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OF THE HUMAN BAEP

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Misha J. Davis-Gunter

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# CHARACTERIZATION OF THE I' POTENTIAL OF THE HUMAN BAEP

By

Misha J. Davis-Gunter

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ABSTRACT

CHARACTERIZATION OF THE I' POTENTIAL OF THE HUMAN BAEP

By

Misha J. Davis-Gunter, B.S.

When stimulated, the hair cells of the organ of Corti depolarize, causing the release of neurotransmitters which excite afferent VIIIth nerve dendrites. Excitatory postsynaptic potentials (EPSPs) are generated and, if adequate summation occurs, initiate compound action potentials (CAPs). The EPSP is thought to be the generator potential (GP) for the CAP. Few researchers have studied the GP as the generator of I' of human brainstem auditory evoked potentials (BAEPs). Determining the anatomical origin of I' would enhance the sensitivity of the BAEP in hair cell/dendritic auditory nerve fiber evaluation.

Research on I' as cochlear or neural in origin is mixed. To explore this dilemma, BAEPs were recorded from human subjects using standard and paired-click stimuli. Derived responses revealed forward-masking effects in the form of increased latency and amplitude. Two waveforms, $I^0$ and I', occurred before wave I. $I^0$ and I' are thought to represent the SP and the GP, respectively.

To my parents, Clarence and AuraLee,
my husband, Dawan, and son, Taylor.

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# CHAPTER I

# INTRODUCTION

Brainstem auditory evoked potentials (BAEPs) are now included as a standard protocol for site-of-lesion evaluation in challenging audiometric situations. The non-behavioral nature of this test is the one factor that allows substantial success as a diagnostic tool. The BAEP in particular has proven to be significantly successful for the localization of lower brainstem lesions, the diagnosis of multiple sclerosis, the evaluation of the difficult-to-test patient such as newborns who are targeted as high-risk, determination of neurological status of the comatose patient, as well as effective use in intraoperative monitoring (Hashimoto, Ishiyama, Yoshimoto & Nemoto, 1981).

Individual BAEP components have been investigated extensively in both animals and humans. The nature of this research includes macro- and micro-electrode recordings and conducting recordings prior to and after creating lesions along the auditory brainstem pathways. The results of these studies include the identification of five to seven major components which have been described in terms of their

intercranial anatomical generators (Jewett, 1970; Lev & Sohmer, 1972; Buchwald & Huang, 1975). These studies have enabled investigators to determine the site of lesion with more precision.

Unfortunately, a significant amount of generator site overlap was found within each of the components which in turn jeopardized the value of the BAEP as a diagnostic test for site of lesion (Jewett, 1970; Huang & Buchwald, 1977; Achor & Starr, 1980a). Despite the handicap caused by this overlap, the auditory brainstem response is still quite powerful and is still successfully utilized as a diagnostic tool. To date, researchers continue to conduct studies relative to exact intercranial generator identification which will obviously lead to the enhancement of the auditory brainstem response as a diagnostic tool (Hughes & Fino, 1985).

A number of investigations have been conducted on the BAEP, but few studies have been conducted for the purpose of investigating the auditory EPSP (Furukawa & Ishii, 1967; Flock & Russell, 1973, 1976; Furukawa & Matsuura, 1978; Furukawa, Hayashida & Matsuura, 1978; Furukawa, Kuno & Matsuura, 1982; Siegel & Dallos, 1986; Palmer & Russell, 1986; Sewell, 1990). However, recent investigative methods have led to the recognition of a new potential recorded in

human BAEP. A wave which has been labeled as "I′", the "$I^0$" wave and "BI" (Moore, 1983; Moore & Semela, 1985; Moore, Semela, Rakerd, Robb & Ananthanarayan, 1992; Hughes & Fino, 1980; Hughes, Fino & Gagnon, 1981; Hughes & Fino, 1985; Benito, Falco & Lauro, 1984) has been found to precede wave I in the human BAEP. It seems as if the designator "I′" has become the most popular in the literature and will therefore be referred to as such throughout this paper.

Investigators have seen the emergence and easy identification of this potential through the use of piezoelectric earphones (Hughes & Fino, 1980) or shielded earphones (Moore et al., 1992). I′ has a documented latency of approximately one millisecond (Moore et al., 1992; Benito et al., 1984) and is reported to be quite easily recorded at high intensities. Although researchers have only recently begun experimentation into the precise characterization and optimal recording parameters of this potential, there is still debate relative to a specific anatomical generator site.

Is I′ a derivative of the hair cells of the cochlea, representing a receptor potential, or a product of the distal most portion of afferent VIIIth nerve dendritic extensions, representing excitatory postsynaptic potentials? Of the two receptor potentials, the cochlear microphonic

(CM) and the summating potential (SP), the CM has been ruled out due the failure of I′ to change polarity in response to clicks of opposite polarity (Hughes & Fino, 1980). However, the contributions of the summating potential itself cannot be ruled out at this time. Even as the SP is usually recorded as a negative potential, whereas I′ is recorded as a positive potential, representation from this receptor potential is still uncertain.

On the other hand, it has been hypothesized that I′ represents the summed excitatory post synaptic activity originating at the distal portion of afferent auditory nerve dendrites. This EPSP activity represents the initial generation of the compound action potential (CAP) and is known also as the generator potential (GP). In other words, the EPSP is thought to be the GP (Furukawa, 1986; Dallos & Cheatham, 1974). In order to obtain an enhanced comprehension of EPSP activity, it is necessary to elaborate on the process of synaptic transmission.

The fluid in the human body can be divided into two groups: intracellular and extracellular. The wall that separates the intracellular and extracellular fluid is the plasma membrane of the cell. Relative to this membrane, there are primarily three substances that are responsible for maintaining an outside positive charge and an inside

negative charge: sodium ($Na^+$), potassium ($K^+$) and Chloride ($Cl^-$). In essence, the plasma membrane acts as a selectively permeable barrier to the diffusion of these ions. Synaptic potentials are the result of brief alterations in the electrical properties of membrane potentials.

Action potentials (AP) are all or none events; this simply means that only if the membrane potential reaches a critical threshold value, then an AP will be generated. In order to achieve this state of activation, the membrane potential must be made less negative (depolarized) by reducing the charge separation across the membrane. This state is achieved with the influx of positively charged potassium ions which in turn causes an outward current of negatively charged chloride ions. Within a certain range of membrane potentials, only generator potentials are produced which initiate inward-outward current flow. As mentioned above, only when these generator potentials summate will an AP be generated.

A summation of the above process of synaptic transmission when applied to the hair cells of the cochlea and auditory nerve fibers of the VIIIth nerve is as follows: Vibrations of the basilar membrane are caused by mechano-electrical stimuli. This leads to the bending of the hair

cell stereocilia, an action which opens ionic channels initiating K+ current which finally causes the depolarization of the hair cell. The depolarization which takes place inside the hair cells may be recorded as electrical potentials, such as the CM and SP. The depolarization in the hair cells initiates the release of neurotransmitter substance(s) which excites the dendrites of type I afferent auditory nerve fibers,
leading to the generation of post synaptic potentials. If the post synaptic potential is excitatory (EPSP) and if it reaches the threshold of the membrane, it will then lead to the generation of AP's (Dallos, 1984).

In the review of the literature on auditory nerve potentials, it is interesting to note that there exists no thorough characterization of the EPSP generator potential. As noted above, the depolarization of the hair cells leads to the release of neurotransmitters. The excitation caused by these substance(s) leads to the generation of EPSP which, if of adequate magnitude, leads to the generation AP's. Evidently, the EPSP plays a crucial role in neuronal propagation but is continually overlooked in descriptions of auditory transmission. Adequate characterization of the EPSP will undoubtedly lead to a more sensitive index of the functioning of the hair cell/auditory nerve complex.

The purpose of this experimental investigation is to obtain information which differentiates between the receptor potential, SP, and the neural activity, EPSP, as the anatomical generator site of I′ in humans. In order to extract the GP from other cochlear potentials, specific stimulus parameter combinations were developed. The "paired-click" stimulus paradigm was used for this purpose. This stimulus combination is derived from what is known about the stimulus related properties of the receptor potential and action potential components, and the underlying electrophysiological processes generating these components and the GP.

Essentially, the specific stimulus paradigm involves BAEP recordings which were evoked with standard-click stimuli and BAEP recordings which were evoked with paired-click stimuli. An off-line subtraction of standard-click responses from paired-click responses revealed a "derived response." Ideally, this derived response may be suggestive of the anatomical site of generation of I′.

The goals of this study include further characterization of I′ in the following manner:

1. Characterization of the mean amplitude and the mean latency of wave I′ in standard BAEP recordings.

2. Development of a specific paired-click stimulus paradigm in which successful differentiation can be made between the cochlear potential, SP, and the neural component, EPSP, as a I′ generator.

3. Specificity of I′ morphological characteristics that result from the paired-click stimuli observed in derived BAEP responses.

4. Characterization of the derived paired-click BAEP response compared to the regular paired-click BAEP response.

# CHAPTER II

# REVIEW OF THE LITERATURE

## Introduction

Accurate site-of-lesion abilities of electrophysiological testing in humans has led to a recent increase of researcher attention. In relation to brainstem auditory evoked potentials (BAEPs) it is quite evident that in order to achieve improved diagnostic accuracy, the precise determination of anatomical generators is essential. The recent discovery of the potential I′ -- occurring before Jewett labeled Wave I of the BAEP -- has created the need for more research relative to the anatomical origin of this wave. As a generator candidate, the summed activity of excitatory post synaptic potentials (EPSPs) in afferent dendrites of the auditory nerve have been suggested. Unfortunately, there is a limited amount of data available relative to the generation site for this potential.

Researchers are engaged in a debate as to whether I′ is a representation of the cochlear summating potential (SP: A receptor potential which is generated by the hair cells

within the cochlea) or an excitatory post synaptic potential (EPSP-like component, a generator potential recorded as a gross potential using surface recordings) originating within the distal most portion of the VIIIth cranial nerve. Successful recordings of EPSP's have been accomplished in the guinea pig (Palmer & Russell, 1986; Siegel & Dallos, 1986) and in many other animals. Through pharmacological manipulation, the summed activity of a residual "EPSP-like" potential has been successfully separated from other gross cochlear potential components in the guinea pig (Xi, Dolan & Nuttall, 1989) and the cat (Moore, Caird, Klinke & Lowenheim, 1988). The purpose of this investigation is to determine whether presumed summed EPSP activity is reflected in the BAEP I' potential or another yet unknown potential.

In order to identify the generator potential (GP) originating within the dendrites of afferent spiral ganglion fibers in the human auditory brainstem response, stimulus parameter specification and manipulation have been suggested. Therefore, the "Paired-Click" stimulus paradigm was derived based on what is known about the stimulus response characteristics, the underlying electro-physiological processes generating the cochlear potentials (i.e., the cochlear microphonic, CM; summating potential, SP; and the compound action potential, CAP), and the GP.

The following sections will include a discussion of synaptic transmission characteristics, excitatory post synaptic potentials (EPSPs), the integration of signals, micro-electrode and surface electrode recordings of the EPSP, the "paired-click" stimulus paradigm, electro-physiological features the receptor potentials and VIIIth nerve fibers, and neurotransmitters of the VIIIth nerve. Furthermore, this review of the literature will include both published and unpublished data that are relevant to this experimental study and which may help substantiate the importance of this type of investigation.

## Synaptic Transmission

Within the auditory nervous system the process of synaptic transmission allows humans to transmit acoustic signals to the brain for interpretation and, eventually, the coordination of appropriate responses. It is important to note that this relay of information is not accomplished through neurons which are physically in direct contact with one another. This process is achieved through a chemical process which involves the release of neurotransmitters into the spaces (synaptic clefts) between neuronal structures. The type of presynaptic/postsynaptic coupling most commonly observed is axon to dendrite (axo-dendritic) but can also involve axon to soma (axon-somatic) or axon to axon (axo-axonic) junctures (Matthews, 1986).

The neurons which comprise the VIIIth nerve are called spiral ganglion cells and can be described as complex cells with long fibrous extensions for receiving and transmitting information. The soma, or cell body, constitutes a relatively small portion of the neuron while a tangle of profusely branched processes, called dendrites, extend out from the soma to receive signals from nearby excited neurons. The direction of activity propagation is dendrite to soma to the axons (also extending from the soma) which terminate with synaptic terminals or boutons. The boutons contain synaptic vesicles (membrane-bound structures) filled with the chemical neurotransmitters necessary to perpetuate communication with the next neuron.

The space between an axo-dendritic synaptic juncture is the synaptic cleft. This space is bordered by the presynaptic membrane of the axons of one neuron and the postsynaptic membrane of the dendrites of another neuron. It is now generally accepted that the release of the neurotransmitters is accomplished by the fusion of the vesicle with the membrane of the bouton at specific "release sites," so that the contents of the vesicle are released into the synaptic cleft. If the signal at the level of the synaptic terminal was of appropriate magnitude to cause an adequate amount of neurotransmitter release into the presynaptic cleft, then the dendritic communication (in this

instance) would result in the perpetuation of the signal.

As mentioned above, the signal perpetuated is an electrical one and can be observed by measuring the changes in electrical potential during neuronal activation. A small delay follows the stimulation of neuronal dendrites after which a sudden increase can be observed in membrane potential. The potential moves in a positive direction, meaning the cell is becoming less negative or more positive, a process known as depolarization. However, this change in membrane potential is transient and soon begins to return toward its resting value; in other words the cell becomes more negative or less positive, a process known as repolarization. The cell's membrane potential may extend beyond (or become more negative than) its resting state potential for a brief period of time before returning to its actual resting state potential, a process known as hyperpolarization. At this point, the cell experiences a brief state during which consecutive stimulation will not evoke another stimulatory reaction, a state known as the refractory period. The sudden change in membrane potential represents the signal which is transmitted through the nervous system and is called the action potential (AP). It is important to note that during chemical synaptic transmission the AP does not cross over into the synaptic cleft, yet it is the neurotransmitters which bind with the

receptors on the postsynaptic membrane which (if strong enough) causes the initiation of postsynaptic potentials (Matthews, 1986).

There are two types of postsynaptic potentials. If the neurotransmitter brings the membrane potential of the postsynaptic cell toward the threshold for firing an action potential and thus tends to excite the postsynaptic cell, then it is said to be an excitatory postsynaptic potential (EPSP). Inhibitory synapses are those cases in which the neurotransmitter acts to keep the membrane potential of the postsynaptic cell more negative than the threshold potential because it tends to prevent the postsynaptic neuron from firing an AP. In this instance the postsynaptic cell is "inhibited" by the release of inhibitory neurotransmitters and is referred to as an inhibitory postsynaptic potential (IPSP). Whether either of these two potentials is evoked depends upon transmitter type and postsynaptic binding site reactions which differ for each cell. The nature of this study involves, specifically, spiral ganglion cell responses evoked via auditory stimulation. Because of the fact that the electrophysiological make-up of these cell structures are mostly excitatory in response to acoustic stimulation of appropriate frequency and intensity, I will examine in detail only the excitatory postsynaptic potential (Pickles, 1988).

## Excitatory Postsynaptic Potentials (EPSP)

Extracellular as well as intracellular recordings, although commonly limited to animals, have become quite popular clinically as diagnostic tools. Specific potentials can be recorded extracellularly in humans; this has created the need for signal averaging techniques that reveal enhanced potentials via separation from any artifacts or unwanted noise. Those potentials are evoked via acoustic stimulation and retrieved remotely by precisely located electrodes on the human head. The results involve a complex representation of electrical activity initiated within the inner ear. This complex representation of potentials include the summating potential (SP), the cochlear microphonic (CM) and the compound action potential (CAP).

The CAP is simply the summation of successive single action potentials of spiral ganglion cell fibers. A single AP propagated through a spiral ganglion cell typically produces only a small depolarization of the postsynaptic cell. This type of response to a presynaptic AP is called an excitatory postsynaptic potential (EPSP). A single EPSP is usually much too small to reach the cellular threshold of the activated cell. However, if a second AP arrives at the presynaptic terminal before the postsynaptic effect of a first AP has disappeared, the second EPSP will sum with the first to produce a larger peak postsynaptic depolarization.

Thus, if a series of AP's arrive in the presynaptic terminal in rapid sequence, the individual EPSPs may add up to a depolarization value that will reach the cell's threshold. This type of summation of sequential postsynaptic effects of an individual presynaptic input is called temporal summation. The importance of this mechanism is evident as it allows even a weak excitatory synaptic input to cause a neuron to fire an action potential. An action potential may also be triggered through spatial summation, a process in which an action potential is triggered among spatially distinct inputs onto a single postsynaptic cell (Matthews, 1986).

