This is to certify that the

dissertation entitled

Characterization of an EPSP-Like Potential in the Ventral-Cord of Cricket, Acheta domesticus

presented by

Kamakshi Venkatarao

has been accepted towards fulfillment of the requirements for

Ph.D. degree in Audiology & Speech Sciences

Major professor

Date 4-02-1992

CHARACTERIZATION OF AN EPSP-LIKE POTENTIAL IN THE VENTRAL-CORD OF CRICKET, ACHETA DOMESTICUS

By

Kamakshi Venkatarao

A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements for
the degree of

DOCTOR OF PHILOSOPHY

Department of Audiology and Speech Sciences

1992

ABSTRACT

CHARACTERIZATION OF AN EPSP-LIKE POTENTIAL IN THE VENTRAL-CORD OF CRICKET, ACHETA DOMESTICUS

BY

KAMAKSHI VENKATARAO

In the mammalian auditory system the neuro-transmitter(s), released from the hair cells in the cochlea by mechano-electrical stimulation, excite type I afferent auditory nerve fibers, which can be recorded as post-synaptic potentials. If these potentials are excitatory they are called excitatory post-synaptic potentials (EPSP), and if large enough, will initiate action potentials (AP). A review of the literature has indicated that few investigations have addressed questions about the EPSPs generated by auditory afferent dendrites of the hair cell/auditory nerve synapse. The EPSP is the generator potential which leads to the generation of the AP. In fact, the auditory EPSP is an integral part of the coding mechanism and, if well characterized, may be used as a sensitive index of hair cell-auditory nerve functioning.

Among the studies which have investigated the EPSP, there is a controversy as to whether the recorded EPSP is truly a post-synaptic potential or a summating potential (DC shift of the hair cells of the cochlea in response to basilar

membrane motion). To address this question, house crickets (Acheta domesticus, invertebrates which do not possess a cochlea) were used as experimental subjects. Gross potential recordings were made to identify EPSP-like potentials from the terminal ganglion, which is the first synapse in the cercal hearing system of the cricket, located in the cricket's ventral-cord.

To identify and characterize the EPSP-like potential several experiments were conducted in which various stimulus parameters, tetrodotoxin (a neurotoxin) and kynurenic acid (an excitatory amino-acid antagonist) were used. Results indicated that it is possible to identify and characterize the EPSP-like potential at intensity levels below the threshold for the AP, at frequencies below 2000 Hz, with a repetition rate of 3.1/sec and a rise-decay time between 1.5 and 6.0 ms. Tetrodotoxin significantly reduced the AP but not the EPSP-like potential. Kynurenic acid reduced the amplitude of the EPSP-like potential within a few minutes of its application. It is concluded that the EPSP-like potential is a true neural response and is an integral part of the coding mechanism for neural transmission within the crickets cercal system.

Dedication

To my dear husband Gopal, daughter sushmita, and son Tejas, for their ever present love and support.

ACKNOWLEDGEMENTS

I would like to express my deepest appreciation to my major professor and chairperson of my dissertation committee Dr. Ernest. J. Moore for his knowledge, understanding, inspiration, intellectual stimuluation, invaluable help, and for the positive role he played as my mentor. I would like to sincerely thank the members of my dissertation committee Dr. Jerry. L. Punch; Dr. John. B. Eulenberg; and Dr. John. I. Johnson for their help, guidance, support and encouragement. Special appreciation goes to Mr. Randy Robb, for his unflinching technical support. My special thanks to Dr. Connie Osborn and Dr. Charles Lowrie who contributed their time, effort and expertise to this study. I once again wish to thank Dr. Ernest. J. Moore, past chairperson and Dr. Michael Casby, present chairperson of the Department of Audiology and Speech Sciences, Michigan State University for providing the facilities and financial support which allowed me to undertake this venture. Finally my very special thanks go to my husband Gopal, daughter Sushmita, and son Tejas for their constant encouragement, support and understanding.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF SYMBOLS AND ABBREVIATIONS

Action Potential (AP) = The cell membrane of neurons when excited undergoes permeability changes which produce an electromechanical all-or-none disturbance that is actively propagated along the length of the axon. The associated voltage change is termed a spike or action potential.

Compound action potential (CAP) = CAP is the sum of the action potentials of many individual neurons which are firing nearly simultaneously to acoustic stimulation.

Excitatory post synaptic potentials (EPSPs) and inhibitory post-synaptic potentials (IPSPs) = The electrical effect at the post-synaptic membrane site is dependent upon the neuro-transmitter substance released by the pre-synaptic membrane. Two types of activity exist, excitatory and inhibitory. For the excitatory post-synaptic activity the transmitter produces a local depolarization by increasing membrane permeabiltiy for K^+ and Na^+. For the inhibitory post-synaptic potentials the transmitter increases the permeability to K^+ and Cl^- causing hyperpolarization and a decrease in neuron excitability. EPSPs are also referred to as generator potentials, and if large enough will generate action potentials.

EPSP-like potential = Excitatory post-synaptic potentials that are not recorded intracellularly or in a single unit, but as a gross potential from surface recordings.

Gross potential = A gross potential is interpreted as the summed effect of massed action potentials travelling in a volley down the auditory nerve. Electrodes that are too large to record from a single neural element can record summed neural activity produced by the simultaneous activation of many neurons.

No = No is the neural negative wave preceding the EPSP-like potential.
Po = Po is the neural positive wave preceding the EPSP-like potential and following the No potential.

N1 = N1 is the first dominant negative wave of the gross neural response recorded to suprathreshold acoustic stimulation.

N1/P1/N2 complex = The complex consists of the first dominant negative potential, followed by the first positive potential and the second negative potential generated by the nerves during acoustic stimulation at suprathreshold levels.

I' = I' is a positive wave which precedes the wave I potential in humans. It may correspond to the Po potential in animals.

Cochlear microphonic (CM) = The deflection of the basilar membrane in response to a sound produces a microphonic potential in outer hair cells, which follows the movement of the basilar membrane and occurs in the chain of events before an EPSP or an action potential.

Summating potential (SP) = SP is a baseline shift in the positive or negative voltage resulting from stimulation of the cochlear partition and can be recorded as a direct current electrical response from the cochlea. It is also considered as a receptor potential generated by the cochlea hair cells.

Chapter I

INTRODUCTION

Synaptic potentials are produced by brief alterations in the electrical properties of the membrane potential. The membrane potential is maintained primarily by three ionic species, Na^+, K^+ and Cl^-. The membrane is able to maintain a separation of charge which is positive outside and is negative inside, since it acts as a permeability barrier to the diffusion of ions. The separation of charge is responsible for the resting negative membrane potential (Kandel, 1985).

To generate an action potential, the membrane potential must be depolarized or made less negative by reducing the charge separation across the membrane. Within a certain range of membrane potentials, only generator potentials are evoked, and a small inward-outward current produces a small depolarization/repolarization. If the inward-outward current is larger, the depolarization/repolarization is, of course, larger. As an active process, the all-or-none action potential (AP) is generated only when the membrane potential reaches a critical or threshold level (Koester, 1985).

Applying the concept of generalized electrical signaling properties of nerve cells to auditory afferent nerve fibers, we know that hair cells, which are receptors of mechano-electrical stimuli in the organ of Corti, release neuro-transmitter(s) which activate the peripheral dendrites of the auditory nerve type I spiral ganglion fibers.

The release of the neuro-transmitter substance is preceded by a number of stages (Dallos, 1984). Briefly, vibrations of the basilar membrane of the cochlea, in response to mechano-electrical stimuli, lead to the bending of hair cell stereocilia. This bending of the stereocilia opens ionic channels for passage of ions, the most likely candidate is K^+, which releases intracellular stores of Ca^{2+} and brings about depolarization of the hair cells. The depolarization of the hair cells may be recorded as electrical potentials, namely, cochlear microphonic (CM), and summating potential (SP). The depolarization in the hair cells results in the release of a neuro-transmitter substance. The neuro-transmitter substance(s) in turn excite the dendrites of the type I auditory afferent fibers, leading to generation of post-synaptic potentials. If the post-synaptic potential is excitatory (EPSP), and if it reaches threshold, this will lead to the generation of APs (Dallos, 1984; Siegel and Dallos, 1986).

In reviewing the literature on auditory nerve potentials, it is of interest to note that identification and

characterization of the EPSP generator potential, which leads to the generation of the AP is not addressed. In other words, depolarization of hair cells and the subsequent release of a neuro-transmitter substance(s) does not lead to an AP, but rather, leads to the generation of an EPSP, which if adequate, leads to the generation of an AP. Thus, the EPSP is an important step in the generation of the AP and should not be ignored. In fact, if the EPSP is adequately characterized, it may prove to be a more sensitive index of the activity of the output of the hair cell rather than the AP of the auditory nerve.

There have been only a few studies which have investigated the auditory EPSP in animals (Furukawa and Ishii, 1967; Flock and Russell, 1973, 1976; Furukawa, 1978; Furukawa and Matsuura, 1978; Furukawa, Hayashida and Matsuura, 1978; Furukawa, Kuno and Matsuura, 1982; Siegel and Dallos, 1986; Palmer and Russell, 1986; Sewell, 1990). In humans, Hughes and his colleagues (Hughes and Fino, 1980; Hughes, Fino and Gagnon, 1981; Hughes and Fino, 1985) have successfully recorded an early wave called "I'", a potential which precedes wave I of the auditory brain stem response (ABR) which is thought to be an EPSP. They have suggested that the generator for I' may be the most distal part of the auditory nerve. Benito, Falco and Lauro (1984) have confirmed the presence of a wave before wave I with a latency of approximately 1.0 msec, which they called the "I^{O}" wave. Moore and Semela (1985) have also recorded a

potential which precedes wave I of the ABR in humans which they called "BI". It has been determined, however, that I', I^{O} and BI are the same response (Moore, Semela, Rakerd, Robb and Ananthnarayan, 1991). I' is therefore the designation of choice since it enjoys precedence in the literature.

