypes	3291	1	3291.45	0.13		NS
	Between intensities	41529	1	1528.95	1.60	0.22	NS
	Genotype-intensity-interaction	148269	1	148268.00	5.70	0.031	S

S Indicates significance at P = .05 level
NS Indicates non-significance at P = .05 level

Table 113. Summary of genotype (BEW and WBA) x intensity x ANOVA of BSER for waves I-IV fot the left ear.

Wave	Source	SS	DF	MS	F	P	S/NS
I	Between genotypes	141172	1	141171	0.75		NS
	Between intensities	201600	1	201599	1.08	0.32	NS
	Genotype-intensity-interaction	51450	1	51450	0.27		NS
II	Between genotypes	406473	1	406472	4.01	0.07	NS
	Between intensities	312631	1	312630	3.09	0.10	NS
	Genotype-intensity-interaction	274694	1	274691	2.71	0.13	NS
III	Between genotypes	439040	1	439039	1.84	0.20	NS
	Between intensities	92829	1	92828	0.39		NS
	Genotype-intensity-interaction	63431	1	63431	0.27		NS
IV	Between genotypes	43376	1	43375	0.47		NS
	Between intensities	97689	1	97689	1.06	0.32	NS
	Genotype-intensity-interaction	150134	1	150134	1.62	0.22	NS

S = Indicates significance at P = .05 level
NS = Indicates non-significance at P = .05 level

Appendix W. Summary of ANOVA for differences between ears for waves I-IV of the BEW and the WBA.

Table 114. Summary of Ear x intensity x amplitude x ANOVA of BSER for waves I-IV of the BEW.

Wave	Source	SS	DF	MS	F
I	Between ears	7801	1	7801	0.05
	Between intensities	261061	1	261061	1.81
	Ear-intensity-interaction	38281	1	38281	0.27
	Error	2164070	15	144271	
II	Between ears	433651	1	433651	3.70
	Between intensities	197011	1	197011	1.70
	Ear-intensity-interaction	272611	1	272611	2.35
	Error	1737290	15	115819	
III	Between ears	8611	1	8611	0.92
	Between intensities	2761	1	2761	0.029
	Ear-intensity-interaction	22781	1	22781	0.242
	Error	1410120	15	94008	
IV	Between ears	117413	1	117413	2.31
	Between intensities	36159	1	36159	0.71
	Error	50836	1	50836	

Indicates non-significance at P = .05 level with $F_{1,15} = 4.54$

Table 115. Summary of Ear x intensity x amplitude x ANOVA of BSER of waves I-IV of WBA

Wave	Source	SS	DF	MS	F
I	Between ears	22968	1	22698	0.66
	Between intensities	52	1	52.08	0.008
	Ear-intensity-interaction	37968	1	37968	0.61
	Error	494883	8	61860	
II	Between ears	9627*	1	9626	0.35
	Between intensities	10890*	1	10890	0.39
	Ear-intensity-interaction	5802	1	5801	0.21
III	Between ears	374533	1	374533	2.07
	Between intensities	373536	1	373536	2.06
	Ear-intensity-interaction	8533	1	8533	0.05
	Error	1450600	8	181325	
IV	Between ears	91002	1	91002	2.62
	Between intensities	77602	1	77602	2.24
	Ear-intensity-interaction	2852	1	2852	0.082
	Error	277616	8	34702	

Indicates non-significance at P = .05 level with $F_{1,8} = 5.32$

* corrected values since responses are from only two animals.

Appendix X. Linear regression amplitude values for waves I, II, III and IV.

Table 116. Correlation values for amplitude for all waves (R/E).

Genotype	I	II	III	IV
Agouti	0.99	0.94	0.98	0.81
Cream	0.98	0.99	0.98	0.97
BEW	0.99	0.96	0.96	0.54
WBA	-0.24	0.97	0.86	0.86

Table 117. Intercepts for waves I-IV for all genotypes (R/E).

Genotype	I	II	III	IV
Agouti	7.8	13	12	20
Cream	5.1	5.6	4.8	10
BEW	9.8	10	15	97
WBA	413	12	561	12

Table 118 Slope for waves I-IV for all genotypes (R/E)

Genotype	I	II	III	IV
Agouti	0.02	0.05	0.02	0.009
Cream	0.02	0.02	0.035	0.02
BEW	0.02	0.02	0.03	0.006
WBA	0.002	0.02	0.02	0.02

Table 119. Correlation for waves I-IV for all genotypes (L/E).

Genotype	I	II	III	IV
Agouti	0.98	o.94	0.96	0.96
Cream	0.95	0.96	0.98	0.98
BEW	0.67	0.046	0.92	0.89
WBA	0.91	0.91	0.97	0.93

Table 120. Intercept for waves I-IV for all genotypes (L/E).

Genotype	I	II	III	IV
Agouti	9.2	5	7	4
Cream	12	13	8	7
BEW	15	232	71	18
WBA	6	13	9	7

Table 121. Slope for waves I-IV for all genotypes (L/E).

Genotype	I	II	III	IV
Agouti	0.02	0.02	0.03	0.03
Cream	0.02	0.015	0.03	0.02
BEW	0.02	0.001	0.02	0.02
WBA	0.006	0.001	0.03	0.02

Appendix Y. Relative amplitude ratio for waves III/I for all genotypes

Table 122. III/I amplitude ratio for all genotypes (R/E)

III/I	25	35	45	55	65	75
Agouti	1.62	2.00	2.39	2.73	3.50	2.75
Cream	1.48	1.57	2.84	6.20	2.87	3.90
BEW			2.88	3.85	4.21	3.47
WBA				3.40	4.90	5.00

Table 123. III/I amplitude ratio for all genotypes (L/E)

III/I	25	35	45	55	65	75
Agouti	1.03	1.66	3.16	3.59	5.30	4.20
Cream	1.03	1.68	3.47	3.03	5.12	3.02
BEW			5.00	0.49	2.05	1.72
WBA				1.77	4.11	3.90

Appendix Z. Difference between Wh-locus and E-locus with respect to wave amplitude as revealed by Chi-square

Table 124. Difference between Wh-locus and E-locus with respect to wave amplitude as revealed by Chi-square.

Right Ear			Left Ear		
Parameter	X2	P	Parameter	X2	P
Wave I	2.57	0.11	Wave I	14.86	0.001*
Wave II	37.57	0.00	Wave II	16.62	0.00*
Wave III	29.76	0.52	Wave III	1.44	0.23
Wave IV	103.37	0.00	Wave IV	3.49	0.52

* Significant.