## Integration of Signals

As mentioned above, the successful generation of an AP depends upon the membrane potential reaching its threshold value. Whereas EPSPs cause depolarization and movement toward the achievement of threshold, one EPSP is far from enough to reach this critical level. EPSPs can, therefore, summate both temporally and spatially, thus enabling threshold achievement and spike generation. In some cases, the cell bodies of neurons cannot trigger an AP; in most of these instances the threshold for spike generation is high. However, the threshold of the trigger zone which is located in the initial segment of the axon, in these same cells, is relatively low. This zone is known as the integrative

component of the neuron as it is this region which sums the excitatory and inhibitory inputs. The cell will discharge only if the excitation exceeds the inhibition at the trigger zone by the cell's critical threshold level.

The understanding of the EPSP is obviously crucial to the coding mechanism of the auditory nerve. Unfortunately, there is a limited amount of research relative to EPSP generation. A thorough review of relevant literature has not been able to distinguish adequately whether or not the EPSP is identifiable in evoked potential recordings. This may be due to the fact that optimal EPSP recording parameters have not yet been established. This fact alone might have led to the masking of the EPSP by components such as the SP, CM, the CAP, and/or noise.

Being able to substantiate the identification of the EPSP in evoked responses is crucial to this investigation. Therefore, I will review the published and unpublished literature which has been successful in their attempt, whether extracellular or intracellular, to record the EPSP (or EPSP-like potential).

## Micro-Electrode Recordings of the EPSP

Furukawa and colleagues (Furukawa and Ishii, 1967; Furukawa, 1978; Furukawa, Hayashida and Matsuura, 1978;

Furukawa and Matsuura, 1978; Furukawa, Kuno and Matsuura, 1982; Furukawa and Matsuura, 1985; Furukawa, 1986) conducted intracellular recordings from afferent eighth nerve fibers in the goldfish and documented potential variations which they called the generator potential (GP). EPSPs which were evoked successively in response to acoustic stimulation displayed synaptic depression or a marked decrease in size. However, the cochlear microphonic displayed no signs of decrease. The amplitude of successive EPSPs was decreased by a fixed ratio to the preceding one; the rundown was thought to be attributed to the depletion of transmitter quanta from the release sites. The above results suggest that the GP is the EPSP. However, even as complete adaptation had been achieved through continual tonal stimulation, a new discharge of EPSPs (of varied amplitude depending on increment size) was observed as intensity was increased. These findings suggested that the presynaptic sites in hair cells might be divided into many tiny sectors and that each sector has a different sensitivity for the release.

Dallos and Cheatham (1974) described generator potentials as a graded type of response, or one which grows in amplitude with increased intensity. The investigators also indicated that the GP is necessary to initiate the all-or-none APs within auditory nerve fibers. Finally, they

presumed that generator potentials arise from the unmyelinated portion of spiral ganglion neurons.

Flock and Russell (1973, 1976) recorded postsynaptic action by electrically stimulating the efferent nerves of the lateral line organ in the Burbot Lota Lota. Along with the IPSP, there was a decrease in the resistance of the hair cell membrane and an increase in the microphonic potential. The increase of the microphonic potential was greater at larger amplitudes of mechanical stimulation. Mechanically stimulating afferent nerve fibers revealed small spontaneous and evoked EPSPs. Furthermore, the EPSPs were reduced in amplitude for the duration of the IPSP.

Palmer and Russell (1986) investigated the high frequency limits of phase-locking in the inner hair cells of guinea pigs. Their investigation included spontaneous and acoustically evoked nerve impulses recorded extracellularly. These recordings revealed spontaneous and acoustically evoked fluctuations in membrane potentials. The investigators deemed these fluctuations to be similar in description to the EPSPs which have been documented within afferent nerve fibers in the goldfish (Furukawa & Ishii, 1967; Furukawa & Matsuura, 1978) and in the lateral line organs of the Burbot Lota Lota (Flock & Russell, 1976).

Siegel and Dallos (1986) conducted microelectrode recordings through the inner hair cell regions of the organ of Corti. Intracellular as well as extracellular recordings were elicited from afferent terminals of guinea pigs. Recordings revealed spontaneous activity which ranged from 1.3 to 136 spikes/s, with "highly irregular interspike intervals." Temporal characteristics of this activity, including phase-locking and onset/offset transients, strongly suggested the peripheral dendrites and/or terminals of type I spiral ganglion cells as the recording sites. A number of recordings revealed neuronal penetration. Some of these instances revealed EPSPs which failed to elicit an AP. Preliminary assessment of the slopes of the rising phase of the EPSPs which did not trigger discharge spikes were smaller than those which elicited discharge spikes.

Sewell (1990) studied the EPSP and AP in afferent fibers innervating hair cells of the lateral line organ in Xenopus laevis (African clawed toad). Through micropipette investigative methods, discharge rates were studied within hair cell clusters called neuromasts. Perfusion of the synapse with a solution containing cobalt -- and subsequent application of another solution containing manganese -- had the effect of diminishing the discharge rate by reducing the occurrence of the EPSPs for as long as the solution was present. These findings suggest that the diminishing effect

of cobalt, an agent that blocks voltage dependent transmitter release, affects the EPSP. This, in turn, indicates that the spontaneous discharge in these afferent fibers is caused by voltage-dependent release of neurotransmitters from the hair cells.

## Surface Electrode Recordings of the EPSP

Hughes and Fino (1980) and Hughes, Fino and Gagnon (1981) used human subjects to record auditory brainstem responses using piezoelectric earphones. The construction of these earphones allows the emission of a much smaller magnetic field which essentially eliminates the stimulus artifact. The elimination of the stimulation artifact revealed an early wavelet preceding wave I which they called I′. Characterization of this wave included a latency of approximately 1.1 msec with an amplitude of .008 μV. These investigators concluded that I′ was not a representation of the CM because its polarity was not changed in relation to opposite stimulus polarity. They interpreted their findings to suggest that I′ is related to the postsynaptic potential arising in the afferent terminals of the eighth nerve fibers in response to the depolarization initiated by chemical transmitters.

Benito, Falco and Lauro (1984) analyzed the auditory brainstem response and described a wave before wave I having

an approximate latency of 1 msec. They called this newly discovered potential wave "o" and deemed it easily observable at high intensities.

Hughes and Fino (1985) included data from an experimental study that is relevant to the identification of anatomical generators of the human BAEP. Amplitude and latency distributions were plotted throughout the scalp for each wave of the ABR as recorded in 20 nonpathalogical ears. The maps for the potential I′ were consistent with the hypothesis that the anatomical generator might be the distal most portion of the eighth nerve.

Moore and Semela (1985) recorded CAPs using surface electrodes in human subjects. The consistent identification of a positive peak was labeled as "BI." This peak was recorded with a latency of 0.6 to 1.2 ms as well as having an amplitude of 30 to 70 nV. Varying stimulus parameters revealed BI as a graded potential which was thought to be representative of the post-synaptic region of the cochlea.

Moore and colleagues (Moore, Caird, Klinke & Lowenheim, 1988; and Moore, Caird, Lowenheim & Klinke, 1989) studied the I′-like potential elicited through the round window of cats and gerbils. They labeled this I′-like potential "Po." Response characteristics included reduced latency and

increased amplitude with increasing stimulus intensity. The behavior of these potentials closely approximated the behavior of wave I and wave II of the BAEP. Furthermore, the intracochlear infusion of TTX revealed a reduction in amplitude of the N1/N2 complex of the CAP and ABR waves but not Po. However, when an amino acid antagonist kynurenic acid (KYNA) or L-glutamic acid diethyl ester (GDEE) was infused after the application of TTX, Po also decreased in amplitude as well. These results suggest two important concepts: The first is that Po is a post-synaptic potential (it is generated after neurotransmitter release) since it appears to be affected by a drug which blocks transmitter release. Secondly, even as it appears to be a neural potential, it could not be the AP because it is not affected by TTX which is known to block fast sodium channels.

Dolan, Xi, and Nuttall (1989) and Xi, Dolan, and Nuttall (1989) recorded whole-nerve APs (CAPs) from the round window of guinea pigs before and after the application of tetrodotoxin (TTX) using tone bursts. Their findings were similar to those of Moore and colleagues (1988, 1989). Results indicated that the application of TTX was an effective means of abolishing the cochlear AP complex (N1-P1-N2), an action which left a residual negative potential. Post application of TTX did not reveal any alterations in the cochlear receptor potentials (SP and CM). Furthermore,

the subsequent application of kainic acid (KA) eliminated the CAP as well as the residual negative potential, leaving the receptor potentials intact. The investigators postulated that this negative potential was a derivative of the unmyelinated portion of the dendrites of the VIIIth nerve which represents summed EPSP activity and the depolarization of the afferent dendrites.

Moore, Semela, Rakerd, Robb and Ananthanarayan (1992) documented their recordings of the wave I′ as they attempted to characterize further this newly discovered potential. They reported an onset latency of approximately 0.83 ms for clicks of alternating, condensation and rarefaction polarity. The latency of I′ was observed to decrease as intensity increased, and its function was said to have approximated that of waves I and II. These investigators agree with the conclusions of Hughes and Fino (1980) who suggested that I′ might be a representation of far-field summed electric currents which are derived from the dendritic structures of the auditory nerve in the form of EPSP. Furthermore, Moore et al. recorded I′ using a shielded dynamic earphone which contributes to successful recordings through the use of low-noise gold electrodes, electrode impedance values of <1.5 K Ohms, short electrode leads, low noise (<10 nV) preamplification, a dwell-time of 10 μs and total data points of 1,000, the high sampling rate

of 100 KHz and averaging 2,048 responses. Their findings suggest that I′ is a neural response; however, they do note the unlikely possibilities of it representing a vestibular response or the cochlear potential SP. Their work also rules out the possibility of the CM as the source of wave I′ because of the non-inverting polarity for rarefaction and condensation clicks and the failure of I′ to be eliminated when using clicks of alternating polarity.

Based on the findings discussed above, it was decided that in order to identify summed EPSP activity from afferent auditory nerve dendrites in the human BAEP, stimulus parameters should be utilized. In order to extract I′ from other potentials, as mentioned above, a specific stimulus combination was used. This combination is derived from what is known about the stimulus response characteristics of the cochlear receptor potentials (CM & SP) and the EPSP. Precise definition of the electrophysiologic processes responsible for their generation is necessary in order to achieve a successful stimulus paradigm. The "paired-click" stimulus paradigm in relation to these processes will be discussed in the following section.

## The "Paired-Click" Stimulus Paradigm

The CAP is a gross response which is recorded from the spiral ganglion neurons of the VIIIth nerve. This gross

response is generated by the summation of underlying response patterns of single-unit action potentials (Antoli-Candela & Kiang, 1978). Action potentials are "all-or-none" responses which include electrophysiologic characteristics revealing a refractoriness to preceding stimuli. Two refractory mechanism are observed: (1) The absolute refractory period and (2) The relative refractory period. Most importantly, the former of these refractory periods characteristic of auditory nerve AP's was cited as 1 ms by Eggermont (1985) or 1.2 ms as cited by Ozdamar & Dallos (1978). The absolute refractory period of AP makes it impossible for discharge activity to be "re-initiated" within this time frame. The latter refractory period is characterized by an exponentially decreasing threshold for the AP to a resting level with a time constant of 4-5 ms (Eggermont, 1985), making it very difficult (but not impossible) for successive discharges to be initiated.

EPSPs, however, are graded responses, which means they increase in amplitude as a function of intensity (Furukawa, 1986). The EPSP is not characterized by refractory mechanisms in response to preceding stimuli but is regulated by specific adaptation phenomena which develop in the transmitter release process (Furukawa et al., 1978) as well as other stages of synaptic transmission (Eggermont, 1985). Because the AP follows the GP, adaptation is reflected in

the later responses as well (Eggermont & Odenthal, 1974). The adaptation processes as defined by Eggermont (1985) are short-term adaptation, long-term adaptation, and auditory fatigue. Eggermont (1985) also states that these adaptation processes develop at a slower rate than the refractory mechanisms. Both of these characteristics should be thought of as individual processes, thus allowing the distinction of refractory-related alterations on the CAP from adaptation related regulations on both the EPSP and the CAP.

Based on these electrophysiologic features, a stimulus which is transient in nature which is presented within 1 ms of a preceding stimulus should fall within the range of the absolute refractory period. This should inhibit the generation of an AP. By making the first of the two stimuli transient as well, effects due to short-term adaptation seen in the response to the second stimulus will be minimized. Unfortunately, the reflection of adaptation in this response can not be ruled out in an absolute manner. Furthermore, the amount of transmitter quanta which remains at the presynaptic site -- after the first stimulus is emitted -- is directly related to the amplitude of the EPSP to the second stimulus. If the results of this stimulation pattern results in the complete depletion of transmitter quanta, the generation of an EPSP may not be possible. Still, it is hypothesized that the second of a paired-click stimulus --

which is initiated within the time range of the absolute refractory period (1 ms) of the first stimulus -- will give rise to EPSP activity in afferent spiral ganglion dendrites. Results derived from this hypothesis include evoked EPSP's observed without the presence of the CAP, thus allowing the summation of their activity to be recorded as the GP in the human BAEP. (Moore et al., 1989). The reader is referred to chapter III (the "Instrumentation and Procedures" section) for a more detailed discussion of the "paired-click" stimulus paradigm and to view exact composition of the stimuli, interstimulus interval measurements and appropriate derived response calculations.

## Electrophysiologic Characteristics

In order to obtain a functional understanding of auditory nerve electrophysiology and its relationship to the "paired-click" stimulus paradigm, basic anatomical findings will first be reviewed. The auditory nerve is comprised of a spiral of tonotopically organized spiral ganglion cells. There are 35,000 afferent fibers in man and 50,000 in the auditory nerve of the cat (Pickles, 1988). There are two types of spiral ganglion cells which are neurons whose axons form the auditory nerve. The first group of cells are known as Type I cells and innervate inner hair cells, whereas the second type, Type II cells, innervate outer hair cells. The

average inner hair cell is innervated by at least 20 Type I cells, a fact which signifies the existence of a certain amount of redundancy within these cells. Microelectrode recordings of Type II cells have revealed a relatively small amount of information about their electrophysiological characteristics due to the minute diameter of their axons. The coiled trunk of the auditory nerve is tonotopically organized with fibers innervating the basal or high frequency region of the cochlea being on the outside of the roll and fibers innervating apical or low-frequency cochlear regions on the inside (Javel, 1986).

Developing a stimulus paradigm which has the capability of defining I′ as a cochlear or neural response necessitates precise definition of the electrophysiologic characteristics associated with the EPSP and CAP. Spontaneous discharges, "primary-like" response patterns, refractory mechanisms, auditory adaptation processes, and forward masking are all areas in which researchers have been able to ascertain a considerable amount of information. For the purpose of an enhanced comprehension as well as validation of this study, all of these areas will be discussed in the following section.

Close examination of auditory nerve fibers reveals a non-uniform distribution of spontaneous discharges. This

spontaneous activity is associated with the leakage or random release of neurotransmitter from the hair cells of the cochlea and appears to have little or nothing to do with the auditory nerve itself. There exists one population of fibers with low (<1 spike per second) spontaneous discharge rates and one or more populations with high (>20 spikes per second) spontaneous rates. Some researchers even differentiate between a third group of fibers with medium (1-20 spikes per second) spontaneous discharge rates. There is a systematic correlation between spontaneous discharge rate and threshold sensitivity. Fibers with high spontaneous discharge rates always have the lowest threshold to sound. Additionally, fibers with low spontaneous rates usually have high thresholds but can have low thresholds to sound.

The role of spontaneous discharge rates in auditory processing is unclear. Even as many fibers have high spontaneous rates, these discharges are not interpreted as acoustic stimuli by the brain, and so they "are not heard." This fact has led researchers to believe that the role of these discharge rates and tinnitus may be related. It has been hypothesized that disease or illness may disrupt spontaneous discharge rates and be interpreted by the brain as sound even though no sound exists.

Response properties include sustained discharges in response to tones of appropriate frequency and intensity. The first action potential occurs 1 to 3 msec after the wave of pressure contacts the eardrum. The amount of delay is dependent upon the location in the cochlea where the fiber is located. Thus, fibers connected to the basal end of the cochlea result in shorter delay than those connecting with the apical end.