Moore and his colleagues (Moore, Caird, Klinke and Löwenheim 1988a and 1988b; Klinke, Caird, Löwenheim and Moore, 1988; and Moore, Caird, Löwenheim and Klinke, 1989) have recorded an I'-like potential in the cat and gerbil, called Po. Using an experimental paradigm in which intracochlear infusion of tetrodotoxin (TTX) was used, they found a reduction in the amplitude of the N1/P1/N2 complex (first negative, first positive and second negative potential) of the AP, but not Po. TTX is known to block Na^{+} channels which serve as precursors for generation of the AP. When kynurenic acid (KYNA) or L-glutamic acid diethyl ester (GDEE) was infused into the cochlea following TTX, Po diminished (KYNA and GDEE are antagonists to certain neuro-transmitter substances). These results would appear to indicate that Po is a post-synaptic potential whose appearance is related to the kinetics of a neuro-transmitter substance. Thus, Po is perhaps a neural response and is not a part of the AP response, since it is not affected by TTX.

Another group of investigators (Xi, Dolan and Nuttal, 1989; Dolan, Xi and Nuttall, 1989) have reported similar results. These investigators found that in guinea pigs, TTX abolished

the AP, leaving a residual slow negative "EPSP-like" potential, without altering the CM or the SP. However, when kainic acid was infused into the cochlea the EPSP-like potential was eliminated, leaving the CM and SP intact. They indicate that the origin of the EPSP-like potential may be the unmyelinated dendrites innervating inner hair cells, and these potentials may reflect summed EPSPs from depolarization of afferent dendrites of the auditory nerve.

Since in vertebrates there are concerns as to whether Po (or the equivalent EPSP-like potential preceding AP) is a true neural response or a SP, it would be of interest to determine if an EPSP-like potential exists in an invertebrate preparation, such as house cricket (Acheta domesticus). The cricket may serve as a model of mammalian hearing since it does not possess a cochlea, and thus, the SP should not be present.

Most work on cricket audition has focused on its role in intraspecies communication. Acoustic communication mediates many important social functions including mating courtship, inter-male aggression, inter-male spacing and detection of predators. Sound reception in the cricket is accomplished by two structurally and neurophysiologically different mechano-receptors (Pumphrey and Rawdon-Smith, 1936; Edwards and Palka, 1974; Counter, 1976), namely, the tympanal organ in the tibia of the prothoracic legs and the cerci located on the abdominal appendages.

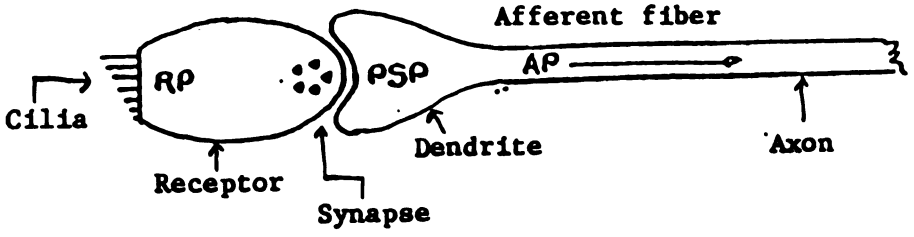
The abdominal cercus is of interest to us since it appears that the response in the cercal nerve is similar to that of the mammalian eighth nerve (Pumphrey and Rawdon-Smith, 1936). The analogy between the mammalian auditory system and the cricket cercal hearing system is schematically represented in figure I-1. Figure I-1A represents synaptic transmission in the mammalian cochlea-afferent auditory complex, while figure I-1B depicts the synaptic transmission in the cricket cercal hearing system. Each hair cell contains freely articulated cilia, while each cercus is covered with freely articulated trichoid sensilla or hair mechano-receptors. There are hundreds of mechano-receptors on each cercus, which respond to light puffs of air and to sound of adequate intensity over a broad frequency spectrum. A similar mechanism of response is obtained from mammalian hair cells. Each of the sensilla are singly innervated by dendritic processes of cercal sensory nerves while each inner hair cell is innervated by several afferent fibers. The axons of the cercal nerve synapse ipsilaterally on branches of a few giant interneurons in the fifth abdominal ganglion (5AG) (Gnatzy, 1976). The axons of the auditory nerve synapse at the spiral ganglion. A group of ten giant interneurons project from the 5AG on the right and left sides, and run anteriorly in the ventral nerve cord, synapsing along the way to the prothoracic brain. Auditory nerve fibers synapse at the level of the cochlear nucleus as they course through the brain stem on their way to the auditory cortex

Figure I-1A: Schematic representation of the mammalian hair-cell auditory afferent synapse. RP = receptor potential, PSP = post-synaptic potential, AP = action potential, receptor = hair cell.

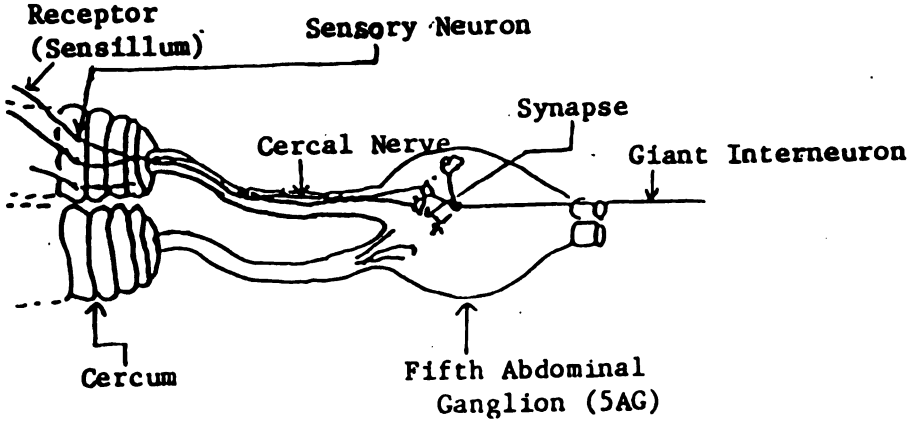
Figure I-1B: Schematic representation of the receptor afferent fiber in the cricket cercal hearing system.

Figure I-1A & 1B

A. Mammalian Hair Cell-Auditory Afferent System



B. Cricket Cercal Hearing System



Auditory stimulation of the cerci evokes correlated spike impulses in the cercal nerve and giant post-synaptic interneurones (Edwards and Palka, 1974). Auditory stimulation (with appropriate parameters) of the mammalian ear produces synchronous responses from the auditory afferent nerve fibers. Electro-physiological recordings also indicate that the cercal auditory system can decode stimulus information by narrow tuning in individual cells and by synchronous discharge patterns (Counter, 1976a).

A thorough review of the results of the literature failed to reveal any information about the characterization of the EPSP in the cercal hearing system of crickets. Thus if we are to understand electrophysiological events within the cercal hearing system, there is a need to characterize the EPSP in crickets. Of interest to us also is the nature of the neuro-transmitter substance(s) at the cercal nerve/ interneuron synapses in the 5AG.

The purpose of this investigation was to characterize the EPSP-like post-synaptic potentials in the cricket. The term EPSP-like is used instead of EPSP since we are making gross potential recordings and there is no evidence that the slow negative potential is a true EPSP. In so doing, the first step was to identify a N1/P1/N2 complex of the cricket auditory system. Thus first prominent negative deflection was designated as N1, the positive peak following N1 was designated P1. If there was a negative peak following P1 it

was designated as N2. Careful observations were made to identify consistent negative-positive waves prior to N1, and they were designated as No and Po, respectively. In the presence of N1, it was not possible to identify the EPSP-like potential. In order to identify the EPSP-like potential, various stimulus parameters and a neurotoxic substance were used. Further, a broad spectrum excitatory amino acid antagonist was used in order to diminish the No-Po/EPSP-like potentials since it was surmised that they were due to the action of the neuro-transmitter substance utilized by the afferent auditory fibers in the fifth abdominal ganglion.

Questions

The experimental investigation was designed specifically to answer the following questions:

1. Is it possible to identify an EPSP-like potential in extracellular recordings of the ventral cord of Acheta domesticus, whose different components can be designated as No-Po/EPSP-like potentials?

2. Following identification of the No-Po/EPSP-like potentials, what stimulus parameters such as frequency, intensity and time best characterize the post-synaptic potentials using latency and amplitude functions?

3. Using pharmacologic agents such as tetrodotoxin and kynurenic acid, how do they assist in the identification of the No-Po/EPSP-like potentials?

Chapter II

REVIEW OF LITERATURE

Introduction

The purpose of this investigation was to characterize the auditory excitatory post synaptic potential (EPSP or EPSP-like) which can be recorded from the fifth abdominal ganglion of the house cricket (Acheta domesticus). In order to accomplish this goal, the EPSP-like response and the compound action potential (CAP) were recorded using gross potential recordings. The EPSP-like potential was identified and characterized using various stimulus parameters. Given, however, that the EPSP is normally masked by the much larger AP, a neurotoxin tetrodotoxin (TTX), was used to suppress the action potential (AP). Upon suppression kynurenic acid (KYNA), a broad spectrum excitatory amino acid antagonist, was used to investigate its effect on the EPSP-like potential.

In the following review, I will discuss the various electrical potentials and their underlying mechanisms, the auditory EPSP, neuro-transmitters in the mammalian inner ear, the cricket auditory system and the analogy between the cricket and mammalian auditory system. The aim of this

chapter is to integrate all of the relevant information that already exists and build a strong basis for the conduct of this investigation.

Synaptic Transmission

Usually in the nervous system, the formation of a conducting pathway does not involve direct anatomical continuity between one neuron and another, or between a neuron and an effector organ. An axon gives rise to many expanded terminal branches known as pre-synaptic terminal boutons. The post-synaptic component of the synapse is usually a dendrite (axodendritic synapse), body (axosomatic synapse), or part of another axon (axoaxonic synapse). At the sites of apposition, both the pre-synaptic and post-synaptic membranes exhibit a plaque of electron-dense material on their cytoplasmic surfaces. Beneath this plaque on the pre-synaptic membrane and within the pre-synaptic bouton, there are vesicles ranging in diameter from 10 to 50 nM and often numerous mitochondria. Synapses can be electrically or chemically mediated (Kandel, 1985).