According to the previous description of synaptic transmission, when EPSP magnitude -- through summation processes -- reaches the threshold of a nerve fiber an AP is generated. Auditory nerve fibers are excitatory for the duration of a tone of appropriate frequency and intensity in the absence of other acoustic stimuli. After the onset firing rate (or spike), the average fiber responds for 5 to 10 msec. When acoustic stimulation is eliminated, there is an abrupt cessation of driven response, after which the discharge rate returns to their previous spontaneous rate. This type of response pattern is typical of most auditory nerve fibers and is described as a "primary-like" response.

It is interesting to note that there is a limit to how often a neuron can be "re-excited" or, in other words, how often a nerve impulse can be propagated down the nerve. The reader is referred to Figure II-1 for a diagram of the

absolute and relative refractory periods of the auditory CAP. During the absolute refractory period of the nerve impulse, no new discharge can occur because the flow of ions must reach a resting state before they can move again. Characterization of the absolute refractory period in terms of duration is crucial to success of the paradigm employed in this study. As earlier noted, this absolute refractory period has been measured by Eggermont (1985) as 1.0 ms or 1.2 ms by Ozdamar et al. (1978). It is similarly as difficult, but not impossible, for a new discharge to occur within the relative refractory period. Again, it is the generation of the CAP which is governed by these refractory mechanisms.

On the other hand, the EPSP is a response which is not regulated by these refractory mechanisms; however, it is regulated by adaptation characteristics. Adaptation is defined as "a perstimulatory change in the firing probability of a neuron." This perstimulatory change is reflected in responses which were elicited by stimuli of moderate to high intensities. In general, there are two types of adaptation models in the literature. The first type considers the probability of release (p) to depend on the time and on stimulus intensity. The other adaptation model assumes the "p" to be a constant. Because the second type of model (multi-stage transmitter release) has

Figure II-1: Schematic representation of the absolute and relative refractory periods of the CAP.

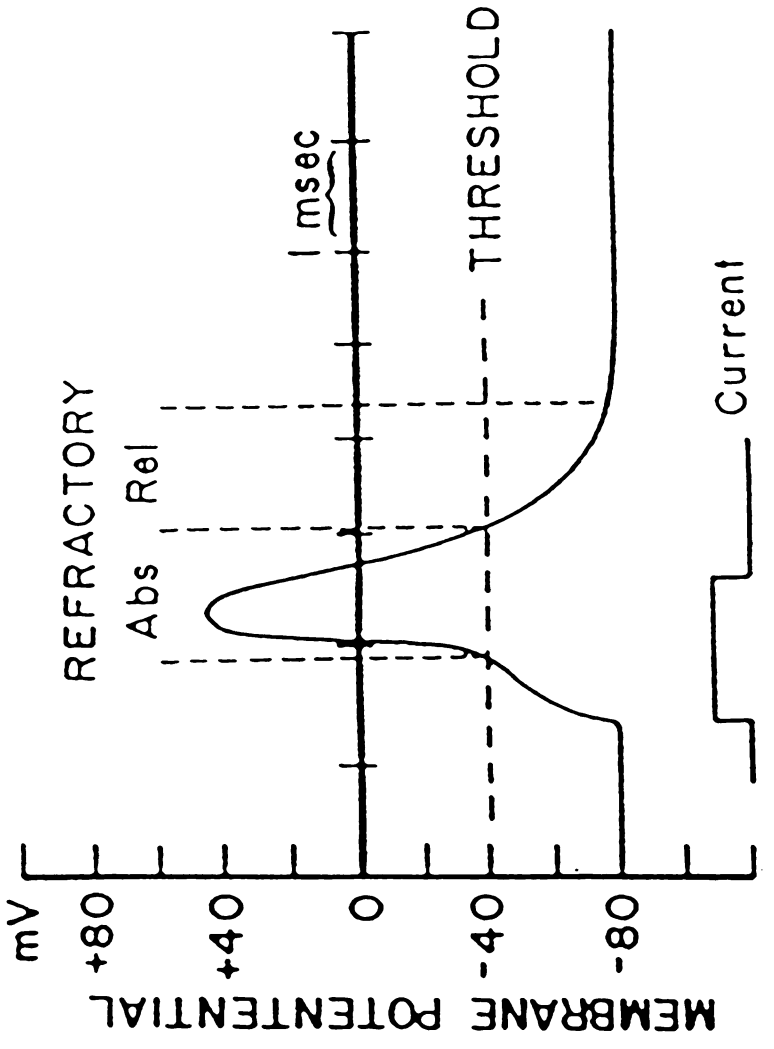


Figure II-1: Schematic representation of the absolute and relative refractory periods of the CAP. Adapted from Durrant and Lovrinic (1984).

proven to be the most commonly accepted in current literature, it will be focused on in this study.

Furukawa et al. (1978) recorded EPSPs in goldfish S1 auditory nerve fibers and concluded that the transmitter release process in the synapse is the most likely candidate for adaptation processes. Furukawa (1986) used a "multiple release site model" to explain auditory adaptation. The reader is referred to Figure II-2 to view a schematic representation of a multiple release site model, as described by Furukawa (1978). A summary of this model as related to adaptation is as follows: Transmitter substances exist in an ordered array of release sites. All of these release sites are initially occupied. Those release sites with the lowest threshold for activation are released by a stimulus of a certain level. From a general store, these transmitter release sites are refilled. This refilling process requires a certain amount of time. Non-activated release sites do not undergo transmitter depletion and can, thus, be recruited at an immediate rate by increments of stimulus intensity.

Eggermont (1985) also described a multi-stage transmitter release model in which he assumes that the release of transmitter substances released from hair cells can occupy receptor sites on nerve fiber dendrites at a very

Figure II-2: Schematic representation of a multiple release site model as an explanation for adaptation.

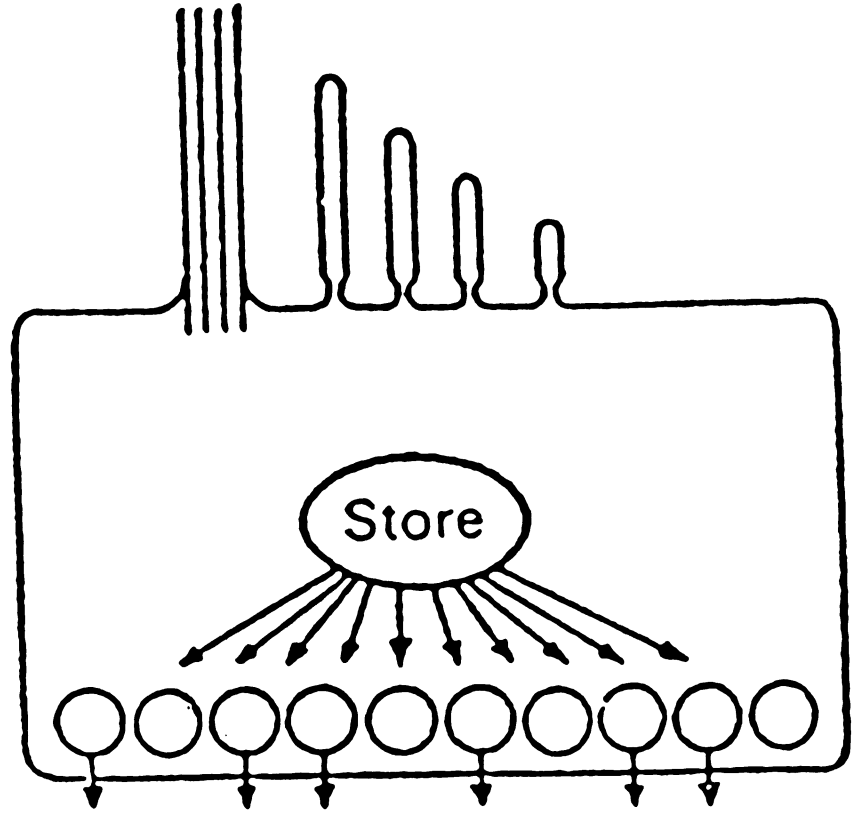


Figure II-2: Schematic representation of a multiple release site model as an explanation for adaptation. Adapted from Furukawa (1986).

rapid rate. Occupied receptor sites are said to be activated for only a short period of time, after which they transform (at a slower rate) into a state of insensitivity relative to transmitter activation. Enzymatic action then liberates occupied receptor sites (representing recovery from a state of insensitivity) at a certain rate. This model includes assumptions that the transmitter combination with receptor molecules causes a conformational change, such as to open $Na^{+}$ channels. After the enzymatic breaking of the bond, the receptor is in a state of insensitivity from which recovery is accomplished at a certain rate.

Upon comparison of Furukawa's (1986) and Eggermont's (1985) multi-stage transmitter release models it becomes apparent that, unlike Furukawa, Eggermont considers post-synaptic mechanisms as contributing to adaptation. Eggermont describes the transfusion of transmitter through the synaptic cleft toward the post-synaptic membrane, eventually occupying receptor sites. This occupation results in changes in membrane permeability for certain ions. These membrane currents are reflected in the EPSP's. Eggermont describes the firing probability of a neuron to be proportional to the size of the EPSP.

Adaptation is best described as increasing with intensity. This increase produces a net response decrease

from the initial peak to the steady state response. Eggermont separates adaptation characteristics into three categories: Short-term adaptation which is observed in the range of 15-60 ms (depending upon the animal) with a recovery rate of 30-100 ms; long-term adaptation observed with a time range including tens of seconds, with a recovery rate of 1-30 s; and auditory fatigue being observed in 1-30 s with a recovery rate of 1-3 min. Short-term adaptation is characterized by the amount of decay from the initial peak to the steady state response. Short-term adaptation is determined by the transformation of active into inactive receptor sites at the postsynaptic membrane and vice versa. Long-term or perstimulatory adaptation, if present, is characterized by the amount of decay of the steady state response. This adaptation process is thought to be regulated by the transfer of transmitter substance from the general store to the vesical array. Finally, auditory fatigue differs from auditory adaptation processes in that it involves the long-term recovery of response amplitude to test tones after the stimulation of the nerve by a "fatiguing" tone. The amount of auditory fatigue depends upon the duration and intensity of the fatiguing tone and on the level of the test tone. Auditory fatigue is thought to be a result of metabolic processes in the hair cells which have the effect of limiting the rate in neurotransmitter is produced (Eggermont, 1985).

Among the first models of peripheral auditory adaptation was a study that was based on the results of a double-click experiment. This experiment entailed the comparison of N1 (of the CAP's N1/P1/N2 complex) amplitude in response to the first click to N1 amplitude of the second. Investigators of this time deemed this recovery function a reflection of properties characteristic of adaptation. But the definition of adaptation entails "perstimulatory" effects, making the results of that experiment invalid for adaptation but valid for forward masking. Adaptation and forward-masking are closely related but surely not identical processes. However, adaptation models can be used to explain forward-masking paradigms. Because of this close relationship, forward-masking will discussed briefly in the following section.

As mentioned above, adaptation and forward-masking are certainly not identical processes; however, adaptation processes can be used to explain forward-masking paradigms. For example, experiments in which CAPs are used to study adaptation processes are in fact based on paradigms of forward-masking. In general, there exists two types of these experimental paradigms. The first type entails repetitive click or tone pip series which results in the amplitude of the AP to decrease as a function of stimulus number and generally reaches a steady-state value after

approximately 5 stimuli (Eggermont, 1974). The second type involves the comparison of AP amplitude in response to a click or tone pip, before and after the presentation of an adapting (or masking) tone. These data suggest AP amplitude increases with increased amounts of time between masker offset and test tone onset. In comparing the data from perstimulatory adaptation processes with the data from forward-masking paradigms, it is observed that the recovery time is approximately 2 1/2 times larger than that of the former processes (Eggermont, 1985).

## Neurotransmitters of the VIIIth Nerve

Since the predominant acceptance of the theory of chemically mediated synaptic transmission, investigators have become quite occupied with the identification and characterization of neurotransmitters. However, neurotransmitters of the auditory nerve have received only moderate attention. Current literature suggests glutamate (GLU) or aspartate (ASP) as a neurotransmitter within the auditory nerve. Wenthold (1985) suggested the following as evidence: The presence of GLU and ASP in VIIIth nerve terminals; the presence of enzymes which have the capability of synthesizing GLU and ASP in auditory nerve terminals; the release of GLU and ASP from auditory nerve fibers; and the presence of GLU and ASP receptors on post synaptic neurons in the cochlear nucleus. Because studies of this nature are

quite complicated due to the difficulty found in adequately demonstrating the criteria of specific release, the support for GLU and ASP as neurotransmitters of the auditory nerve is considered circumstantial. However, the data supporting these chemicals as neurotransmitters are common among several laboratories in that none of the results have all been consistent. This fact makes them both promising candidates.

# CHAPTER III

## INSTRUMENTATION AND PROCEDURE

A description of auditory characteristics of human subjects used within this investigation, selection criteria and procedural variables will be discussed in this chapter. The instrumentation involved can be organized and examined in the following sections: Stimulus Generation, Analog, Digital and Display set up.

Subject Criteria & Relevant Equipment: The data were collected from the left ears of three informed and consenting female volunteers. The age range of the subjects was from 17 to 23 years (mean: 19). These individuals had no personal or family history of significant neurological or otological disorders such as hearing loss, tinnitus, dizziness and noise exposure, or active upper respiratory infection. The otoscopic screening was negative for excessive cerumen and tympanic membrane retraction and/or bulging for each subject. The hearing sensitivity of all of the subjects was within normal limits (re: ANSI s3.6, 1989 specifications). The criteria for establishing normal hearing included a thorough audiological evaluation.

Conventional audiometric techniques revealed normal pure tone thresholds (i.e., air conduction thresholds were less than 25 dB HL bilaterally at all octave frequencies from 250 Hz through 8000 Hz, and bone conduction thresholds were within + or - 5dB of respective air conduction thresholds). Speech recognition thresholds and word recognition scores were commensurate with pure tone thresholds bilaterally for each of the subjects.

Additionally, immittance measures revealed Type A tympanograms characterized by points of maximum compliance between + or - 50 mm $H_20$. Ipsilateral and contralateral acoustic reflex thresholds in each ear were 95 dB HTL or better at the octave frequencies from .25 through 2 kHz. No reflex decay at 1 KHz was noted in either ear of any of the subjects. Impedance measurements were performed on the Grason-Stadler Middle Ear Analyzer. The audiometric testing discussed above was performed using an audiometer that was calibrated to S3.6-1989 specifications in a double-walled suite that met ANSI standards for acceptable levels of background noise in an audiometric testing facility.

Stimulus Generation Section: A block diagram of the stimulus generation set-up and electrode montage used in this experimental study is included in Figure III-1. The generation of stimuli consisted of the following components:

Figure III-1: Block diagram of the instrumentation.