It is generally accepted that within chemical synapses the AP does not cross the synaptic cleft, but instead, causes the release of a neuro-transmitter at pre-synaptic terminals. The release of a transmitter requires the entry of extracellular calcium ions into the pre-synaptic terminal upon arrival of the AP. The transmitter molecule then

crosses the gap, binds to receptors on the postsynaptic membrane, which then gives rise to post-synaptic potentials. There are at least two kinds of post-synaptic potentials. One is similar to the spike potential, which tends to decrease the resting potential. Hence, it is a depolarizing post-synaptic potential and is referred to as an excitatory post synaptic potential (EPSP). The other one tends to increase the resting membrane potential, and thus it is a hyperpolarizing post-synaptic potential referred to as an inhibitory post-synaptic potential (IPSP). EPSP and IPSP depend on the transmitter receptors and are present in different proportions in different cells.

Excitatory Post Synaptic Potentials (EPSP)

During excitation a potential appears in recordings from the post-synaptic cell which is a sign of post-synaptic depolarization. This slow potential change that appears prior to the AP is called the EPSP. It consists of a depolarization of the membrane and can best be seen when the extent of depolarization is insufficient to give rise to an AP. When the membrane potential falls to a critical value the post-synaptic neuron fires, and an AP appears superimposed on the EPSP. Temporal summation of EPSPs is possible. If two subliminal volleys are transmitted over the same nerve, each volley produces an effect which is manifested by an EPSP. The EPSPs will then sum, and if a critical level of depolarization is reached in time, an AP

will be initiated (Eccles, 1959).

Characteristics of the EPSP

The EPSP is monophasic and nonpropagating, and as mentioned above, represents a depolarization of the post-synaptic neuron. The EPSP from a neuron usually has no maintained plateau; the potential returns to rest once the peak is reached. Unlike the AP, the EPSP is not an all-or-none response, since it can be augmented by increasing the intensity of the input stimulus. EPSPs of different inputs can sum on a post-synaptic cell to produce a greater depolarization (Eccles, 1959). These characteristics show that the EPSP is produced by a process which is fundamentally different from that of the AP.

One difference is that the movement of sodium (Na^+) and potassium (K^+) during the AP is somewhat sequential; during the EPSP it is simultaneous. The differences in ion movement during the AP and EPSP can be explained by differences in the molecular properties of the ion channels responsible for these two signals. During the AP, membrane depolarization leads to the opening of two independent channels first, one selective for sodium, and then another selective for potassium. To produce the EPSP, the transmitter opens a special channel whose size and shape allow both Na^+ and K^+ to pass with nearly equal permeability. It may also allow larger cations, but anions

are excluded. This cation selectivity suggests that the channel has a negative charge at its mouth that attracts a variety of cations below a certain size, but repels anions because of their different charge.

Another difference between an AP and an EPSP is that the increase in Na^+ influx which produces the AP is regenerative, whereas Na^+ influx produced by the EPSP is not regenerative. The Na^+ and K^+ channels responsible for the AP are voltage sensitive, since both are opened by depolarization and closed by hyperpolarization. The opening of the channel responsible for the EPSP is not controlled by voltage, but rather, depends on the concentration of a specific chemical transmitter. As a result, the depolarization produced by the transmitter does not lead to further increases in the number of channels that are open, which explains to some extent why EPSPs are small and additive as compared to an AP. Furthermore, the channels opened by the transmitter for an EPSP differ pharmacologically from those opened by the AP. Thus, the influx of Na^+ produced by an EPSP is not blocked by TTX, while the influx of sodium through the voltage-gated Na^+ channel responsible for the AP is blocked (Kandel and Siegelbaum, 1985).

Integration of Signals

In order to generate an AP, the membrane potential has to reach a threshold critical for spike generation. EPSPs drive the membrane potential toward threshold; however, the amount of depolarization that is brought about by a single EPSP is far less compared to what is required for an AP. EPSPs have the property of summing spatially and temporally, enabling the membrane potential to reach threshold for spike generation (Eccles,1959). The cell bodies of some neurons cannot generate an AP, thus in those neurons in which bodies can trigger an AP, the threshold for spike generation in the cell body is usually high, but in the trigger zone which is in the initial segment of the axon, it is relatively low. The trigger zone is called the integrative component of the neuron since at this region, the excitatory and inhibitory inputs are summed. The cell will fire only if the excitation exceeds the inhibition at the trigger zone by a critical value.

Evolution of Ears

In order to receive sound and vibration, a variety of sense organs have developed in vertebrates and invertebrates. When an organ is specialized so that it preferentially responds to sound, it is then considered an auditory receptor. Animals that have some type of an auditory organ

include the Arthropods among invertebrates, and most creatures derived from bony fishes among vertebrates (Dallos, 1984). The fundamental building block of all ears is the mechanoreceptor sensory cell. In invertebrates this cell is a primary sensory cell that possess a single modified cilium. The arthropod ear may consist of only a single receptor cell or as many as several thousand. These sensory cells are incorporated within a variety of auditory accessory structures.

Accessory structures are developed to convert airborne sounds to mechanical displacements and to deliver these to the mechanosensitive receptive cells. In vertebrates the receptor itself is not part of the sensory neuron, but is a specialized epithelial receptor cell. In this system the sensory neuron receives its information from the receptor, generally via a synaptic path. The sensory cell in all vertebrate auditory organs is a hair cell, its function being to convert relative displacements between cilia and the cell body into the outflow of electrical activity.

Transduction in the Inner Ear

The auditory periphery is basically a transduction mechanism in which acoustic signals are transformed into neural impulses that are processed by the auditory peripheral nervous system. The auditory system has a conductive mechanism, and hydromechanical sensorineural system. The

conductive mechanism consists of the external ear and the middle ear. The external ear is composed of the pinna, external auditory meatus, and the tympanic membrane which conducts acoustic signals to the middle ear. The acoustic resonant properties of the outer ear, in particular the size and shape of the ear canal, can affect the spectrum of sound that reaches the tympanic membrane. The middle ear is an air-filled cavity containing a chain of three ossicles, the malleus, the incus and the stapes, connecting the tympanic membrane to the oval window. The middle ear transmits sound energy from the external auditory meatus to the cochlea through the vibrations of the three ossicles.

The cochlea is a fluid-filled chamber that is divided into three compartments, scala vestibuli, scala tympani and scala media. The division is made superiorly by Reissner's membrane and inferiorly by the basilar membrane. The cochlea is coiled in a spiral shape along its length from the base to the apex. At the base there are two openings into the middle ear. The stapes fits into the oval window which opens into the scala vestibuli, the round window opens into the scala tympani. The scala vestibuli and scala tympani communicate at the apex and are filled with perilymph. The scala media contains endolymph, a fluid of different ionic composition. These fluid-filled compartments comprise the hydromechanical parts of the system. The auditory transducer is the spiral organ of Corti, which is located on top of the basilar membrane, the

latter which divides the scala media from scala tympani (Pickles, 1982).

The sensory hair cells of the spiral organ of Corti convert mechanical energy into electrical energy and cause an outflow of synaptic transmitter(s). Thus, hair cells may be thought of as transducers (Pickles, 1982). Identification of the chemical properties of cochlear sensory cells and neurons, and their neuro-transmitters, remain a major goal for understanding cochlear function.

Briefly, the process of transduction includes the following stages. As a result of the vibratory motion of the basilar membrane, internal deformations of the organ of Corti transmit mechanical energy to the hair cells. The most commonly accepted concept (Dallos, 1984) is that this transmission occurs due to a relative motion between the tectorial membrane and the matrix of the organ of Corti cells that contains the sensory cells. This relative motion translates into a bending of the stereocilia and some form of deformation of the apical pole of the hair cells, resulting in the generation of receptor potentials, this is the first stage of transduction. However, the actual site of the receptive region has not been identified, although there have been various speculations. Portions of the infranuclear segment of the cell are specialized as the presynaptic region, where presynaptic structures such as vesicles are presumed to store synaptic transmitters.

It is considered that the release of synaptic transmitters is controlled by receptor currents generated at the apical pole of the cell (Hudspeth and Corey, 1977; Dallos, 1984). The release of these transmitters by such currents constitutes the next stage of transduction. The chemical transmitters that are presumed to diffuse across the synaptic cleft between the cell body and the afferent nerve endings depolarize the postsynaptic membrane. The resulting postsynaptic potential is conducted to the electrically excitable portion of the nerve fiber, where it initiates a train of APs.

The nerve fibers within the organ of Corti are classically divided into two main classes: afferents and efferents. Afferents refer to the neurons which carry electrical activity from hair cells to the brain. Efferents refer to the neurons located in the brain stem which carry electrical activity from the brain stem to the cochlea. The afferent system can further be divided into two subtypes, the dendrites of the large myelinated spiral ganglion cells (90 to 95% of the total population; Spoendlin, 1972) are radially connected with the inner hair cells (IHC) and are called type I fibers, while the dendrites of the small type II ganglion neurons are spirally connected with the outer hair cells (OHC), one neuron is in contact with a large number of cells in the three rows of OHC. Similarly, the efferent system also has two subtypes, the lateral system, originating essentially in the ipsilateral superior olive,

terminating below the IHC and forming synapses with the radial auditory dendrites. The medial efferent system arising mainly in the contralateral trapezoid body of the brain stem makes direct synapses with OHC bases (Pujol and Lenoir, 1986).

Synapses at the IHC Level

The afferent innervation of IHC is provided by fibers of type I myelinated ganglion cells (Kiang et al., 1982). These fibers are called dendrites when they lose their myelin sheath upon entering the organ of Corti. They course radially from the habenula perforata to contact the basal pole of the IHC. Each IHC is connected with at least 20 afferent dendrites. In all species, a constant and typical synaptic junction has been described. This synapse is characterized by pre- and post-synaptic membrane densities, and pre-synaptic specialization which is generally a synapatic body surrounded by microvesicles. The synaptic cleft is a narrow gap which is approximately 10 nM in diameter in the cat (Liberman, 1980) and 20 nM in diameter in humans (Nadol, 1983).

The question as to the identity of the neuro-transmitter acting between hair cells and afferent fibers, as well as efferent terminals, has attracted a great deal of interest. The incorporation of afferent transmitters into the postsynaptic membrane causes a depolarization, leading to

EPSPs which have been recorded in afferent nerve fibers (Furukawa and Ishii, 1967; Flock and Russell, 1976; Furukawa, 1978; Furukawa and Matsuura, 1978; Furukawa, Hayashida and Matsuura, 1978; Palmer and Russell, 1986; Siegel and Dallos, 1986; Sewell, 1990). The most likely transmitters have been identified as L-glutamate and L-aspartate or a closely amino acid type (Bobbin and Thompson, 1978; Guth and Melamed, 1982; Wenthold, 1985; Klinke, 1986). However, identity of the receptor subtypes and ion channels involved in the process need to be further investigated.

Auditory EPSP

Gross potential recordings of the auditory system are of particular interest since they can be recorded in human subjects as well as experimental animals. Intracellular recordings are made only in experimental animals. Signal averaging techniques are typically used to enhance the recordings relative to random noise. Sound-evoked voltage reponses recorded by electrodes placed at a close distance to the cochlea in animals and humans are a complex summation of electrical activity emanating from the inner ear. The response includes the summating potential (SP), cochlear microphonic (CM), and the whole nerve compound action potential (CAP).

The CAP is a summation of synchronous discharges of auditory nerve fibers, presumably each of which is preceded or

accompanied by an EPSP (Siegel and Dallos, 1986). The EPSP is believed to be the result of hair cell transmitter, opening ligand-activated ion channels in the auditory nerve dendrites that synapse on inner hair cells (Furukawa and Matsuura, 1978). In the mammalian auditory system the peripheral processes of the afferent fibers that proceed from the habenula perforata to the inner hair cell are unmyelinated (Spoendlin, 1974). The EPSP generated at the synapse below the IHC would thus produce a graded potential that propagates passively in the unmyelinated peripheral process until reaching the area of the habenula perforata where myelination begins. At the point of myelination, the EPSP evokes all-or-none spike or AP discharges.

A thorough review of the literature in auditory physiology indicates that EPSP generation has not been extensively investigated. Only a few studies have identified the EPSP in evoked potential recordings. One possible reason could be that we do not yet know the optimum stimulus parameters and recording sites that elicit the best EPSP responses. Since in the past optimum settings have not been used, the EPSP may be masked by the more dominant components such as the SP, CM and the CAP.

It is important to mention, however, that depolarization of the hair cells (reflected as CM and SP) perhaps leads to neuro-transmitter release from the hair cells on to the dendrites of the auditory afferent fibers, resulting in

excitation in the nerve fibers and reflected as an EPSP. The EPSP, if adequate, will lead to AP generation. Thus, it would appear that the EPSP is an integral part of the coding mechanism of auditory nerve activity. If we can successfully characterize the EPSP, it may contribute significantly to our basic understanding of auditory neurophysiology. I shall now briefly discuss those studies that have successfully recorded the EPSP (or EPSP-Like potentials) by using intracellular or extracellular recordings.

Furukawa and colleagues (Furukawa and Ishii, 1967; Furukawa 1978; Furukawa and Matsuura, 1978; Furukawa, Hayashida and Matsuura, 1978; Furukawa, 1981; Furukawa, Kuno and Matsuura, 1982; Furukawa and Matsuura, 1985) recorded subthreshold potential variations in the goldfish, which they called the generator potential (GP). The GP was found to be graded in size, and when its magnitude was adequate, initiated spike or AP potentials. The GP was found to have a time course not much different from that of the AP, and its presence was evident only when it was not obscured by a spike potential. With continuous stimulation there was a rapid decline of the GP and the spike potentials, but not of the microphonic potentials. There was a delay of 0.6-0.8 ms between the microphonic potentials and the GP. These features suggested that the GP is the excitatory postsynaptic potential (EPSP). An interesting observation was that even after the EPSP had been completely adapted to a continuous sound, a vigorous

discharge of new EPSP was observed when the intensity of the sound was increased. On application of a step decrement in the sound intensity, the amplitude of the EPSP showed a decremental response in which the amplitude was reduced, but soon returned to a new steady level appropriate to the decreased sound intensity. Statistical analysis revealed that the decrease in the mean quantal content of the neurotransmitter during step decrement and an increase in the mean quantal content during step increment was directly associated with the readily available store (which was proportional to the intensity of the stimulus), rather than the mean probability of release.

Dallos and Cheatham (1974) described generator potentials as graded electrical responses that mediate the initiation of all-or-none discharges in the fibers of the auditory nerve. The generator potentials presumably arise from the unmyelinated dendritic portion of the neurons. They suggest that these potentials have not been identified experimentally with certainty, however, postulate that a negative component of the average SP might be a gross, remote, manifestation of the generator potential.

Flock and Russell (1976) electrically stimulated the efferent nerve fibers of the lateral line hair cells in the fresh water cod, the burbot Lota lota and recorded the IPSP intracellularly. The IPSP were accompanied by a decrease in the resistance of the hair cell membrane and an increase in

the intracellular receptor potential. Recordings from afferent nerve fibers to mechanical stimulation indicated small spontaneous and evoked EPSPs. The EPSPs were reduced in amplitude for the duration of the IPSP.

Palmer and Russell (1986), while investigating the high frequency limit of phase locking in the hair cells and the cochlear nerve of the guinea pig, found spontaneous and acoustically evoked voltage fluctuations in afferent nerve fibers. The time difference between the onsets of hair cell and post synaptic potentials was found to be 0.83 ms, and was attributed to the synaptic delay at the hair cell/afferent nerve fiber junction.

Siegel and Dallos (1986) recorded from the afferent fibers innervating the IHC in guinea pigs. They could record spontaneous activity ranging from 1.3 to 136 spikes/sec with highly irregular interspike intervals. They also found that in some instances there were EPSPs which failed to elicit APs. A preliminary assessment of the slopes of the rising phase of the EPSPs indicated that the EPSPs that did not trigger all-or-none spikes had a slower rising phase than those EPSPs that triggered spikes.

Sewell (1990) investigated the EPSP and the AP in the afferent fibers of the lateral line organ of Xenopus laevis (African clawed toads). He used micropipette recordings to study the discharge rates in the afferent nerve fibers

innervating the hair cells organized into discrete clusters called neuromasts. Perfusion of the synapse with a solution containing cobalt and manganese diminished the discharge rate in the afferent fibers for the period of time over which the agents were present. This supports the hypothesis that cobalt, an agent blocking voltage dependent transmitter release, affects the spontaneous EPSP, an indication that the spontaneous discharge in afferent fibers is due to voltage-dependent release of the transmitter from hair cells.

Surface Electrode Recordings of the EPSP

Hughes and Fino (1980) and Hughes, Fino and Gagnon (1981) recorded auditory brain stem responses (ABR) in humans using piezoelectric earphone. They identified an early scalp positive deflection at approximately 1.1 ms which they called I'. They concluded that I' was not a part of CM, since it did not change in polarity for clicks of opposite polarity. They stated that I' was more specifically related to the post-synaptic potential arising in the afferent terminals of the eighth nerve fibers in response to the depolarization brought about by the action of chemical transmitters.

Benito, Falco, and Lauro (1984), investigating brain-stem auditory evoked potentials, demonstrated a wave before wave

I with a latency of approximately 1.0 msec. The response was more readily identifiable at high intensity levels, which they called "I^{O}".

Hughes and Fino (1985) presented data indicating the possible generator of I'. They plotted distributions of amplitude and latency throughout the scalp for each of the positive and negative waves of the ABR in 20 normal ears. The isopotential maps for I' were consistent with the suggestion that the generator may be the distal part of the eighth nerve.

Moore and Semela (1985) recorded the whole nerve AP using surface electrodes in humans. They have consistently identified a positive peak (which they initially designated as BI) with a latency of 0.6 to 1.2 msec and an amplitude of 30 to 70 nV. When they repeated several stimulus parameters along the dimensions of frequency, intensity and time, a consistently graded potential was recorded and was thought to be generated by the post synaptic region of the cochlea.

Moore, Caird, Klinke and Löwenheim (1988a and 1988b), Moore, Caird, Löwenheim and Klinke (1989) and Klinke, Caird, Löwenheim and Moore (1989) recorded the ABR and the I'-like potential from the round window of cats and gerbils, and called it "Po". They found that when the intensity of the stimulus increased the Po latency decreased and its amplitude increased. The latency and intensity of Po varied

in a

and

used

in t

wave

acid

diet

TTX

mas

tim

mas

is

The

wou

to

aff

per

not

sod

Xi,

(198

(198

the

indi

leav

the

the

in a manner which closely paralleled the behavior of wave I and II of the ABR. In an interesting paradigm in which they used intracochlear infusion of TTX, they found a reduction in the amplitude of the N1/N2 complex of the CAP and ABR waves, but not Po. However, when a broad-spectrum amino acid antagonist kynurenic acid (KYNA) or L-glutamic acid diethyl ester (GDEE) was infused in the cochlea following TTX, Po diminished along with the N1/N2 complex. Forward masking experiments showed Po to have a different masking time-decay function than both N1 and CM. Simultaneous masking with high-and low-pass filtered noise showed that Po is generated at the high frequency end of the cochlea. These results reveal several concepts: First, these data would appear to indicate that Po is a potential that is due to perhaps transmitter release (post-synaptic), since it is affected by drugs acting on the synapse. Secondly, it is perhaps a neural potential but is not the AP, since it is not affected by TTX, which is known to block fast-acting sodium channels.

Xi, Dolan, and Nuttall (1989) and Dolan, Xi, and Nuttall (1989) have reported results similar to those of Moore et al., (1988, 1989). They used tone bursts as stimuli to record the CAP from the round window of guinea pigs. Results indicated that TTX abolished the N1-P1-N2 (AP complex), leaving a residual slow negative potential without altering the CM or the SP. Kainic acid (KA) eliminated the AP and the slow negative potential, leaving the CM and SP intact.

From the results obtained, the investigators postulated that the origin of the slow negative potential may be the responses from the unmyelinated dendrites innervating the inner hair cells, and this may reflect summed EPSPs and depolarization of the afferent dendrites. The slow negative potential is referred to as the EPSP-like potential.