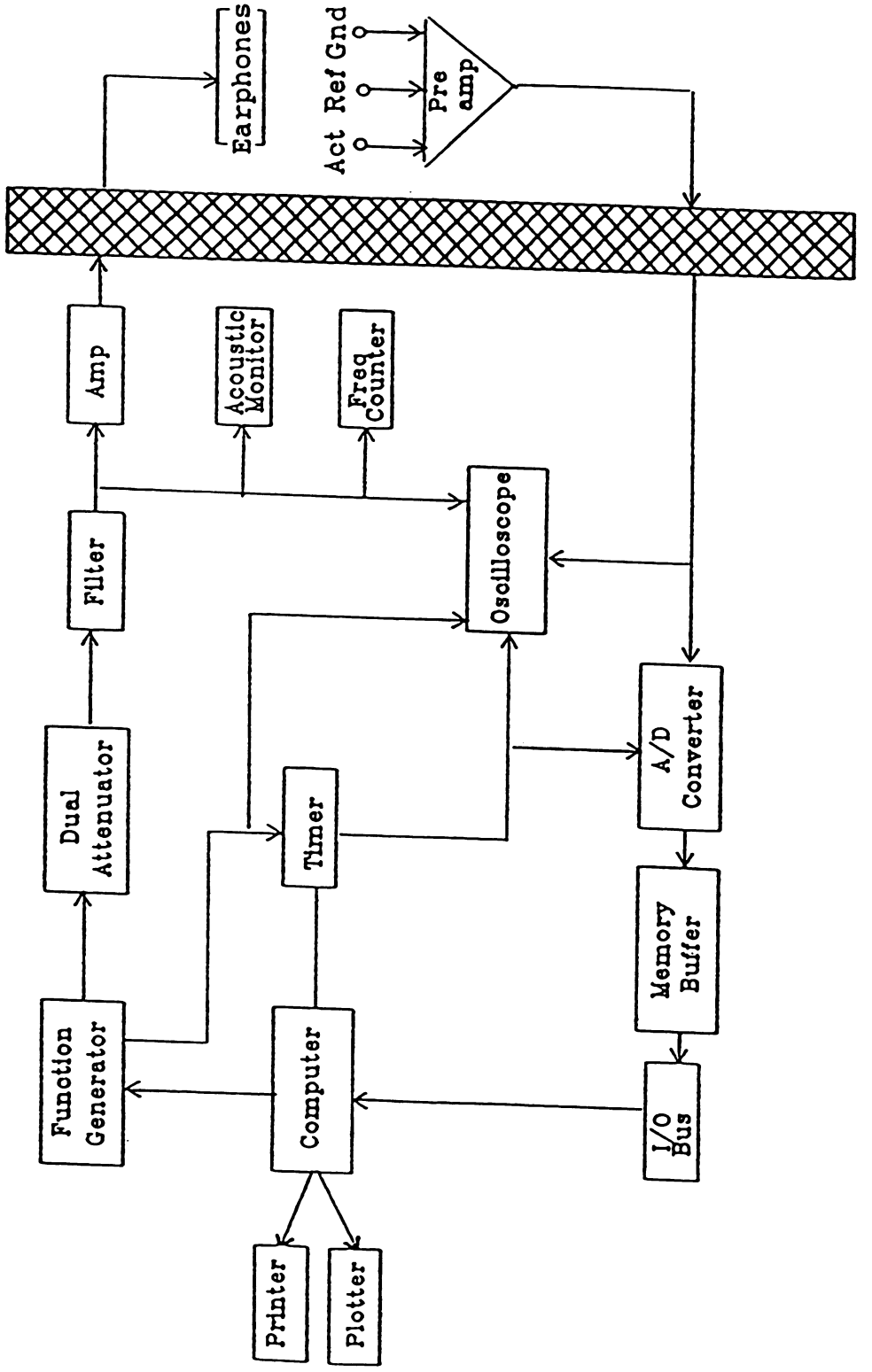


Figure III-1: Block diagram of the intrumentation.

1. Computer: IBM PC-AT #80286
2. Power source: Modular Instruments, Inc., or $MI^2$
3. Dual function generator: $MI^2$ #208
4. Dual Attenuator: $MI^2$ #108
5. Filter: Frequency Devices, Low pass filter #901F
6. Amplifier: Technics power amplifier SE-9060
7. Data controller timer: $MI^2$ #214
8. Madsen MSH-300 shielded headphones

In essence, single- and double-click stimuli were generated by a custom written software program which was run on the IBM personal computer system. These stimuli were then directed to the earphones from the function generator through the dual attenuator, low-pass filter and finally through an amplifier. The purpose of the data controller timer was to create the simultaneous or delayed initiation of the averager with the onset of the stimulus. The headphones served as a transduction device to convert electrical energy to acoustic stimulation.

Analog Section: The components of the analog section include the following equipment for the purpose of response recording:

1. Three Grass gold cup electrodes
2. Preamplifier and Filter: Data, Inc., 2124 Mod 2

As the stimuli were generated, the responses in the form of bioelectric activity were recorded. The electrodes channeled this activity to the AC coupled preamplifier for differential amplification which was set to $1.8 \times 10^5$ KV. Filter settings were set from $1 \times 10^2$ to $3 \times 10^3$ Hz.

Digital Section: The equipment listed within this section was employed for the purpose of response processing:

1. Analog/Digital Converter: $MI^2$ #202
2. Input/Output Bus: $MI^2$

After the subject responses were differentially amplified and filtered, the analog responses were digitized by the analog/digital converter. This piece of equipment systematically sampled the subject responses and created digits which approximated the value of the activity at each particular data point. Next, the responses were channeled into the memory buffer as well as the input/output bus where they were averaged a total of 2,048 times. The sweep time was 10 ms, with a dwell time of 10 us, sample rate of $1 \times 10^5$ KHz and $1 \times 10^3$ data points.

Display Section: The instrumentation included in this section served the purpose of response visualization:

1. Monitor: IBM enhanced color monitor #5154001

2. Oscilloscope: Teletronix D15

3. Frequency counter: Hewlett Packard #5314A

4. Printer: IBM Proprinter II

The IBM monitor revealed a rough visual representation of the recorded subject responses. The oscilloscope is comprised of four individual channels. The first of the channels was used to display the double- and/or single-click stimuli that were generated by the function generator. The second channel displayed the trigger pulse, and the third displayed the click stimuli post- differential amplification. Finally, the fourth channel was set to display the electrophysiologic activity of the subject. The frequency counter monitored the repetition rate of the signal. The printer created hard copies of the analog data collected from the human subjects.

## Equipment Calibration

It is necessary to specify stimulus parameters with precision as well as a great amount of detail in order to document a relationship with the data. For this purpose the stimuli were calibrated in the following domains: 1) phase, 2) rate, 3) intensity, 4) duration, 5) rise & decay time, and 6) frequency. Reference should be made to Figure III-2 for a schematic of the equipment used in the calibration process. This equipment is listed below:

Figure III-2: Block diagram of the calibration system.

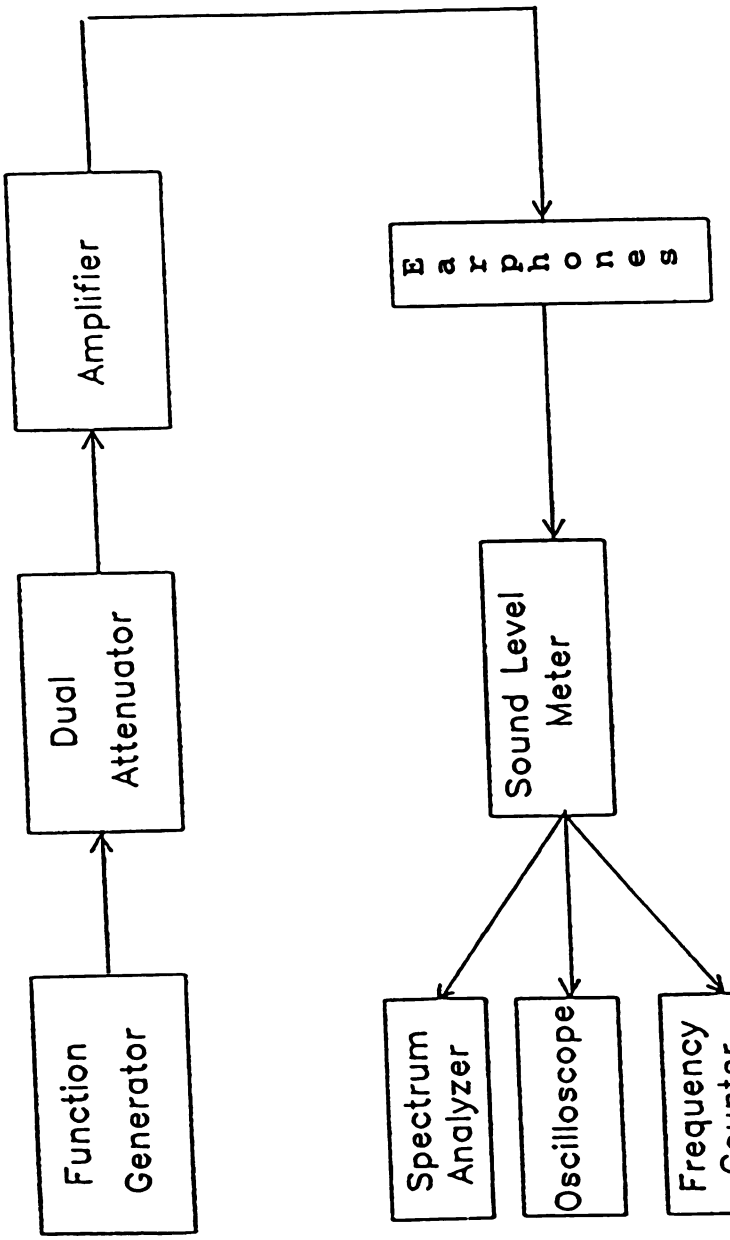


Figure III-2: Block diagram of the calibration system.

1. Function Generator: $MI^2$ #208
2. Dual Attenuator: $MI^2$ #108
3. Amplifier: Technics power amplifier SE-9060
4. Oscilloscope: Textronix D15
5. Sound Pressure Level Meter: Larson & Davis
6. Frequency Counter: Hewlett Packard #5314A
7. Madsen MSH-300 shielded headphones

A 1 KHz sine wave with a peak-to-peak amplitude of 100 mV as measured on the oscilloscope was generated by the function generator and was then channeled through the attenuator. The final routing of this sine wave continued to the amplifier, earphone and finally coupled to the sound level meter. The intensity of the sine wave was adjusted at the level of the function generator until a level of 80 dB nHL was accomplished as measured at the earphone. This level then was referred to as 105 dB P.E. SPL.

The dual attenuator was connected to the oscilloscope which was used for the measurement and monitoring of the phase, rise & decay time and duration of the stimuli. The output of the dual attenuator was also connected with a frequency counter that monitored the repetition rate of the signal. The frequency content of the stimuli was analyzed using the spectrum analyzer.

## Materials and Procedures

The subjects were first prepared for the auditory brainstem response by clearing the areas of electrode site of any excess oils and debris with alcohol swabs. To reduce electrode impedances, the electrode sites were then scrubbed with Omni-prep using a cotton swab. Three Grass gold-cup surface electrodes filled with Medi-trace conducting solution were used to record the bioelectric responses. One electrode was then applied to the subject's vertex (Cz - active), a second gold-cup clip-on electrode was applied to the ipsilateral earlobe (A1 - reference), the third was applied to the subjects forehead (Fpz - ground). As cited in the work of Moore et al. (1992), the wires of the electrodes were braided to enhance the recording of the wave I'. Inter-electrode impedance was kept at or below 1.0 K Ohms throughout all recordings. Electrode impedances were measured and monitored by a battery operated Grass EZM5A impedance meter.

The subjects were situated in a reclining chair with instructions to remain as immobile and relaxed as possible while the experiment was in progress. A blanket was also made available to insure no fluctuations within their recordings due to reduced bodily temperature. The shielded earphones were placed over the subject's ears, and the electrode impedances were rechecked. If the impedances

remained within acceptable limits, as per the guidelines of this investigation, the electrodes were then attached to the preamplifier. If inter-electrode impedance values were elevated, the subject was re-prepared with Omni prep and electrodes were reapplied until acceptable values were obtained. All of the data used in this experiment were recorded from within a sound-treated room.

The stimuli used for both the "standard-" and "paired-click" ABRs were generated at the level of the function generator and introduced to the subject via the left earphone at an intensity of 105 dB P.E. SPL. Each response, initiated by the varying click stimuli, was of alternating polarity in order to eliminate the effects of the cochlear microphonic in the subjects' responses. There was only one independent variable, referred to as "Delta t", within the paired-click stimulus paradigm. As mentioned in the introduction (Chapter I) of this paper, the "paired-click" stimulus paradigm is derived from specific stimulus-related properties of the receptor potential and action potential components and the underlying electrophysiological processes generating these components and the GP. The reader is referred to "The Paired-Click Stimulus" section within Chapter II of this paper for a schematic representation of the above stimuli. At this time, only the basic parameters of the paired-click stimulus paradigm will be described in

the following section.

## Paired-Click Stimulus Paradigm

The paired-click stimuli paradigm involves the manipulation of BAEP traces which were elicited with "standard-click" stimuli and BAEP traces elicited with "paired-click" stimuli. In essence, BAEP responses generated by standard clicks were used for subtraction from ABR responses evoked by paired-click stimuli. The composition of the standard-click stimuli is simple relative to structural components; however, it is important to note that the paired-click stimuli is comprised of two separate clicks: "paired-click 1" (PC1) and "paired-click 2" (PC2). The reader is referred to Figure III-3 for a schematic representation of stimulus composition of the "paired-click" stimulus paradigm. The independent variable represents the amount of time between "PC1" and "PC2" of the paired-click stimuli. The subtraction which was obtained off-line therefore resulted in a "derived response." See Table III-1 for a list of subtraction pairs and corresponding derived responses. The subtraction of these wave forms was done with the expectation that the derived response would actually be representative of the second click ("PC2") of the paired-click BAEP. Specifically, this representation would then reveal the GP for the appropriate paired-click intervals (i.e., 4.0 ms, 2.0 ms, 1.0 ms, 0.8 ms, 0.4 ms, 0.2

Figure III-3: Stimulus components of the "paired-click" stimulus paradigm.

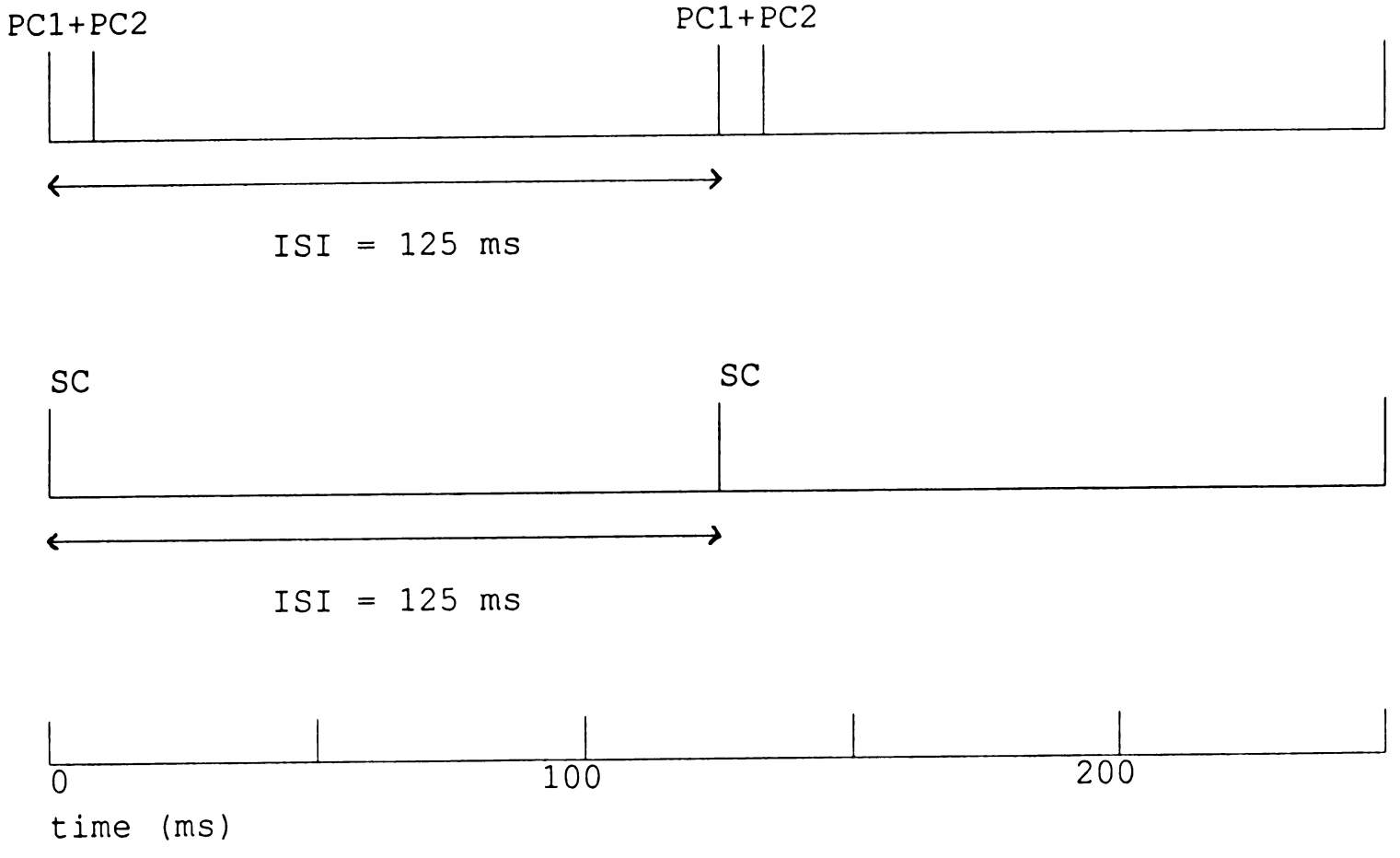


Figure III-3: Stimuli components of the "Paired-Click" Stimulus Paradigm.