Glutamate as the Neuro-Transmitter in the Inner Ear Afferent System

Excitatory amino acids mediate perhaps as many as four distinct receptor systems (Cotman and Iversen, 1987; Watkins and Olverman, 1987). Three of these, the N-methyl-D-aspartate (NMDA), Kainate (K) and Quisqualate (Q) receptors, are named after artificial agonists that preferentially activate a single receptor subtype. Recently one other type of receptor that has been described is the α-amino-3-hydroxy-5-methyl-4-isoxazole-proprionic acid (AMPA), in which the agonist activates an intrinsic channel similar to the kainate receptor (Barnard and Henley, 1990). Selective NMDA antagonists are 3-3(2-carboxypiperazine-4-yl-propyl-1-phosphonic acid) (CPP) and D-2-phosphonopentanoic acid (D-AP5). Both K and Q receptors are unaffected by these NMDA antagonists.

Recently the discovery of the Quinoxalinediones (Qu), a series of potent and selective antagonists at K and Q amino acid receptors has opened the possibility for extensive

studies of these non-NMDA receptor subtypes (Honore et al., 1988). Some of the potent non-NMDA receptor antagonists used so far are, 6,7-dinitro-quinoxaline-2,3-dione (DNQX), 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) and 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo quinoxaline (NBQX), among several others. Of the described compounds acting at the Qu site, 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo quinoxaline (NBQX) has been found to be more potent than either DNQX or CNQX (Sheardown et al., 1990). The affinity of NBQX for the K receptors was about 30 times less than its affinity for Q receptors, but was found to have a very low affinity for the NMDA receptors. The fourth class of the excitatory amino acid receptor is defined by the antagonist action of L-2-aminophosphonobutyric acid (L-AP4). There are no potent antagonist for this class of receptor.

Bobbin and Thompson (1978), Bobbin (1979), Comis and Leng (1979), and Bobbin, Bledsoe and Chihal (1981) investigated the effects of various putative neuro-transmitter on afferent cochlear transmission in the guinea pig, and suggested glutamate and aspartate as the afferent NT. They also support the contention that the post-synaptic receptor is not of the NMDA type, but rather it may be a kainate or quisqualate receptor subtype.

There have been a number of investigators (Jenison and Bobbin, 1985; Pujol, Lenoir, Robertson, Eybalin and Johnstone, 1985; Jenison, Winbery and Bobbin, 1986; and

Bobbin and Ceasar, 1987) who have injected within the guinea pig cochlea several non-NMDA antagonists such as kynurenic acid, γ-D-glutamylamino-methylsulfonic acid, etc. The non-NMDA antagonists were found to reduce the magnitude of the Compound AP of the cochlear nerve. Puel, Bobbin, and Fallon (1988) examined the effect of 2-amino-4-phosphonobutyric acid (2-APB) on the cochlear potentials of guinea pigs, and found that it had no effect on the auditory nerve potentials.

Littman et al (1989) investigated the effect of various Qu, i.e., DNQX, CNQX, 6,7-dichloro-3-hydroxy-2-quinoxalinecarboxylic acid (DHQX), and 3-hydroxy 2-quinoxalinecarboxylic acid (3HOC), on cochlear potentials in guinea pig. They found dose-related suppression of the CAP, a prolongation of N1 latency at suprathreshold levels, elevation of CAP threshold and a decrease in N1 latency at CAP threshold. None of the drugs had any effect on CM or SP.

All the above studies have one trait in common, i.e., they have all investigated the effect of the various antagonists on the single nerve fiber AP, or CAP. The AP, however, is not the first post-synaptic potential that can be recorded from the inner ear. As was stated above, an AP is not generated if the EPSP does not reach threshold. Thus, it would be of interest to examine the effect several of these various antagonists on the EPSP.

There have been only a few studies which have investigated the effects of glutamate antagonists on the EPSP. As cited previously, Moore et al.,(1988, 1989), Xi et al.,(1989), and Dolan et al.,(1989) have successfully demonstrated that with TTX administration it is possible to diminish the amplitude of the AP. A reduction in amplitude is not seen for the slow EPSP-like response, which remains until an amino acid competitive antagonist is administered. However, all the animals used in these studies were mammals, and thus, they possess a cochlea. It has been argued that the remaining slow negative potential could be a part of the receptor potentials from the hair cells in the cochlea namely SP. A method which may clarify this ambiguity is to identify the EPSP in an animal model which does not posess a cochlea. Hence, I propose using the house cricket (Acheta domesticus) as a model to characterize the auditory EPSP-like potentials in the fifth abdominal ganglion, which is the first synapse in the cercal hearing system of crickets.

General Features of House Cricket (Acheta domesticus)

The body of the cricket is rather slender, and is divided into three main parts, the head, thorax and abdomen. Mean body length of medium males is approximately 15.9 mm, and that of females is about 15.7 mm. The tegmina nearly reaches the end of the abdomen; hind wings are either short and covered by tegmina, or extend considerably beyond the wings. The posterior femur is short and slender and the

ovipositor is slightly longer than the posterior femur. Their general color is brown and head has four dark reddish brown transverse bars (The New Encyclopedia Britannica, 1984).

House crickets are essentially domiciliary, but may cause economic damage. Rearing the house crickets for fish bait and pet food is a large industry in some parts of the United States. Several of the species are used extensively in experimental laboratory studies of insect physiology.

Classification

The house cricket is classified as follows (Randell, 1964): Superphylum, Arthropoda; Phylum, Entoma; Class, Insecta; Order, Grylloptera; Suborder, Gryllodae, Superfamily, Grylloidae, Family, Gryllidae; Subfamily, Gryllinae; Genus, Acheta Fabricius; Species, Acheta domesticus; Common name, House cricket (cricket).

Crickets are often said to exhibit programmed behavior. The nervous system controls the behavior which detects, decides and reacts by triggering appropriate responses. It includes input elements, such as the sense organs, eyes and ears which sense changes in the surroundings, decision centers which evaluate the many inputs in order to choose a response and output elements which direct that response.

The nervous system is an organized network of individual nerve cells. The neurons are of three functional types, sensory neurons, interneurons and motor neurons. The individual nerve cells are similar to that of humans, but are fewer in number. They have neurons clustered together in a CNS into which sensory nerves extend, and from which motor signals are sent. The brain is in the head capsule and the nerve cord is ventral.

The basic anatomical unit of the the cricket nervous system is the ganglion, and almost every major segment begins with a mass of nerve cells in an embryonic ganglion. The three main ganglia in the head are the protocerebrum, the deutocerebrum and the tritocerebrum, all of which are fused to form the brain, or supraesophageal ganglion. The remainder of the ganglionic chain lies below the alimentary canal against the ventral body surface and consists of the prothoracic, mesothoracic and metathoracic ganglia. The abdomen has five ganglia; the fifth abdominal ganglion (5AG) is of special interest to us, and will be discussed in greater detail later.

The Cricket Auditory System

Crickets have a highly specialized auditory system capable of analyzing different temporal and spectral characteristics of sound in their environment. The auditory system mediates

specific phonotactic responses of female crickets to the calling song of conspecific males, acoustic interaction between males, and escape reactions of both males and females to sounds produced by certain predators.

Sound reception in the cricket is accomplished by two structurally different mechano-receptors (Pumphrey and Rawdon-Smith, 1936; Edwards and Palka, 1974; Counter, 1976). One is the tympanal organ in the tibia of the prothoracic legs, while the other system is located on the abdominal appendages of cerci, which arise from the embryonic terminal abdominal segment.

Tympanal Receptors

Tympanal organs involved in the transmission of sound to the body of the cricket and in its conduction to auditory structures include the tympanal membranes, spiracles and trachea (Pumphrey and Rawdon-Smith, 1936; Edwards and Palka, 1974; Oldfield, 1988). In order to excite receptor cells, sound pressures act on mechanical sound receptors. There are three potential sound receiving structures in the ear, the anterior tympanal membrane (ATM), the posterior tympanal membrane (PTM), and the partition between the anterior and the posterior tracheal branches. Movement of each of these air-exposed structures may cause movement of receptor cells relative to a group of supporting cells.

The receptor cells are positioned on the anterior tracheal branch (ATB) without direct connection to either tympanal membrane. Each auditory receptor is tuned to a specific sound frequency. The frequency of sound to which the receptors are tuned increases progressively from the proximal to the distal ends of the arrays. The range of sound frequencies represented in the auditory organ encompasses those used for acoustic communication, with an emphasis on specific biological importance. This emphasis of specific frequencies is achieved by the presence of several receptors tuned to the same sound frequency.

The auditory receptors have individual axons that project from the auditory organ to the primary auditory neuropile in the CNS (Pumphrey and Rawdon-Smith, 1936; Katsuki and Suga, 1960; Oldfield, 1988). These axons are several millimeters in length and enter the CNS at the level of the prothoracic ganglion. The ascending interneurons have terminal arborizations in the subesophageal ganglion.

The Cercal Receptors

The function of the cercal receptors in the class insecta was first investigated by Pumphrey and Rawdon-Smith (1936). They described the cercus as a primitive hearing organ, analogous to the hair structures in the mammalian cochlea in both manner of innervation and function. Pumphrey and Rawdon-Smith also found similarity in the cercal receptors

among the insects.

Anatomy of the Cerci

The cerci of the crickets are paired segmented sensory organs located at the tip of the abdomen. They are equipped with numerous sense organs, especially mechanoreceptive sensilla (see figure II-1).

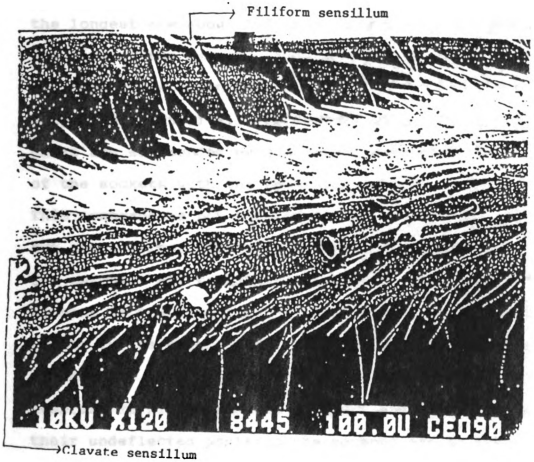
The sensilla and sensory neurons are formed from epidermal cells and lie beneath the cuticle. The cerci, the sensilla, cercal nerve innervation and the terminal abdominal ganglion are somewhat similar among different types of crickets. They are not very different from the cockroach as related to features and functions. Giant interneurones with considerable morphologic and functional similarities are present in crickets, cockroaches and locusts (Boyan and Ball, 1989). Hence, anatomical and physiologic description of cercal hearing in Acheta domesticus is sometimes also reinforced by investigations that have dealt with insects other than Acheta domesticus.