Table III-1: Subtraction pairs and corresponding derived responses.

# Table III-1: Subtraction pairs and corresponding derived responses.

| Delta "t" (ms) | Subtraction Pairs | Derived Responses |
|---|---|---|
| 4 | 4.0 t ABR - SC ABR | D/ 4.0 |
| 2 | 2.0 t ABR - SC ABR | D/ 2.0 |
| 1 | 1.0 t ABR - SC ABR | D/ 1.0 |
| 0.8 | 0.8 t ABR - SC ABR | D/ 0.8 |
| 0.4 | 0.4 t ABR - SC ABR | D/ 0.4 |
| 0.2 | 0.2 t ABR - SC ABR | D/ 0.2 |
| 0.1 | 0.1 t ABR - SC ABR | D/ 0.1 |

ms, 0.1 ms).

In order to arrive at an accurate derived response, measures were taken to ensure that the standard-click of the ABR and click I of the paired-click were identical. Interstimulus intervals were kept at 125 ms for both the standard- and paired-click recording conditions to minimize adaptation effects. See Table III-2 for a listing of interstimulus intervals for standard-click and paired-click measurements. It is important to note that latency effects are not expected for an ISI> 128 ms (Eggermont & Odenthal, 1974). Furthermore, click intensities were set to 80 dB nHL.

Table III-2: Interstimulus intervals for standard-click and paired-click measurements.

Table III-2: Interstimulus intervals are kept at 125 ms for both the PC1-PC1 and SC-SC interval. The PC1-PC2 ISI is set at 8.0-0.1 ms. PC, paired-click; SC, standard-click; ISI, interstimulus interval.

# TABLE III-2: Interstimulus intervals for standard-click and paired-click measurements.

| Condition | ISI | Minimum ISI | Maximum ISI |
|---|---|---|---|
| SC ABR | SC - SC: 125 ms | 125 ms | 125 ms |
| 4.0 t ABR | PC1 - PC1: 125 ms | PC1 - PC2: 4.0 ms | PC2 - PC1: 121 ms |
| 2.0 t ABR | PC1 - PC1: 125 ms | PC1 - PC2: 2.0 ms | PC2 - PC1: 123 ms |
| 1.0 t ABR | PC1 - PC1: 125 ms | PC1 - PC2: 1.0 ms | PC2 - PC1: 124 ms |
| 0.8 t ABR | PC1 -PC1: 125 ms | PC1 - PC2: 0.8 ms | PC2 - PC1: 124.2 ms |
| 0.4 t ABR | PC1 - PC1: 125 ms | PC1 - PC2: 0.4 ms | PC2 - PC1: 124.6 ms |
| 0.2 t ABR | PC1 - PC1: 125 ms | PC1 - PC2: 0.2 ms | PC2 - PC1: 124.8 ms |
| 0.1 t ABR | PC1 - PC1: 125 ms | PC1 - PC2: 0.1 ms | PC2 - PC1: 124.9 ms |

# CHAPTER IV

# RESULTS

The proposed goals of the investigation are as follows:

1. Characterization of the mean amplitude and the mean latency of wave I′ in standard BAEP recordings.

2. Development of a specific stimulus paired-click paradigm in which successful differentiation can be made between the cochlear potential, SP, and the neural component, EPSP, as a I′ generator.

3. Specificity of I′ morphological characteristics, that result from the paired-click stimuli observed in derived BAEP responses.

4. Characterization of the derived paired-click BAEP response compared to the regular paired-click BAEP response.

In order to achieve the above objectives, specific

stimulus parameters were chosen and BAEP responses were recorded, on four separate occasions, from three human subjects. The recordings from these subjects resulted in twelve sets of data. Individual subject data series encompassed the recording of one BAEP using a standard-click stimulus, followed by the recording of seven BAEP's with the paired-click stimulus, using Delta "t" intervals including 4.0 ms, 2.0 ms, 1.0 ms, 0.8 ms, 0.4 ms, 0.2 ms and 0.1 ms. The subtraction of the standard-click response from the paired-click response was done to achieve seven derived responses, which constituted the final sample of data.

Statistical Analysis: BAEP responses were measured from the onset of the clicks at the earphone to the most prominent peak for all of the waves discussed in this study; namely, waves $I^0$, I' and I. Peak-to-peak amplitude measurements were made from the first positive peak to the next negative trough of the respective waves. The mean, standard deviation, and range were computed for the amplitude and latency of waves $I^0$, I' and I, for the BAEPs recorded with the standard-click stimulus and the derived BAEPs. Descriptive statistics were applied whenever it was appropriate.

This chapter presents data for each of the research goals listed above. This is followed by a presentation and

brief discussion of all relevant data in order to specify quantitative and descriptive analysis in terms of latency, amplitude and morphology.

**Goal #1: Characterization of the mean amplitude and the mean latency of wave I' in standard BAEP recordings.**

Since the primary focus of this investigation was on developing a paradigm which was capable of successfully extracting out the cochlear potential, SP, from the neural potential, EPSP, the characterization of I' was analyzed as related to mean latency and amplitude, recorded by standard-click BAEP's. Additionally, the latency and amplitude of wave I was analyzed for the purpose of comparing the response characteristics of I', whose anatomical generator is unknown but suspected to be the unmyelinated, afferent VIIIth nerve fibers, with those of wave I, whose anatomical generator has been established as the most distal portions of afferent VIIIth nerve fibers. Common methods of characterization -- such as varying polarity, intensity, frequency and temporal parameters -- were not utilized in order to more efficiently achieve the more primary goal.

Subjects: The left ear of three female subjects were tested in the investigation a total of four times.

Responses obtained from each subject, relative to their amplitude and latency measurements, were found to be consistent and within normal limits relative to their morphological make-up, latency and amplitude characteristics.

Procedure: The stimulus used to elicit regular BAEP responses consisted of single clicks which were generated by one of the dual function generators and channeled to a shielded Madsen MSH-300 earphone. These single clicks had a duration of 200 us, a repetition rate of 11.1/s and an intensity of 105 dB P.E. SPL. The electrophysiologic activity was averaged a total of 2,048 times using an analysis time of 10 ms, and no trigger delay. A sixty second period of rest was provided between runs to avoid neural fatigue. Three gold cup surface electrodes were used to collect subject responses in the form of bioelectric activity. An electrode was placed at the vertex (Cz - Active), one placed on the forehead (Fpz - Ground) and another one was placed on the left ear lobe (A1 - Reference). The bioelectric activity was then channelled to the A/D converter for data sampling, and numerical values that approximated the amount of activity were assigned at each particular data point. Visual display of the responses was accomplished through the use of an oscilloscope, printer and plotter. A software program was run on the personal

computer (IBM) in order to filter the waveforms and calculate latency and amplitude measurements.

Responses: As mentioned above, typical subject BAEP responses that were recorded using the standard-click stimuli were morphologically similar for all subjects. All responses were characterized with a normal occurring series of major positive and negative peaks, labeled by Jewett (1970), with respect to latency and amplitude functions.

A prominent wave I′ was consistently observed within each of the subject's BAEP responses. Figure IV-1 is a diagram of the mean latency values of waves I′ and I as recorded with the standard-click stimuli. Figure IV-1 is followed by Table IV-1 which includes the results of statistical measures of central tendency as well as measures of variance for latency values across subjects.

The mean latency of wave I′ is .97 ms, which is consistent with previously documented I′ latency values (Hughes & Fino, 1980, 1981; Benito et al., 1984; & Moore & Semela, 1985). Wave I mean latency was 1.83 ms which is within normal limits for each subject. The latencies of wave I′ and wave I were characterized by small measures of variance. However, the latency of wave I′ exhibited a slightly larger degree of variance than did the latency of

Figure IV-1: Mean latency for waves I′ and wave I recorded with standard-click stimuli.

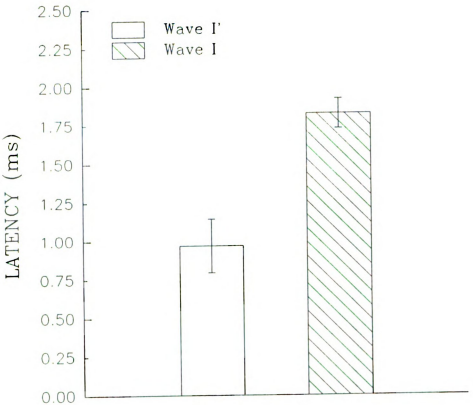


Figure IV–1: Mean latency for waves I' and I recorded with standard–click stimuli.

Table IV-1: Statistical analysis for latency values of waves $I^0$, I′ and I.

Table IV-1: Statistical analysis for latency values (ms). of wave I' and wave I.

| Statistics | Wave I' Latency | Wave I Latency |
|---|---|---|
| Mean | 0.97 | 1.83 |
| SD | 0.18 | 0.1 |
| Range | 0.65 | 0.31 |

wave I. Standard deviation values of 0.18 ms for wave I' and 0.10 ms for wave I revealed a small deviation from the mean throughout the distribution of measurements. A range of .65 ms for I' and .31 ms for wave I are consistent with the small amount of variability. Deviance measures of this size would seem to suggest relatively small differences in documented latency values for successive BAEP recordings.

Figure IV-2 is a bar diagram representing I' and wave I amplitude values as recorded in BAEP responses evoked with the standard-click stimulus. Figure IV-2 is followed by Table IV-2, which displays the results of the statistical analysis of wave I' and wave I amplitude.

Important observations of I' amplitude recorded within this experimental investigation include a mean of 26.94 nV which fall slightly below those measures documented by Moore et al. (1992) and are not consistent with amplitude values documented by Hughes & Fino (1980; 1981). The amplitude values of wave I were characterized by 66.85 nV. Wave I values were within normal limits for each subject. It is important to note that I' and wave I amplitude, in contrast with latency values, include significantly larger measures of variability. Standard deviation measurements were observed as 13.31 nV for I' and 19.2 nV for wave I. This fact again becomes quite apparent as the range of these

Figure IV-2: Mean amplitude for waves I′ and I recorded with standard-click stimuli.

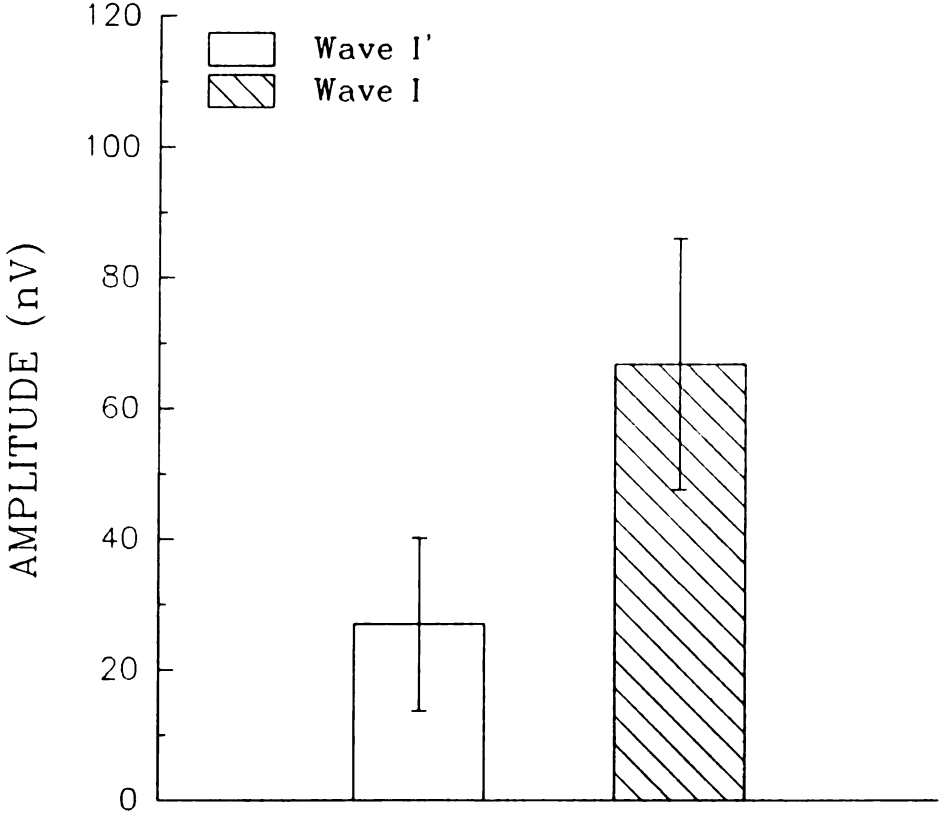


Figure IV-2: Mean amplitude for waves I' and I recorded with standard-click stimuli.

Table IV-2: Statistical analysis for amplitude values of waves $I^0$, I′ and I.

Table IV-2: Statistical analysis for amplitude values (nV). of wave I' and I.

| Statistics | Wave I' Amplitude | Wave I Amplitude |
|---|---|---|
| Mean | 26.94 | 66.85 |
| SD | 13.31 | 19.2 |
| Range | 41.75 | 68.68 |

potentials are considered. Wave I′ had a range of 41.75 nV and wave I amplitude had a range of 68.68 nV, displaying a large amount of variability of amplitude. These findings suggest that the amplitude of waves I′ and I varied from the mean quite often and that individual BAEP recordings reveal a wide range of variability.

Summary: These data suggest that the latency of wave I′ and of wave I is quite consistent, occurring within a relatively small range of time, as recorded with the standard-click stimulus. However, measurements of variability relative to I′ and wave I amplitude reveal larger amounts of deviation from the mean. This suggests that the latency measurements of BAEP recordings are characterized by smaller amounts of deviance from the mean than amplitude values of BAEP responses.

**Goal #2: Development of a specific paired-click stimulus paradigm in which successful differentiation can be made between the cochlear potential, SP, and the neural component, EPSP, as a I′ generator.**

Methods: In order to achieve this goal, a "paired-click" stimulus paradigm was employed. This stimulus paradigm attempts to achieve the differentiation between a cochlear origin and a neural origin of I′ through stimulus parameter

manipulation. Exact stimulus parameters were derived from what is known of the stimulus-related electrophysiological response characteristics of the cochlear potentials (i.e., CM & SP) as well as the neural potentials (i.e., EPSP & CAP). The elimination of the cochlear potential CM was achieved through the use of stimuli with alternating polarity.

The "paired-click" stimulus paradigm consisted of a paired-click stimulus and a standard-click stimulus. The paired-click stimulus encompassed two individual clicks which were identical in structure to one another, as well as to the standard-clicks, but were characterized by a varied time interval between the two clicks. This time interval, also known as Delta "t", was the independent variable within this study and was varied from 4.0 ms, 2.0 ms, 1.0 ms, 0.8 ms, 0.4 ms, 0.2 ms and 0.1 ms. Specifically, the paired-click stimulus paradigm was derived based upon the electrophysiologic processes which are responsible for the generation of the GP and CAP. It is based on the knowledge that the presentation of a stimulus within one second of a preceding one should fall within the refractory periods of the nerve, thus making it almost impossible for the generation of APs. It is important to note the rationale behind choosing the delta "t's" listed above. As Eggermont (1985) stated, the absolute refractory mechanism of a neuron

is approximately 1.0 ms, whereas the relative refractory mechanism is 4 - 5 ms in duration. Because the delta "t" of greatest duration is 4.0 ms, the second of the paired-clicks (PC2) should fall within either the absolute or relative refractory mechanisms of the nerve. In essence, the elimination of the CAP should reveal information relative to the anatomical generator of I'. Even as the generation of the CAP should not be possible, PC2 should still give rise to EPSP's whose summed activity should be able to be recorded as GP. The subtraction of the standard-click BAEP from the paired-click BAEP would result in any differences caused by PC2, thus resulting in a derived response for the appropriate delta "t" and making each of the derived responses, ideally, a representation of the GP.

However, it is important to note that the amount of transmitter quanta left at presynaptic sites will have an effect on GP amplitude which must be taken into consideration. Although making the first of the paired-click stimuli transient will reduce the effects of auditory adaptation, these effects cannot be completely ruled out. These factors are discussed in more detail in chapter V (the discussion section) of this paper.