There are three types of exclusively mechanoreceptive sensilla on the cricket cerci, campaniform sensilla, filiform hairs and clavate hairs. There is also a similarity in the fine structure of the dendrite insertion

Figure II-1: Micrograph of a cercum from one of the crickets used in this investigation. (The electron micrograph was taken by Willard Hooks Jr, Graduate student, Neuro-Audiologic Lab, Department of Audiology and Speech Sciences, Michigan State University).

The long hair structures seen prominently in the picture are the filiform sensilla; the short round ones represent the clavate sensilla.

Figure II-1


Filiform sensillum
10KU X120 8445 100.0U CE090
Clavate sensillum

in the three sensilla (Edwards and Palka, 1974). A striking feature of the campaniform sensilla is their close proximity to the sockets of the filiform hairs (Edwards and Palka, 1974). The campaniform sensilla are evenly distributed over the dorsal and ventral surfaces, and tend to be aggregated in transversely aligned groups.

There are approximately 700 filiform hairs on one cercus; the longest are about 1.5 cm in length and are situated on the base of the cercus. Approximately one-fourth of the filiform hairs which have long shafts are flanked by campaniform sensilla. The sockets of the longer filiform hairs are surrounded by a membrane which connects the base of the sockets with the remaining cuticle of the cercus. This membrane is not equally spread out in each direction, but has a larger extension in the direction of the tip of the cercus. All sockets surrounded by such a membrane can be bent in the direction of its largest extension, which points to the cercus tip. Upon release, deflected sockets immediately return to their undeflected position, since the membrane is highly elastic (Dumpert and Gnatzy, 1977). In their undeflected position the sockets are to some extent inclined towards the cercus tip and form an angle with the surface of the cuticle of about 70°. The amplitude of the receptor potential depends on the direction in which the hair is bent (Gnatzy, 1976).

The filiform hairs on the cercus are arranged in a very

characteristic fashion. Hairs on the dorsal and ventral aspects of the cercus vibrate transversely (T-hairs) to the long axis of the cercus. Hairs on the medial and lateral aspects of the cercus vibrate longitudinally (L-hairs). Bacon and Murphey (1984) extended the description of these mechanoreceptors by showing that each of the two main hair population is composed of two sub-populations, L-hairs are anterior and posterior, T-hairs are lateral and medial.

The club-shaped clavate hair sensilla are gravity receptors. They are situated ventrally on the base of the cerci which deflect differentially according to body position. The distal part of each sensillum consists of a fluid-filled club, which extends from a thin shaft inserted in a cuticular socket on the cercus.

The shaft of the sensilla has an opening that lies about three microns above the vertex of the cuticular wall. This opening is called the ecdysial canal (Gnatzy, 1976). The sensillum on the cerci of crickets is innervated by a single bipolar sensory cell (Gnatzy, 1976). The cell body is located peripherally and gives rise to a distal dendrite at its apex and an axon near its base. Within the proximal portion of the distal sensory process there are numerous branches of the ciliary root called rootlets. More distally, these coalesce to form a single root. The dendrite swells to form the dendrite dilatation, and then constricts markedly at the level of the ciliary base.

Within the dendrite the ciliary root crosses the dendrite dilatation and enters the tubular body.

The tubular body is a highly organized structure consisting of approximately 700 individual microtubules. The tubular body is supposed to play an essential role in the transduction of the mechanical stimuli because of its location at the site to which the stimulus force is transmitted. Microtubules in the sensory dendrite are arranged in different patterns, with free microtubules distributed over the entire length of the dendrite, microtubules in the ciliary transitional region, and densely packed microtubules situated in the tip of the dendrite comprising the tubular body (Erler, 1983).

The oscillating direction of each sensilla is determined by the structure of the insertion points of the hairs (Gnatzy, 1976). The side of depolarization corresponds to that on which the ecdysial canal opens to the outside. During deflection of the sensilla, the cuticular projection at the hair base presses against the membrane surrounding the tubular body, leading to bending of the microtubules of the tubular body.

The axon of the sensory cell surrounded by accompanying glial cells joins the cercus nerve. The sensory cell is surrounded by two glial cells which have their nuclei in the basal region of the sensory cell. The glial cells are

completely enclosed by the innermost of the enveloping cells (Gnatzy, 1976).

The Cercal Nerves

Sensory axons arising from cercal sensilla assemble in groups which coalesce to form two bundles in the base of the cercus. These bundles fuse to form the cercal sensory nerve. More proximally the cercal nerve is variably joined by sensory nerves from neighboring integumentary structures, and a group of neurosecretory axons bound for the rectal wall. The cercal motor nerve which innervates the extrinsic cercal musculature leaves the ganglion close to the cercal sensory nerve, but the two are invariably separate (Edwards and Palka, 1974). Light and electron micrographs indicate that the distal sections of the cercal nerve contains about 10,500 axons (in human, there are about 30,000 axons of the auditory nerve).

The cercal nerve is dominated by fibers in the range of 0.1 to 0.5 microns. Longitudinal sections of the cercal nerve show that individual axons maintain uniform diameter over distances of at least 2.0 mm. Cercal sensory fibers degenerate rapidly after severance from their peripheral cell bodies. Axons appear to travel in spatially associated patterns in the cercal nerve. As they enter the terminal abdominal ganglion, however, they interweave in a complex fashion, raising the possibility that they are sorted according to systematic pattern before terminating in the

region of giant fiber dendrites, the later of which are located in the 5AG (Edwards and Palka, 1974).

Hair Deflection and Stimulus Transmission

With stimulation, the hair shaft moves away from its resting position, in the direction of greatest mobility within the plane. The stimulus is conducted to the dendrite as soon as deflection of the hair shaft begins, the cuticular peg at the base of the hair moves perpendicular to the long axis of the dendrite. This leads to compression of the tubular body (Gnatzy and Tautz, 1980). Since the tubular body is bilaterally symmetrical, the area affected by the pressure is several times larger. The depolarizing stimulus for the receptor cell, which is a local increase in pressure across the membrane, causes an indentation of the dendrite. Thurm, Erler, Godde, Kastrup, Keil, Volker, and Vohwinkel (1983) determined that the movement of the filiform hair base is towards the dendrite, at least in crickets. In these sensilla approximately 1.0 Å is required to initiate a nerve impulse. An adequate increase in pressure across the dendrite induces, after a latency of approximately 50 to 70 µs, an increase of the membrane conductance, thus modulating the receptor current. The sensory signal coded in the conductance change is transmitted by the receptor current to the pacemaker site for cercal nerve impulses. In other words, when the sensilla is deflected in the preferred direction, impulses are generated by a depolarizing receptor

potential, and if threshold is reached, it will lead to action potentials in the axons. The axons travel in spatially associated patterns in the cercal nerve and enter the 5AG where they interweave in a complex fashion. These axons are sorted according to modality before synapsing with the giant fiber dendrite (Edwards and Palka, 1974).

Fifth Abdominal Ganglion (5AG)

The terminal or the fifth abdominal ganglion is recognizably larger than the other abdominal ganglia. The 5AG is embryologically derived from the fusion of five segmental ganglia (seventh through eleventh), and morphologically is found to be a simple structure amenable to cell counts (Gymer and Edwards, 1967). It innervates segments 7 through 10 through dorsal and ventral mixed nerves. The volume of the 5AG is found to increase about 40-fold from hatchling (first instar) to adult. The volume of the adult ganglion was found to be $84.90 \times 10^6 \mu^3$. The total number of cells in the 5AG range from 3400 in the first instar to about 20,000 in the young adult.

The neuropile of the terminal ganglion is dominated by the processes of the giant interneurons, which lie in two lateral tracts interconnected by anterior and posterior commissures. Fibers from the cercal sensory nerve terminate principally in two regions of the terminal ganglion. The major projection of the cercal nerve, in the normal adult

cricket, is to the posterior ipsilateral quadrant where a loosely organized spherical glomerulus appears to contain the majority of the cercal axons. Others terminate along two oblique dorsoventrally oriented lines. All fibers from the cercal nerve which synapse in the terminal ganglion appear to do so on the ipsilateral side (Edwards and Palka, 1974; also see figure I-1).

Bacon and Murphey (1984) investigated afferent projections of the filiform hairs on to the terminal ganglion using staining techniques. They found that the filiform hair afferents project only into the cercal glomerulus, a well defined club shaped region of neuropile. Sections cut through the glomerulus revealed that it was a hollow, shell-like structure. Bacon and Murphey also found a strong correlation between the directional selectivity of a sensory neuron and the position of its terminals within the glomerulus. Lateral-T hair afferents were found to project to the lateral region of the cercal glomerulus. More distal hairs had afferent neurones which projected more anteriorly within this region. The medial-T hairs project to the posterior and dorsomedial regions of the glomerulus. Anterior-L hairs were found to project to a region of the glomerulus that extended from a ventrolateral position and arched up more dorsally towards the midline. Most of the posterior-L hair afferents projected to a hook-shaped region of the glomerulus, although some of them projected contralaterally to the homologous ventromedial region of the

neuropile.

Bacon and Murphey (1984) also examined the structure of the medial giant interneurones (MGI) and lateral giant interneurones (LGI) to determine whether the position of dendrites within the afferent projection is correlated with their known excitatory receptive fields. They recorded simultaneously from MGI and a posterior-L afferent hair afferent to which it appeared to make contact. By triggering the oscilloscope sweep with the action potentials of the sensory neurone, they observed EPSP's up to 1.0 mV in amplitude and at a latency of 3.0 ms. The EPSP's that exhibited slow rise and fall times, were found to be very labile. Consistent with the observations of synaptic connections between single afferents and the interneurones, the receptive fields paralled predictions based on structure.