Responses: Figure IV-3 depicts BAEP responses to a standard-click stimulus, and seven derived responses for one

subject, followed by an 0.8 ms paired-click stimulus. The standard-click BAEP response was characterized by the normally occurring major positive and negative peaks, as mentioned above. Here again, the mean latency value of I′ in response of the standard-click stimulus was .97 ms. Latencies were also characterized by small measures of deviance relative to mean values. I′ latency is discussed in detail in the previous section (Goal #1). Amplitude values of I′, on the other hand, included a mean of 26.94 nV and were characterized with large measures of deviance from the mean. Amplitude values as recorded with the standard-click stimulus are discussed in greater detail in the "Goal #1" section of this paper as well.

However, the derived BAEP responses for each of the Delta "t" values displayed a "shift," or an increase in latency for all of the Jewett labeled positive and negative peaks. Furthermore, these derived responses were characterized by the appearance of two individual peaks, the first of which will be called $I^0$ and the second of which will be referred to as I′ for the remainder of this investigation.

The reader is referred to Figure IV-4 for a schematic representation of mean latency values plotted against Delta "t′s" for waves $I^0$, I′ and I of the derived responses.

Figure IV-3: Standard-click BAEP response followed by seven paired-click derived responses, and an 0.8 ms paired-click.

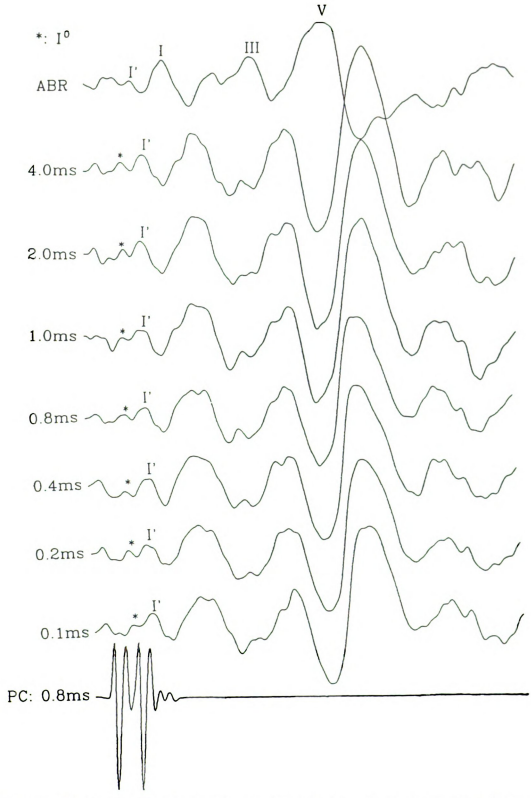


Figure IV-3: BAEP response to standard-click stimuli followed by seven derived responses and an 0.8ms paired-click.

Figure IV-4 is followed by Table IV-3 to view tables a - c for statistical analysis for the latency values of waves $I^0$, I′ and I. The latency for the first occurring of the two peaks, wave $I^0$, was characterized by means ranging from 0.82 to 0.77 ms (0.05) across delta "t′s." The peak occurring after wave $I^0$, I′, was observed with mean latency values ranging from 1.31 to 1.26 ms (0.05) across delta "t′s". Wave I mean latency, as a function of delta "t" ranged from 2.60 to 2.52 ms (0.08) between 4.0 ms - 0.1 ms delta "t′s". Measures of variability include standard deviations ranging from 0.10 to 0.16 ms for wave $I^0$, 0.10 to 0.20 for wave I′, and 0.10 to 0.17 ms for wave I. It is important to note that measures of variability were smallest for the delta "t" values from 4.0 ms to 0.4 ms, and increased outside of this range. Even as scores of variability were consistently small within the above mentioned range, it should be taken into consideration that the identification of wave $I^0$ was, at times, a bit difficult for delta "t′s" 4.0 ms and 0.4 ms.

Figure IV-5 is a diagram of the mean amplitudes as a function of delta "t" for waves $I^0$, I′ and I. Figure IV-5 is followed by Tables IV-4(a - c) for statistical analysis of the amplitude values for waves $I^0$, I′ and I. The means for $I_0$ amplitude ranged from 30.20 to 23.36 nV (6.84) between delta "t" values. Mean amplitudes for the derived responses ranged from 52.03 to 36.37 nV (15.66) for wave I′

Figure IV-4: Mean latency plotted against Delta "t" for waves $I^0$, I′ and I.

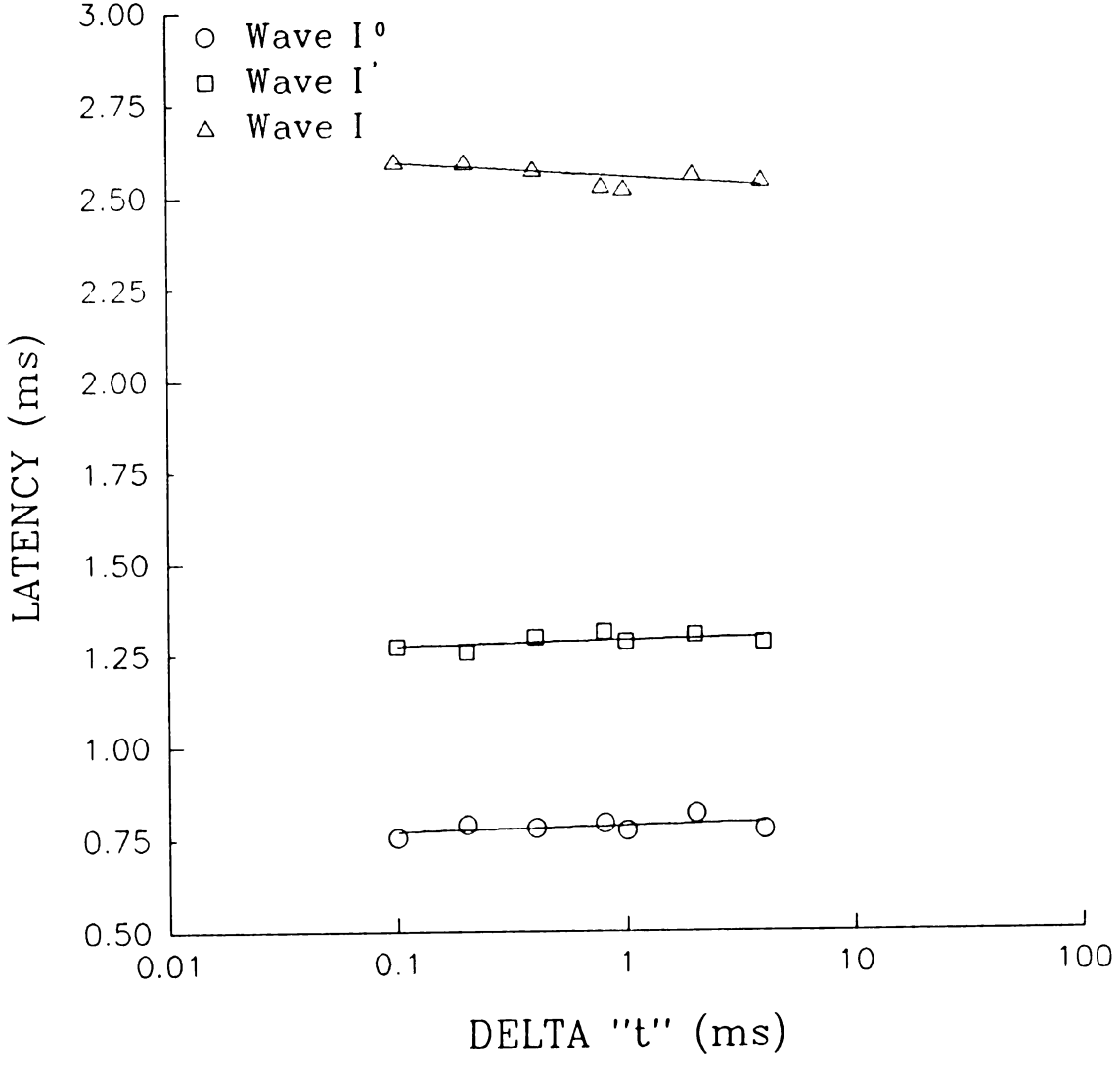


Figure IV–4: Mean latency plotted against Delta "t" for waves $I^0$, I' and I.

Table IV-3a-c: Statistical analysis for latency values of waves $I^0$, I′ and I.

Table IV-3a: Statistical analysis of latency values for wave I$^{0}$.

| Statistic | 4.0 ms | 2.0 ms | 1.0 ms | 0.8 ms | 0.4 ms | 0.2 ms | 0.1 ms |
|---|---|---|---|---|---|---|---|
| Mean | 0.78 | 0.82 | 0.77 | 0.79 | 0.78 | 0.79 | 0.76 |
| SD | 0.16 | 0.1 | 0.15 | 0.14 | 0.16 | 0.12 | 0.14 |
| Range | 0.46 | 0.27 | 0.47 | 0.43 | 0.51 | 0.37 | 0.4 |

Table IV-3b: Statistical analysis of latency values for wave I'.

| Statistic | 4.0 ms | 2.0 ms | 1.0 ms | 0.8 ms | 0.4 ms | 0.2 ms | 0.1 ms |
|---|---|---|---|---|---|---|---|
| Mean | 1.28 | 1.3 | 1.28 | 1.31 | 1.29 | 1.26 | 1.27 |
| SD | 0.16 | 0.1 | 0.13 | 0.14 | 0.15 | 0.16 | 0.2 |
| Range | 0.5 | 0.29 | 0.38 | 0.45 | 0.43 | 0.46 | 0.66 |

Table IV-3c: Statistical analysis of latency values for wave I.

| Statistic | 4.0 ms | 2.0 ms | 1.0 ms | 0.8 ms | 0.4 ms | 0.2 ms | 0.1 ms |
|---|---|---|---|---|---|---|---|
| Mean | 2.55 | 2.56 | 2.52 | 2.53 | 2.58 | 2.59 | 2.6 |
| SD | 0.16 | 0.16 | 0.17 | 0.13 | 0.1 | 0.11 | 0.17 |
| Range | 0.59 | 0.55 | 0.61 | 0.42 | 0.33 | 0.32 | 0.57 |

Figure IV-5: Mean amplitude plotted against Delta "t" for waves $I^0$, I′ and I.

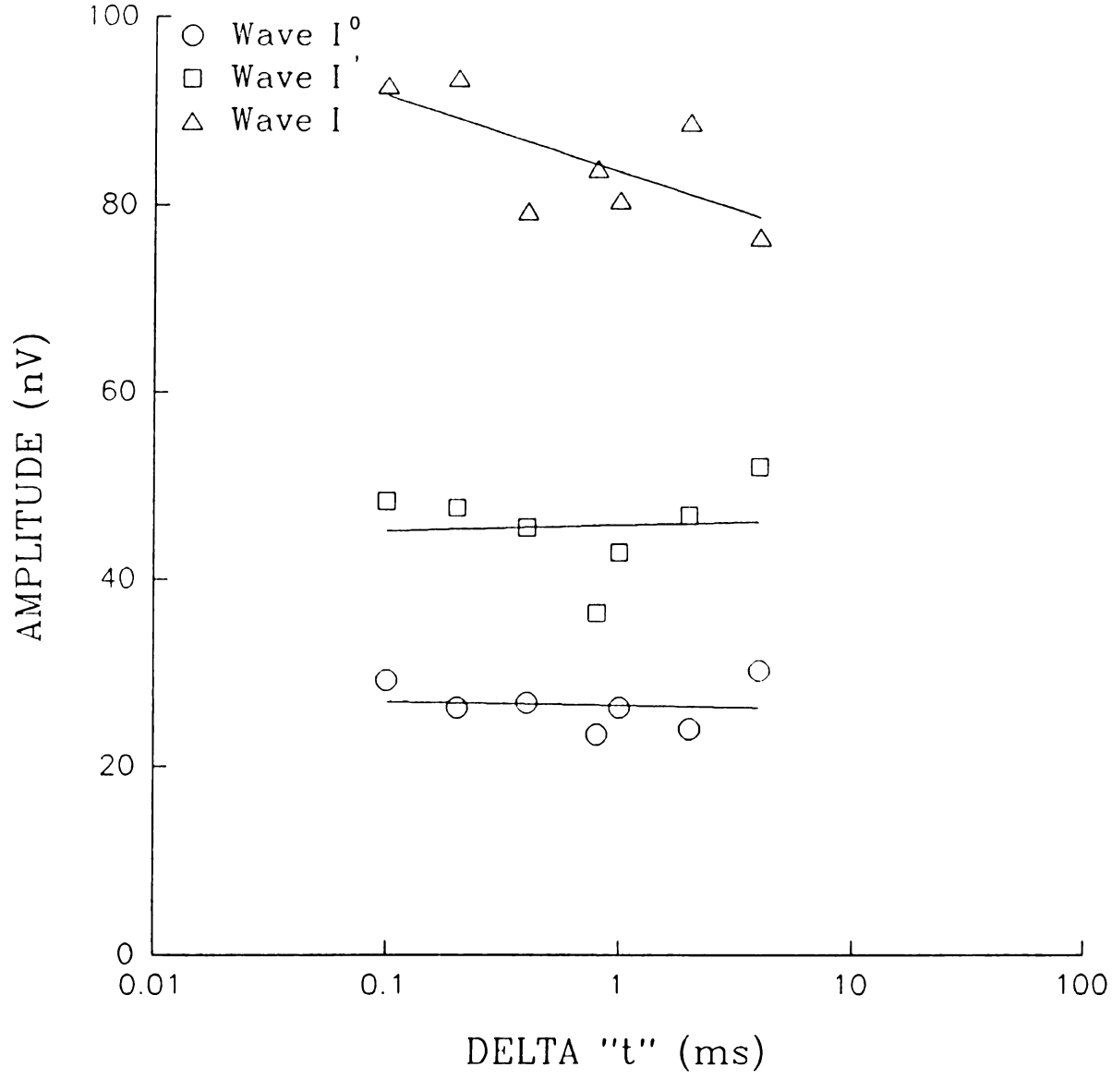


Figure IV-5: Mean amplitude plotted against Delta "t" for waves I , $I'_0$ and I.

Table IV-4a-c: Statistical analysis for amplitude values of waves $I^0$, I' and I.

Table IV-4a: Statistical analysis of amplitude values for wave $I^0$.

| Statistic | 4.0ms | 2.0 ms | 1.0 ms | 0.8 ms | 0.4 ms | 0.2 ms | 0.1 ms |
|---|---|---|---|---|---|---|---|
| Mean | 30.2 | 23.9 | 26.17 | 23.36 | 26.72 | 26.21 | 29.15 |
| SD | 14.7 | 16.43 | 14.64 | 17.68 | 19.68 | 21.28 | 22.51 |
| Range | 39.61 | 48.09 | 44.81 | 55.76 | 59.54 | 69.79 | 68.38 |

Table IV-4b: Statistical analysis of amplitude values for wave I'.

| Statistic | 4.0 ms | 2.0 ms | 1.0 ms | 0.8 ms | 0.4 ms | 0.2 ms | 0.1 ms |
|---|---|---|---|---|---|---|---|
| Mean | 52.03 | 46.81 | 42.89 | 36.37 | 45.5 | 47.61 | 48.37 |
| SD | 14.51 | 16.79 | 16.31 | 15.3 | 16.4 | 17.59 | 15.14 |
| Range | 50.28 | 62.96 | 47.46 | 44.16 | 54.4 | 53.63 | 47.9 |

Table IV-4c: Statistical analysis of amplitude values for wave I.

| Statistic | 4.0 ms | 2.0 ms | 1.0 ms | 0.8 ms | 0.4 ms | 0.2 ms | 0.1 ms |
|---|---|---|---|---|---|---|---|
| Mean | 76.58 | 88.75 | 80.5 | 83.86 | 79.32 | 93.4 | 92.63 |
| SD | 32.83 | 28.85 | 31.62 | 27.84 | 27.77 | 35.67 | 32.02 |
| Range | 100.18 | 104.9 | 102.3 | 86.82 | 88.8 | 111.22 | 115.25 |

and from 93.40 to 76.58 nV (16.82) for wave I. Standard deviations for waves $I^0$, I′ and I ranged from 22.51 to 14.64 nV, 17.59 to 14.51 nV, and 35.67 to 27.77 nV, respectively. Salient features of this statistical analysis suggest smaller amounts of variability for delta "t" values from 2.0 ms to 0.4 ms. As with latency measures of variability, the amount of deviation increased for delta "t" values outside of this range.

Summary: The seven derived responses were characterized by an increase in latency for all the major and minor peaks of the BAEPs. In addition, these derived responses were each characterized by the appearance of two individual peaks, both of which occurred before wave I. The latency values of each of these peaks -- $I^0$, I′ and I -- were characterized by small measures of variability, suggesting a minimal amount of distribution from the mean in successive BAEP recordings. The amplitude of wave $I^0$ was smaller than that of I′ in each of the subject's BAEP responses. When comparing the ranges of latency and amplitude values, it becomes apparent that there was significantly more variability in the latter of the two measurements. Finally, measures of variability relative to latency were smaller for the delta "t" values from 4.0 ms - 0.4 ms but increased for delta "t" values outside of this range. Standard deviation scores were also smallest for the delta "t" values from 2.0 ms to 0.4 ms for

amplitude measures.

**Goal #3: Specificity of I′ morphological characteristics, that result from the paired-click stimuli observed in derived BAEP responses.**

Specific morphologic characteristics became apparent with the examination of the seven derived responses in this study. These characteristics include peak splitting, wave reduction and/or elimination, and peak widening.

Responses: In viewing these derived responses, specific morphological changes were documented. It should be noted that the derived BAEP responses were characterized with the splitting of individual and combinations of the major and minor positive peaks. It was especially common to observe the uppermost portion of wave I of derived BAEPs to split into two and, in some instances, three small peaks. Wave III displayed this splitting in a number of derived responses as well. The widening of all of the major and minor positive peaks was also noted (I - V) throughout all of the derived BAEP responses. In addition to the above morphological characteristics, it was common to observe the disappearance and/ or elimination of the minor peaks. For instance, the disappearance of wave II was a common observation in the derived responses.

Summary: At least three morphologic characteristics can be observed in the derived responses of the paired-click stimulus paradigm. First, peak splitting characteristics of all the major and minor positive BAEP waveforms were observed in derived responses. Secondly, the disappearance of minor positive peaks was noted. Finally, the widening of all major and minor positive peaks became apparent in the derived responses.

**Goal #4: Characterization of the derived paired-click BAEP response compared to the regular paired-click BAEP response.**

Derived responses in this investigation were obtained by subtracting the standard-click BAEP from the paired-click BAEP for each delta "t". Specifically, this subtraction technique would then reveal any effects caused by PC2 and should represent the summation of EPSP, or the GP in the BAEP paired-click responses; thus, making the separation of neural from cochlear within wave I′ obvious. To appreciate the characteristics of the derived response, BAEP responses were examined before the subtraction was performed.

Responses: The reader is referred to Figure IV-6(a-b) in order to compare a 1.0 ms delta "t" paired-click (no subtraction was performed at this level) BAEP response to a

1.0 ms delta "t" derived BAEP response. Clearly, there are significant differences between the two BAEP responses. Figure IV-6a demonstrates a paired-click BAEP before subtraction of the standard-click BAEP was performed. In essence, the effects of both PC1 and PC2 are overlaid upon one another, making it impossible to identify what peaks are the result of what click. The subtraction of the standard-click BAEP from the paired-click BAEP results in any differences between the two stimuli, namely PC2. Thus, the derived response displayed in Figure IV-6b is a result of the separation of the effects of PC2 from the effects of PC1. This makes it possible to identify only the potentials caused by PC2 of the paired-click stimulus. The results of the derived responses are discussed in detail under the "Goal #2" section of the paper. It is also important to note that the derived responses should result in any differences in latency and/or amplitude between the two waveforms.

Summary: The importance of the derived response calculation can be observed through its comparison with an un-subtracted BAEP response. Potentials caused by PC1 and PC2 are essentially overlaid upon one another, making it impossible to separate individual PC effects. Derived responses are necessary in order to identify the effects of PC2 which, ideally, represent summated EPSP activity in the

Figure IV-6a-b: Regular paired-click BAEP response followed by a derived paired-click BAEP response.

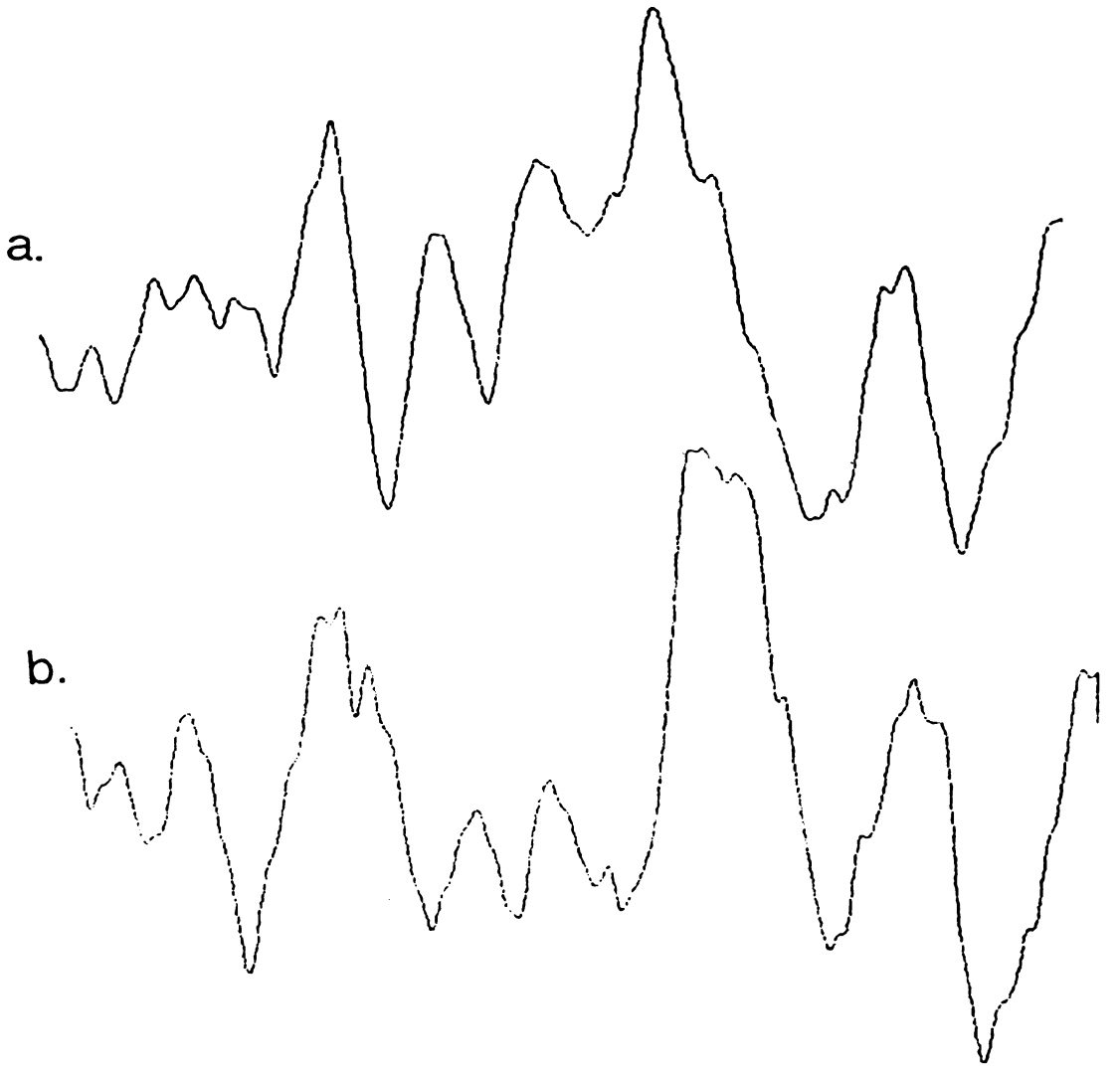


Figure IV-6 a-b: Regular paired-click BAEP response followed by a derived paired-click BAEP response.

form of the GP. The reader should also keep in mind that any latency and/or amplitude differences should be observed in the derived responses (Henry & Price, 1992).

## Possible Limitations

It is important to note any variables, related to the nature of this study, that may produce any type of limiting effects. Possible limitations include the following:

1. An assumption of the "paired-click" stimulus paradigm involves the ability of EPSP activity to be recorded as GP in the human BAEP. Although success has been achieved in studies involving animals, it has yet to be documented in humans.

2. Because Eggermont (1985) states that adaptation effects are unlikely for ISIs greater than 128 ms, and the delta "t" values (or minimum ISIs) range from 4.0 to 1.0 ms, the effects caused by adaptation processes may be too great to detect EPSP activity of this magnitude.

3. As the "paired-click" paradigm involves close structural relationships with forward masking paradigms, the manifestation of these types of effects in the results of this study should be

taken into consideration.

# CHAPTER V

# SUMMARY AND DISCUSSION

The organization of this chapter will include a summary of the study, the summary and discussion of major findings, and recommendations for future research.

## Summary of the Study

The primary goal of this experimental investigation was to gain insight into the anatomical generator of the recently discovered potential I′, of the human BAEP. Although I′ research includes a number of studies where I′ is characterized by latency and amplitude functions, research into the specific anatomical generator remains mixed and inconclusive. A review of the literature revealed data suggesting the cochlear potential, SP, or the neural potential, EPSP, as possible I′ generators. The "paired-click" stimulus paradigm was created on the suggestion that I′ generator identification might be possible through stimulus parameter manipulation.

Specifically, the "paired-click" stimulus paradigm involves BAEP responses to a paired-click stimulus. The

components of the paired-click stimulus, PC1 and PC2, are characterized by time intervals ranging from 4.0 ms, 2.0 ms, 1.0 ms, 0.8 ms, 0.4 ms, 0.2 ms to 0.1 ms and are based upon the duration of the absolute (1.0 ms) and relative (4 - 5 ms) refractory periods of the nerve. Varying these time intervals in separate BAEP recording conditions result in seven different paired-click BAEP responses. Therefore, each of the components of the paired-click stimulus causes separate neurophysiologic effects. PC1 will have the effect of initiating AP's along the auditory nerve, resulting in waves I' and I through V in the BAEP. On the other hand, PC2 is delivered to the auditory system during times when additional AP's cannot be re-initiated. Although PC2 should not initiate AP's, it should still initiate EPSP's whose summated activity should then be able to be recorded in the BAEP as the GP. Finally, a derived response was calculated by subtracting a standard-click BAEP response from individual paired-click BAEP responses. The derived responses were calculated in order to separate the effects of PC1 from the effects of PC2. In other words, each of the seven derived responses, ideally, represent the effects of PC2, or the GP for the appropriate delta "t."

The hypotheses discussed in the following section are based upon the results of I' literature and relevant SP research. Because of the "paired-click" stimulus paradigm's

close relationship with forward-masking, a review of forward-masking literature was completed and applied to the results of this study.

## Summary and Discussion of Major Findings

In the discussion of the major findings of this study, the reader should keep in mind the possible limitations discussed in Chapter IV.

It was observed that the latency values of wave I′ and wave I were quite consistent, occurring within a relatively small range of time, as recorded with the standard-click stimulus. However, measurements of variability relative to wave I′ and wave I amplitude reveal larger amounts of deviation from the mean. This suggests that latency measurements of BAEP recordings are characterized by smaller amounts of deviance from the mean than amplitude values of BAEP responses.

Overall, latency measurements for the waves I′ and I reveal their reliability of measurement through their small standard deviation scores. However, amplitude measurements of variability for wave I′ and wave I revealed just the opposite effect. While I′ amplitude values only approximated the lower end of the values documented by Moore et al. (1992), the amplitude values recorded in this study

were significantly higher than the measurements documented by Hughes & Fino (1980; and 1981).

The amplitude and latency functions have been studied extensively; and it has been demonstrated that as stimulus intensity increases, the latency of the BAEP waves decreases, whereas the amplitude increases (Rossi, Solero & Pira, 1982; Moore, 1971). However, the results of an unpublished doctoral dissertation suggest that the amplitude relationship to intensity commonly accepted is not applicable to all subjects (Amedofu, 1985). The data collected within this study are consistent with that of Amedofu's (1985), as both revealed latency values that are quite consistent across subjects, whereas BAEP amplitude measures are determined by individual characteristics.

Based on the works of Petrie (1960), Buchsbaum & Silverman (1968), and Braden, Haier & Space (1983), it was suggested that individual BAEP amplitude responses be separated into at least two groups (Amedofu, 1985). The first group is designated as "augmenters" and are characterized by BAEP amplitudes that increase as a function of stimulus intensity. The second group, "reducers," are individuals whose BAEP amplitude decreases or remains constant as a function of stimulus intensity. These data are offered as a confirmation of and as an explanation for

the latency values and the highly variable amplitude measures observed in the BAEP responses throughout this study.

In order to assess the success of the "paired-click" stimulus paradigm, the derived BAEP responses were examined. Examination of these derived responses, however, revealed results that were more complex than what had previously been expected. In fact, these derived responses were characterized by the appearance of two individual peaks, designated as wave $I^0$, and wave I′ within this study, both of which occurred before wave I. The latency values of each of these peaks -- $I^0$, I′ and I -- were characterized by small measures of variability, suggesting a minimal amount of distribution from the mean in successive BAEP recordings. The amplitude of wave $I^0$ was smaller than that of I′ in each of the subject's BAEP responses. When comparing the ranges of latency and amplitude values, it becomes apparent that there was significantly more variability in the latter of the two measurements. Morphological characteristics, such as peak widening, were also observed within all major and minor peaks of the derived BAEP responses. There was also an apparent shift in latency for all of the major and minor peaks of the derived responses. Examination of the statistics for latency values for waves $I^0$, I′ and I revealed smaller measures of variability for the delta "t"

values of 4.0 ms to 0.4 ms. Furthermore, the delta "t" values from 2.0 ms to 0.4 ms displayed smaller amounts of deviance from the mean, relative to amplitude measurements, for the three waveforms.

Based upon the above results and the findings of current SP, I′ and forward-masking literature, the following hypotheses were developed: 1) Wave $I^0$ represents the cochlear summating potential in derived BAEP responses; 2) Wave I′ represents the summation of neural excitatory postsynaptic potentials, or the GP in the derived BAEP; 3) Waves I through V remain in the derived BAEP responses because of the summation of PC1 effects in the 35,000 auditory nerve fibers. Each of these hypotheses will be examined individually, in the following section.

Hypothesis #1: Wave $I^0$ represents the cochlear summating potential in derived BAEP responses.

In research using conditions similar to this study, SP amplitudes recorded from the external auditory meatus (EAM) of 48 normal ears stimulated with 116 dB P.E. SPL clicks ranged from 0.82 to 0.02 μV (mean, 0.39; SD, 0.17) (Coats, 1981). Eggermont (1976a) used a 2,000 Hz tone burst to elicit EAM-recorded SP's from 25 normal ears; these data reveal SP amplitude ranges from 6.0 μV to 0.36 μV at 85 dB

HL. Furthermore, SP amplitudes approximately ten times larger than those measured from the EAM were documented by other investigators using promontory methods of recording (Schmidt, Eggermont & Odenthal, 1974; Eggermont, 1976a; Kumagami, Nishida & Baba, 1982; and Gibson, Prasher & Kilkenny, 1983). For example, Gibson et al. (1983) obtained SP amplitudes ranging from 0.5 to 10 $\mu$V (mean, 3.90; SD, 2.66) from 33 normal ears using clicks of 100 dB HL. These data suggest that the further the electrode is from the anatomical originator, the smaller the SP amplitude.

Wave $I^0$ amplitude values reveal amplitudes which fall within and extend beyond the lower end of the SP voltages recorded via the EAM. The reader is again referred to Figure IV-5 and table IV-4a for a diagram of wave $I^0$ amplitude and a statistical analysis of wave $I^0$, respectively. Because one of the waveforms is involved in the derivation (subtraction) process, a direct comparison of the amplitude increase, between the two responses, is not possible (Henry & Price, 1992). Therefore, the smaller amplitudes seen in this study could be explained by the far-field method of recording used to collect the BAEP responses and the lower stimulus intensity used in this study. Also, dissimilarities in sample size and in age and sex distribution of the subjects may account for the differences between previous data and the data collected in

this study. Furthermore, the lack of ability to accurately quantify the amplitude changes, as a result of forward-masking, might also be an explanation for the differences between these data.

Upon examination of the amplitude, plotted as a function of delta "t", it is apparent that there is very little fluctuation. In fact, viewing the standard deviation scores of wave $I^0$ alone reveals small measures of deviation across all of the delta "t" values. This feature is unique for this wave, as measures of variability display a noticeable increase for at least two of the delta "t" values, for waves I′ and I. Continuing to reason in terms of wave $I^0$ representing a receptor potential, this observation becomes important. The amplitude of a receptor potential is directly related to the amount of basilar membrane displacement, which is actually a direct result of the stimulus intensity. Thus, an increase in stimulus intensity would result in an increase in SP amplitude; the opposite would be true of a decrease in stimulus intensity. Since the intensity parameter within this study is kept at 105 P.E. SPL for all BAEP conditions, overt fluctuations in the amplitude of wave $I^0$ amplitude, if it truly represents SP, should not be observed.