Blagburn, Beadle, and Sattelle (1984) used a cobalt injection technique in investigating the synapse between an identified giant interneurone and a filiform hair sensory neurone in the terminal ganglion of cockroach nymphs, which have cerci bearing only two functional filiform hair sensilla. They found synaptic vesicles clustered around bar-shaped pre-synaptic densities. The average length of each bar was found to be 150-300 nM, width was 20-30 nM, and height 30-60 nM, and some thickening of the post synaptic region was also observed. Horse radish peroxidase (HRP)

staining of one giant interneuron showed its dendrites forming synaptic contacts with the large identifiable axon of the lateral filiform hair sensory neurone. The giant interneuron population of the ventral nerve cord comprises a group of ten axons with diameters between 20 and 40 µM. These interneurons are the most prominent interneurons in the cricket nervous systems.

Mendenhall and Murphey (1974) mapped the giant interneurons in the cricket, using the technique of back-filling with cobalt chloride from the axons in the connectives. They found 8-10 giant axons greater than 20 µM in diameter in the connective anterior to the terminal ganglion with, their somata and major dendritic processes in the 5AG. The giant interneurons were arranged in three tracts, a dorso-lateral, a ventro-medial and a ventro-lateral tract. These groups maintained a constant position in the nervous system from the terminal ganglion anteriorly through the thoracic ganglion, with some of the cells projecting as far as the sub-esophageal ganglion. The giant interneurons were found to be derived from one of the five segmental ganglia, and shared several common features. The soma of the giant interneurons were located laterally near the anterior most edge of their respective ganglion. The commissural processes of each interneuron crossed the midline in the same sequential fashion within the boundaries of each primitive segment. Major dendritic arborizations were located in more posterior positions as compared to the soma.

Electrophysiology of the Cercal System

Pumphrey and Rawdon-Smith (1936) investigated the anal cercus of the cricket and the cockroach due to the similarity between cercal nerve responses of the two animals and eighth nerve responses in mammals. They described three important types of cercal hairs. Of the three types, the long slender hairs distributed on the ventral side of the cercus were found to be the most important, since application of petroleum jelly to the ventral surface abolished the nervous response to sound stimulation.

The cercal receptors were found to be extremely sensitive to gross movements of the surrounding air. There was a great deal of similarity between the cercal responses of the cricket and the cockroach, suggesting that the similar kind of cercal receptors mediate the response in both. Physiologically, the response to sound stimuli were found to resemble in many respects the mammalian cochlear nerve. At lower frequencies the response were synchronized with the stimulus frequency. At higher frequencies, synchrony was partial and persisted only during the initial time of stimulation. At lower frequencies, i.e., below 400 Hz, the cerci were found to respond to both phases of the stimulus. At frequencies higher than 600 Hz, the opposite responses were obtained, i.e., one response per two cycles of the stimulus, which was attributed to lengthening of the refractory period.

Edwards and Palka (1974) recorded responses from the abdominal nerve connectives of the cricket to tones, airpuffs, and substrate vibrations. They found substantial ongoing spike activity in the range of 1.0-2.0 mV. The giant fibers found in the connective of the nerve responded to sound stimuli ranging from about 2000 Hz and below, to sub-audio frequencies, with greater sensitivity at lower frequencies, with a peak at approximately 400 to 600 Hz. The preferred direction of a sound source for the giant fibers of each side was approximately at right angles to the shaft of the corresponding cercus. The interneurons were found to respond rather weakly to substrate vibration, with a peak sensitivity to about 2000 Hz.

Rozhkova and Polishchuk (1976) investigated the electrical activity of the connectives between the fourth and fifth abdominal ganglia of crickets in response to stimulation of the cerci using frequencies from 20 Hz to 3.0 KHz. The goal of the investigations was to determine the orientation of the cerci relative to the body and its importance to directional sensitivity. They fixed the cerci in the necessary position with pins impeding movement, and except for the cercal nerves, all other strucutures joining the cerci to the body of the cricket were sectioned. As a result, the giant neurones completely lost sensitivity to the direction of the signal and their diagrams of directional sensitivity became practically uniform. Hence, they concluded that due to deprivation of information of the

position of the cercus, the giant neurones sum the signals of different receptors equally from all directions.

Counter (1976a) made electrophysiologic intra-and extra-cellular recordings of the response patterns of the intact cerci of adult crickets to well-controlled acoustic stimulation. The focus of the investigation was to obtain absolute auditory thresholds for the cercal sensory nerve and the lateral post-ganglionic giant interneuron in crickets, and compare them with the corresponding post-ganglionic thresholds in the cercal system of the adult cockroach. The results indicate a frequency response from 30 to 3000 Hz. It is significant that the pre-synaptic elements are consistently more sensitive than the post-synaptic axons, with the cricket response more sensitive than that of the cockroach. It is also of interest that the sensory cercal nerve of the cricket is capable of sinusoidal synchrony from 300 to 600 Hz, is asynchronous above 600 Hz, but even the most sensitive of the post-ganglionic units were found to be asynchronous above 60 Hz. Acoustic stimulation of the cercal receptors evoked characteristic discharges in several small and a few large fibers anterior to the terminal ganglion. The conduction velocity of large and small fiber populations in the postganglionic connectives was found to be 5.5 and 2.3 M/sec. Spontaneously active elements in the post-ganglionic units in a single connective increased their activity significantly when the cercal nerve contralateral to the

connective under study was severed at a point near the ganglion. Transection of the ipsilateral cercal nerve, however, resulted in a significant reduction in spontaneous spike discharges.

To analyze more precisely the acoustic parameters of the call song of the cricket, Counter (1976b) analyzed spectrally the call song using digital Fourier transformation and measured electrophysiologically the hearing capacity of the cricket's tympanal nerve. The waveform and spectral analyses showed the call song to be a stereotyped chirp of three pulses, each of which had a dominant carrier frequency of 4600 Hz, resulting from the rate at which the individual teeth of the pars stridens are struck by the plectrum. The electrophysiologically determined audiogram of the tympanic nerve responses indicated that the most sensitive portion of the curve corresponds well with the carrier frequency of 4600 Hz.

Tobias and Murphey (1979) investigated the physiology of the receptor sensory neuron and the directional response of cricket giant interneurons to an airpuff stimulus. They made extracellular recordings of the afferent axons of the cercal nerve, intracellular recordings from the medial giant interneuron and extracellular recordings of the giant interneurons. Results were similar to those found by Edwards and Palka (1974), in which there were two populations of mechanoreceptive hairs on cricket cerci,

those that moved in the longitudinal axis (L), and those in the transverse (T) axis. Further, Tobias and Murphey found two subgroups proximal and distal in each of the L and T fibers, based on their unidirectional response curves. The giant interneurons were found to be directional only to an airpuff, but not to acoustic stimuli. They speculate that the directional response properties of the interneurons is the sum of excitatory input via the receptors on one cercus and inhibitory input from the other.

EPSP Recordings

Pichon and Callec (1970) described the oil-gap technique that they used to record extracellularly, excitatory post-synaptic potentials in a single post-synaptic giant axon in the terminal abdominal ganglion of the cockroach. There were several advantages of using the oil-gap technique namely, a low-impedance preparation, a low-noise level, good stability and unequivocal identification of the post synaptic element at the level of which the recordings were made. This permitted efficient irrigation of the ganglion allowing for better pharmacological studies. When extracelluar EPSP recordings were compared with intracellular recordings, the former were smaller and slower; however, the extracellular post-synaptic action potentials were similar or larger than those recorded with intracelluar electrodes.

Using the same technique, Callec, Guillet, Pichon and Boistel (1971) investigated input-output post-synaptic events to stimulation of the cercal mechanoreceptor. Simultaneous recording of pre-synaptic and post-synaptic activity revealed that in most cases, each spike at the receptor initiates an EPSP. There was a delay of about 0.85 ms between the pre-synaptic spike recorded where it enters the ganglion and the rising phase of the corresponding EPSP. The 0.85 ms delay corresponded to the conduction time in the pre-synaptic element and to the synaptic delay.

Using increasing levels of electrical stimulation of the homolateral cercal nerve, there was a smooth increase in the EPSP. When the post synaptic depolarization was larger than 3-10 mV, it gave rise to a full-size 100 to 120 mV AP. Hence in this type of integrative synapse, it was necessary to summate the action of numerous sensory impulses in order to trigger a propagating AP.

It was found that even in the presence of an AP, the EPSP remained, indicating that the site of initiation of the EPSP and of the AP are likely to be differentially located. The investigators studied also the effects of repetitive stimulation at different repetition rates. They obtained data at sub-threshold levels so as to avoid spikes, and isolated the ganglion from spontaneous sensory inputs by severing the cerci. In order to suppress the interference with depression or potentiation, the rest interval between

two volleys was maintained for 3-5 minutes. Repetitive stimulation of pre-synaptic fibers resulted in a reduction of the post-synaptic events. Greater repetition rates resulted in a more rapid decline of amplitude. If the preparation was unstimulated for a period of about 3 to 5 minutes, the EPSP's could be restored.

Boyan and Ball (1989) examined the responses to cercal stimulation of the giant interneuron (GIN1) in the terminal abdominal ganglion of the locust. The goal was to demonstrate that the GIN1 receives input from primary afferents and from a spiking local interneuron. Following dye injection the terminal ganglion was dissected, and the GIN1 was found to have a lateral cell body, an axon which exited the ganglion anteriorly via the contralateral connective, and an extensively branched posteriorly directed dendrite. The response of the GIN1 to wind stimuli directed at the cerci, or electrical stimulation of the cercal nerve, consisted of depolarization supporting spikes, evoked by input from filiform afferents, and a suprathreshold depolarization evoked by input from an interneuron. Simultaneous recording of a filiform afferent intracellularly from its terminals in the ganglion, and extracellularly from its axon in the cercal nerve, showed that spikes are conducted to the ganglion in this afferent. Spikes obtained from the intracellular recordings of a filiform afferent were used to trigger a signal averager into which post-synaptic potentials from GIN1 were led.

Triggering from the spikes in the afferent established that each AP evoked an EPSP 1.4 ms later in GIN1. With mechanical stimulation of the filiform hairs on different parts of the cercus, intracellular recordings from a dendritic branch of GIN1 showed that several filiform hairs evoked an EPSP in the GIN1. The shape of the EPSP evoked varied with respect to both rise time and duration, suggesting that the input synapses for the different afferents were located at different distances from the recording site. The response of GIN1 to electrical shocks applied to the cercal nerve always consisted of an intial EPSP and a spike of constant short latency followed by a delayed spike which was most probably evoked by an interneuron.