The next logical step in the process of supporting the

hypothesis that wave $I^0$ is the cochlear potential SP would be to compare the latency values of this wave with that of documented SP latency values. Unfortunately, current SP literature relative to specific measures of SP onset, peak and rise times is not available at this time. However, most authors generally document SP latency as < 1 ms (Moore, 1983; Hall, 1992). These data are consistent with the mean latency values documented in this study, that range from 0.82 to 0.76 ms. However, as with amplitude, a latency shift or increase is expected as a result of forward-masking effects, making a latency shift analysis impossible (Henry & Price, 1992). Therefore, the quantification of the latency shift caused by forward-masking is not possible. Without this quantification, the true latency value of I′, in the derived BAEPs, cannot be established.

Similar to amplitude, the examination of wave $I^0$ latency, plotted as a function of delta "t", suggests that the latency of wave $I^0$ does not exhibit any significant fluctuations. The cochlear SP is a product of the activation of a specific group of hair cells determined by the frequency of the stimulus. With these properties, successive stimulation of the hair cells by a stimulus that is unchanging, in terms of frequency, should reveal insignificant differences in successive latency measurements. In view of the fact that this study involved

the use of a stimulus that remained constant, these data are considered promising. The latency characteristics of wave $I^0$ are similar to the characteristics of a cochlear potential; and since the CM was canceled and can be ruled out, the only remaining hypothesis is that wave $I^0$ represents SP.

The SP is a presynaptic potential and is, therefore, not affected by adaptation. As discussed in Chapter II of this paper, adaptation and forward-masking are closely related. In fact, the structure of the "paired-click" stimulus paradigm represents a forward-masking paradigm. Given this information, adaptation effects caused by the "paired-click" stimulus paradigm are not expected for wave $I^0$, if it truly reflects SP. A summary of my review of forward-masking literature is discussed below and will be followed by a comparison of these data with wave $I^0$.

In common forward-masking paradigms, the amplitude of the AP to a click or tone presented after a masking tone (or click) increases as the time interval (ISI) between masker offset and click onset increases (McGill & Rosenblith, 1951; Eggermont & Spoor, 1973a & b; Eggermont & Odenthal, 1974; Eggermont, 1974; Abbas, 1979; Abbas & Gorga, 1981; Harris & Dallos, 1979; and Eggermont, 1985). Forward-masking literature also reveals that the response decrement is

independent of the level of the test tone but dependent on the level of the masking tone and obviously on delta "t" (Smith, 1977). It was also documented that frequency does not affect the decrement when the adapting tone level remains constant (Abbas & Gorga, 1981). Furthermore, the longer in duration the masking tone, the more adaptation effects are observed (Smith, 1977; Abbas & Gorga, 1981; and Eggermont, 1985).

Eggermont et al. (1973a) summarized the effects of forward-masking paradigms as being highly dependent on the ISI, stimulus intensity and stimulus duration. It was observed that AP amplitude decreased as ISI decreased and that increased adapting stimulus level and duration were directly related to the amount of decrement. Forward-masking effects also revealed an increase in latency as the ISI decreased; as with amplitude, the increase in latency was directly related to increased stimulus intensity and duration. Finally, Eggermont et al. (1973a) observed the distinct widening of N1 (of the AP complex N1/P1/N2). It was also observed that peak N1 widened as a function of ISI.

If wave $I^0$ is a representation of a neural potential, it would be expected that its amplitude would display a certain amount of decrement as the delta "t" decreased. However, the examination of Figure V-1 does not reveal any

observable decline in amplitude as delta "t" value increases. In the same vein, the manifestation of forward-masking effects within latency characteristics of a neural potential would include a decreasing latency as delta "t" increases. Examination of Figure IV-4 and Table IV-3a does not reveal any overt changes in latency as a function of delta "t". Thus, it would appear that wave $I^0$ does not represent a neural potential.

Hypothesis #2: Wave I' represents the summation of excitatory postsynaptic potentials, or the GP in the derived BAEP.

Wave I has been associated with N1 of the AP complex, and its anatomical generator is thought to be the distal most portion of the VIIIth nerve. For this reason, amplitude and latency measures were documented for wave I and wave I', for increased comparison abilities. This type of analysis should reveal similar functions if I' truly represents a neural potential, namely the GP. In contrast, differences between the functions of the two potentials would reveal just the opposite.

Inspection of the amplitude functions of all three waveforms reveals higher amounts of variability (as a function of delta "t") for waves I' and I than for wave $I^0$.

According to forward-masking literature, the amplitude of a neural potential should increase as the delta "t" increases. Examination of I′ amplitude as a function of delta "t" shows that the results are consistent with these data. Furthermore, if I′ is a neural potential, its amplitude function should also mimic that of wave I but not wave $I^0$. As expected, I′ amplitude seems to increase as a function of delta "t", whereas wave $I^0$ amplitude seems to remain fairly constant. However, the amplitude functions of waves I′ and I are not similar. Figure IV-5 displays a decreasing amplitude for wave I, as delta "t" increases. This observation is inconsistent with all forward-masking literature, which is based upon N1 functions. Viewing the amplitude of I′ plotted against delta "t" reveals a pattern that is not seen in either of the other two waveforms. A distinct decrease in amplitude from 4.0 ms and 0.1 ms, to 0.8 ms is observed. As these data were analyzed and fit with a line characteristic of a regression of the first order, a pattern within delta "t" values might not be made clear. For this reason, the measures of variability were scrutinized. It turns out that, relative to amplitude, standard deviation scores for waves I′ and I were smaller for delta "t" values from 2.0 ms to 0.4 ms. Clearly, observing characteristics of data which are more consistent should reveal patterns or trends that are more consistent and reliable.

Figure V-1 is the result of plotting the amplitude measurements as a function of these four out of seven delta "t" values. It can be seen that the amplitude functions of wave I′ and wave I are quite similar. It is also important to note the dissimilarity between these functions with the amplitude function of wave $I^0$. Thus, it would appear that these data are consistent with forward-masking effects, which are observed in neural, as opposed to cochlear, potentials.

In viewing the latency function of wave I′, a slight increase is observed as delta "t" values increase. This observation is inconsistent with forward-masking literature for neural potentials, as an increasing ISI should result in a decreasing latency. Furthermore, if I′ is a neural potential, its latency function should approximate that of wave I but not wave $I^0$. It turns out that the latency functions of I′ and I are actually inconsistent. An apparent decrease in latency is observed for wave I as delta "t" increases. Similar to amplitude values, the measures of variability were scrutinized in order to reveal patterns that are more reliable. Statistical analysis of this nature revealed smaller standard deviation scores for the delta "t" values from 4.0 ms to 0.4 ms.

Figure V-2 is the result of plotting the latency

Figure V-1: Mean amplitude as a function of four delta "t" values for waves $I^0$, I′ and I.

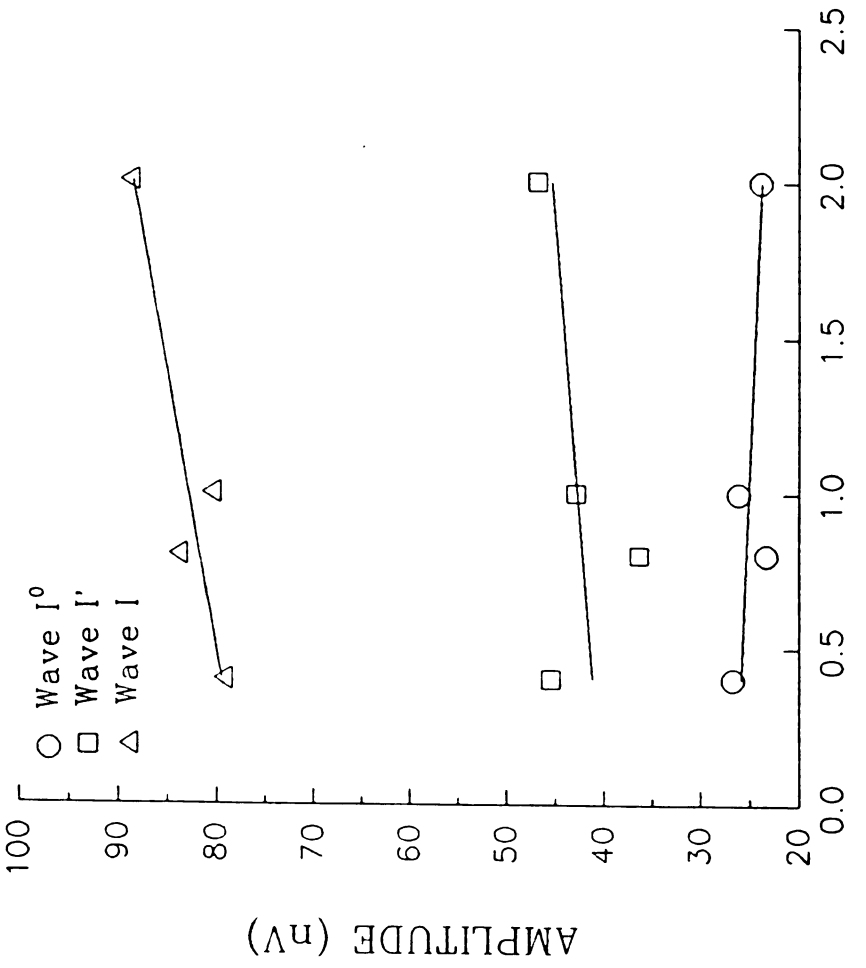


Figure V-1: Mean amplitude as a function of four delta "t" values for waves $I^{o}$, I' and I.

measurements as a function of five delta "t's". Because of the small range in latency measures, the patterns displayed by the waves are similarly small. However, close observation of I' latency reveals a slight, but insignificant, decrease in latency as the delta "t" increases. These data are consistent with adaptation effects as a result of forward-masking. With these properties, these data also support I', in the derived BAEP response, as a neural component. It is also important to note that the differences between the amount of adaptation in an 4.0 ms ISI and a 0.4 ms ISI are most likely small. This could also be the cause for the small changes in latency values across delta "t's".

To understand the morphological behavior of waves I through V in the derived responses, forward-masking literature was, again, consulted. Eggermont (1973a) was the first to describe N1 in both an unadapted and adapted condition with respect to morphology. This investigator used a Gaussian distribution function to describe the shape of an unadapted N1. However, it was noted that a decreasing ISI led to a deviation from the Gaussian distribution curve. A stimulus intensity of 50 dB with an ISI of 4 ms, displayed a broader N1 that was characterized with a "bimodal" or double-peak shape. Eggermont (1973a) explained this phenomenon as the result of N1 being the sum of two

Figure V-2: Mean latency as a function of five delta "t" values for waves $I^0$, I′ and I.

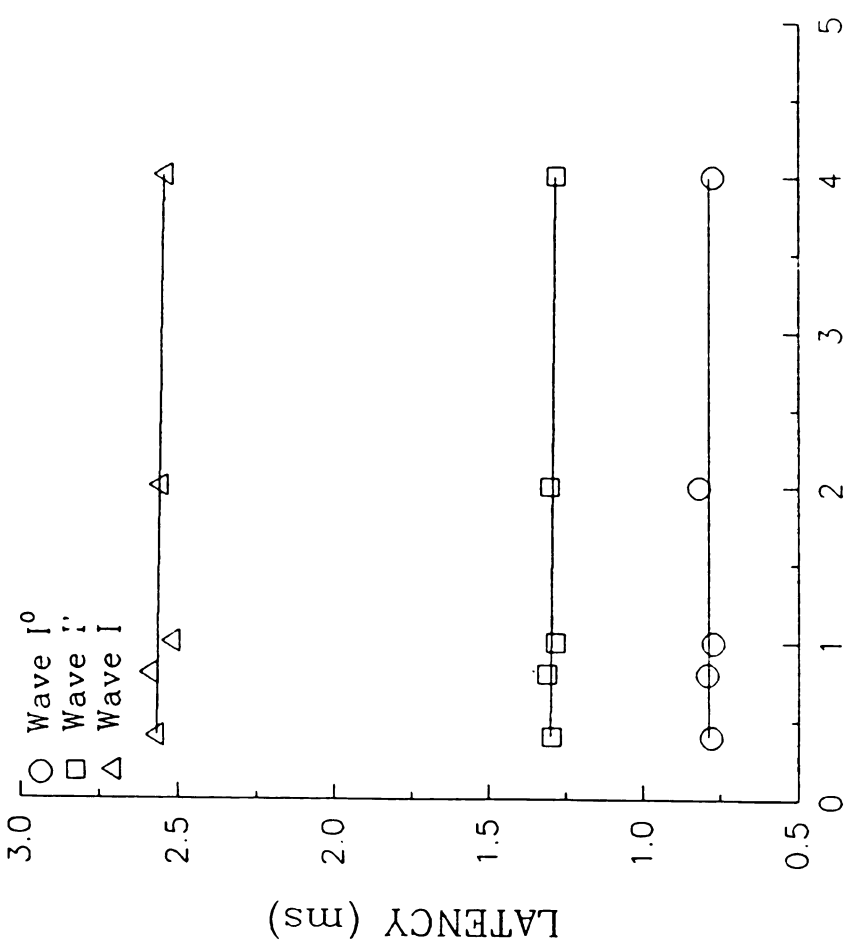


Figure V-2: Mean latency as a function of five delta "t" values for waves $I^{o}$, I' and I.

distributions. Each of the two individual distributions are Gaussian and have approximately the same width but different latencies and amplitudes. These data are consistent with the morphological changes referred to as "peak widening" and "peak splitting" in the derived responses of this study.

Hypothesis #3: Waves I through V remain in the derived BAEP responses because of the summation of PC1 effects in the 35,000 auditory nerve fibers.

According to the basic premise of the "paired-click" stimulus paradigm, PC2 should be delivered during either the absolute or refractory periods of the auditory nerve. This being true, PC2 should not evoke successive APs but should evoke EPSP activity. The calculation of the derived responses was done in order to separate the effects of PC1 from PC2, making it possible to record the GP in the human BAEP. Additional effects, in the form of increased latency and amplitude, were expected in derived responses as well. However, viewing the derived responses revealed peaks I through V. The appearance of these peaks in the derived responses indicates membrane potential fluctuations observed with AP propagation at the time PC2 was initiated. An 0.8 ms delta "t" falls within the absolute refractory period and cannot trigger AP's in the VIIIth nerve; however, peaks I through V remain in 0.8 ms derived responses. Since AP

generation is not possible at this level, it is hypothesized that the residual effects of PC1 summate in the 30,000 nerve fibers, to reveal peaks I through V in derived responses.

## Recommendations for Future Research

Based on the possible limitations and the major findings of this study, the following recommendations for future research were developed:

1) A major limitation of this study was that a direct amplitude and latency shift analysis was not possible, because one of the waves for comparison underwent a derivation process. It is therefore recommended that the effects of PC1 and PC2 be directed into separate channels of the computer and then routed to a digital oscilloscope where individual PC1 and PC2 effects can be more accurately observed.

2) Adaptation literature using ISIs as small as used in this study are unavailable at this time. It is therefore recommended that a "double-click" study, similar to the "paired-click" stimulus paradigm, be developed for the sole purpose of quantifying the decrement seen for PC2 in BAEP responses. Click parameters should mimic those of PC1 and PC2. In order to better observe patterns of amplitude and latency, as a function of delta "t", these values should

range from 5.0 ms to 0.3 ms including a greater number of delta "t" values (e.g., 4.5 ms, 3.5 ms, 3.0 ms, 2.5 ms, etc.) The use of human subjects is a necessity.

3) The use of a small number of subjects but repeated measures were employed in this study in order to establish the reliability and validity of the "paired-click" stimulus paradigm, to record waves $I^0$ and I′. Thus, a larger number of subjects, than was used in this study, might reveal more overt trends or patterns within the data.

4) This study should be done with transtympanic needle electrodes in order to increase the amplitude of waves $I^0$ and I′. This method of recording should allow the investigator to reduce the intensity in small increments, to a point where AP effects are not apparent but EPSP activity remains in BAEP responses.

5) In order to test the clinical applicability of the "paired-click" stimulus paradigm, well defined neural subjects should be tested.

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