Neuro-Transmitters in the Abdominal Ganglion

The chemical nature of neurotransmitter substances in the cricket ganglion relies on biochemical, ultrastructural and electrophysiological data. The six criteria for the identification of transmitter substances have not been satisfied in this preparation, largely due to the difficulties of working with a complex tissue. However, there are several studies which implicate cholinergic mechanism for excitatory synaptic transmission.

Callec and Sattelle (1973) used the oil gap method to record

extracellular potentials from the abdominal ganglion of the cockroach using pharmacologic agents. Mechanical stimulation of cercal receptors was brought about by using a puff of air to initiate depolarization of the post synaptic cell. Greater intensity of stimulation resulted in further depolarization, at times reaching threshold for spike generation. Electrical stimuli also produced EPSP and AP. Synaptic potentials recorded from both whole ganglion and isolated post-synaptic fibers provided similar potentials. The EPSP were smaller in amplitude to AP. Application of acetylcholine on the de-sheathed abdominal ganglion caused a reduction in the amplitude of the EPSP. A number of experiments were conducted in which the acetylcholine was applied to the abdominal ganglion. They indicate that the EPSPs are a sensitive monitor for drug action.

Meyer and Reddy (1985) characterized the central cholinergic binding sites of putative acetylcholine responses (AcHR) in the terminal ganglion of crickets. They conducted binding studies using the muscarinic antagonist L-[H]quinuclidinyl-benzilate (QNB), and the nicotinic antagonist bungarotoxin (BGT). Results indicate that a large population of putative AcHRs in the cricket terminal ganglion specifically binds the QNB, and therefore, is predominantly muscarinic in its pharmacologic profile. There were also a separate class of sites in the terminal ganglion that selectively bind to the nicotinic ligand BGT, and at least a portion of these AcHRs were found in the cercal giant interneuron pathway.

Interestingly, there are no investigations which have addressed the possibility of excitatory amino acids as neuro-transmitters in the terminal ganglion of the house cricket.

In summary, several investigations have addressed the auditory EPSP in humans and laboratory animals. However, due to the controversy as to whether the recordings made are truly EPSPs or receptor potential from the cochlea it is very essential at this point in time to clarify this problem. In this study the house cricket is used as a model to characterize the EPSP, as the cricket does not possess a cochlea, but exhibits the cercal hearing system. The receptors for the cercal hearing system are freely articulated sensilla. There are hundreds of these sensilla on each of the cercum. The sensilla are innervated by sensory neurons whose axons combine to form the cercal nerve, which synapse on to the giant interneurons in the terminal abdominal ganglion and the EPSP's are investigated in this synapse. An analogy is drawn between the cercal hearing system's first synapse and the mammalian first synapse, which is in the hair cell-auditory nerve of the inner ear. In order to be able to use the cricket as a model, it is essential to first establish a reliable technique to record, identify and characterize the EPSPs hence the purpose of the present investigation.

Chapter III

INSTRUMENTATION AND PROCEDURE

The instrumentation employed in this investigation can be basically discussed under the following sections: stimulus generating, analog, digital and display sections.

Stimulus generating section: The stimulus generating section consists of the following components:

- .Computer (IBM PC-AT 80286)
- .Power source (Modular Instruments, Inc, or MI^2)
- .Dual function generator (MI^2 208)
- .Dual attenuator (MI^2 108)
- .Filter (Frequency devices, Low pass filter 901F)
- .Amplifier (Technics power amplifier, SE-9060)
- .Level discriminator (MI^2 104)
- .Data controller timer (MI^2 214)
- .Speaker (Realistic Minimus-3.5)

A simplified block diagram of the instrumentation shows the experimental set-up used in this investigation (Figure III-1). Basically, tone bursts were generated by a custom-written software program, and were fed to the speaker from the function generator, dual attenuator, low-pass filter, and amplifier. The data controller timer was used to

Figure III-1: Block diagram of the instrumentation

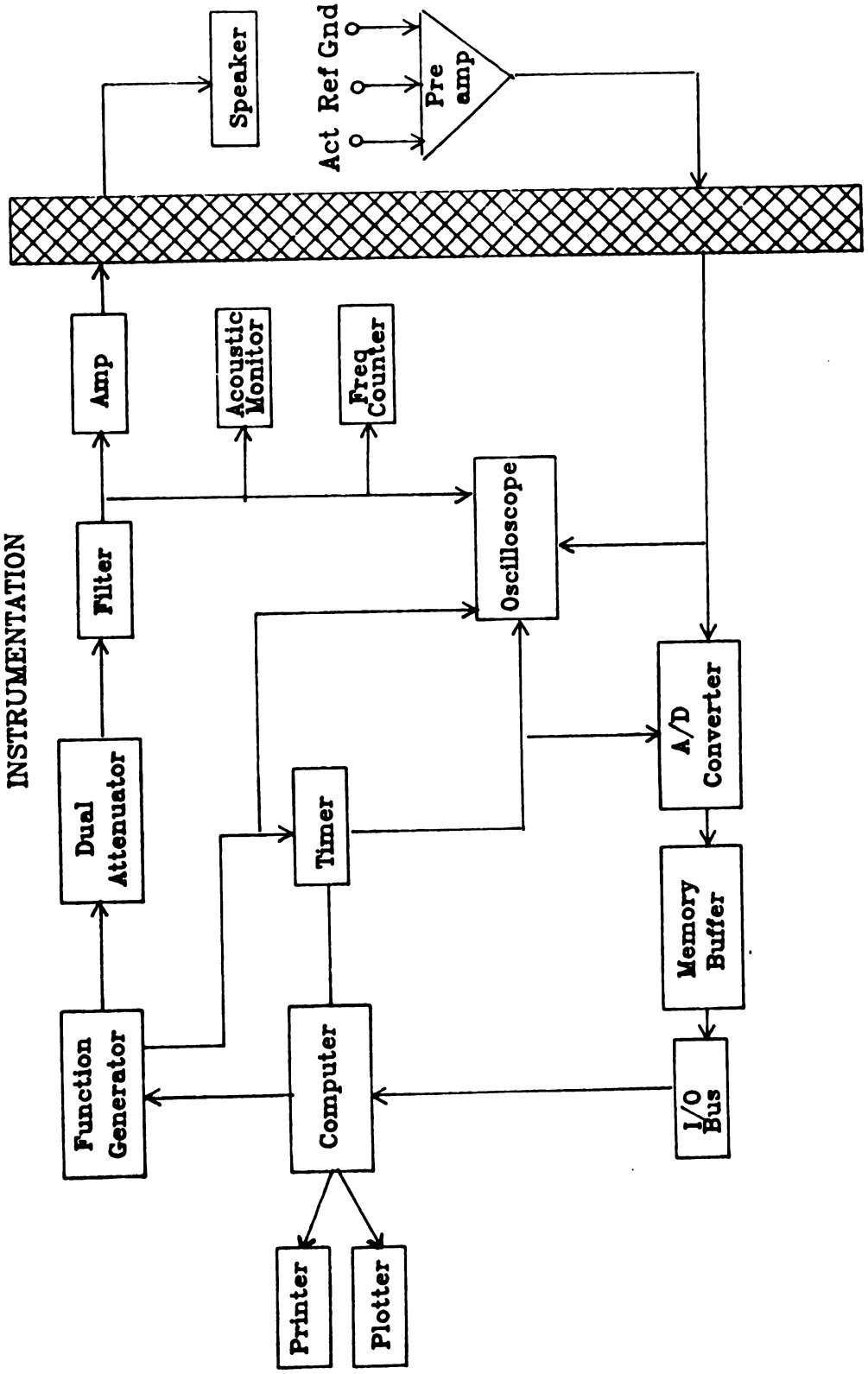


FIGURE III-1
INSTRUMENTATION

activate the averager simultaneously or with a delay if needed, with the stimulus onset. The speaker was used to transduce electric energy to acoustic energy in order to provide acoustic stimulation.

Analog section: This section basically includes the following equipment for response recording:

.Three silver wire electrodes (silver-silver chlorided wire, 270 μM in diameter).

.Preamplifier and filter (Data Inc, 2124 Mod 2)

Following elicitation of the bio-electric activity by the stimulus, the responses were collected and recorded. The electrodes detected the responses which were routed to the pre-amplifier for differential amplification. The amplification factor was set at 5×10^4, which resulted in a gain of 94 dB. The responses were filtered from 30-3000 Hz to enhance the quality of the averaged evoked potentials, and by setting the system to maximally reject any interfering random signals.

Digital section: This section includes the following equipment for processing responses:

.Analog/Digital converter (MI^2 202)

.Input/output bus (MI^2)

Following differential amplification and filtering, the analog responses obtained from the crickets were converted to digital form by the A/D converter, which sampled the activity and generated integers that approximated the value

of the activity at the instant of sampling. This was done rapidly, at regular intervals. The responses were then passed through the memory buffer and input/output bus, and averaged 256 times, for a sweep time of 20 ms, a dwell time of 10 μs, sample rate of 1×10^5 Hz and 2×10^3 data points.

Display section: The following equipment were used for visualizing the stimuli and the recorded responses.

- .Monitor (IBM enhanced color monitor, 5154001)
- .Oscilloscope (Textronix D15)
- .Frequency counter (Hewlett Packard 5314A)
- .Plotter (IBM 6180)

The oscilloscope consists of four channels. The first channel is used to display the tone bursts generated by the function generator, the second displays the trigger pulse, the third displays the tone bursts after amplification and the fourth channel displays the raw electrophysiologic activity from the crickets. The frequency counter is used as a monitor to check for the repetition rate of the signal. The plotter connected to the computer was used to print the analog wave forms obtained from the crickets.

Calibration of Equipment

In order to understand the relation between stimulus parameters and responses, it is essential to specify precisely the stimuli used. Hence, the stimulus was calibrated in the intensity, rate, duration, phase, rise-decay time and frequency domains. Figure III-2 shows the instrumentation used for calibration of the equipment. A description of the procedure adopted for calibration is described below:

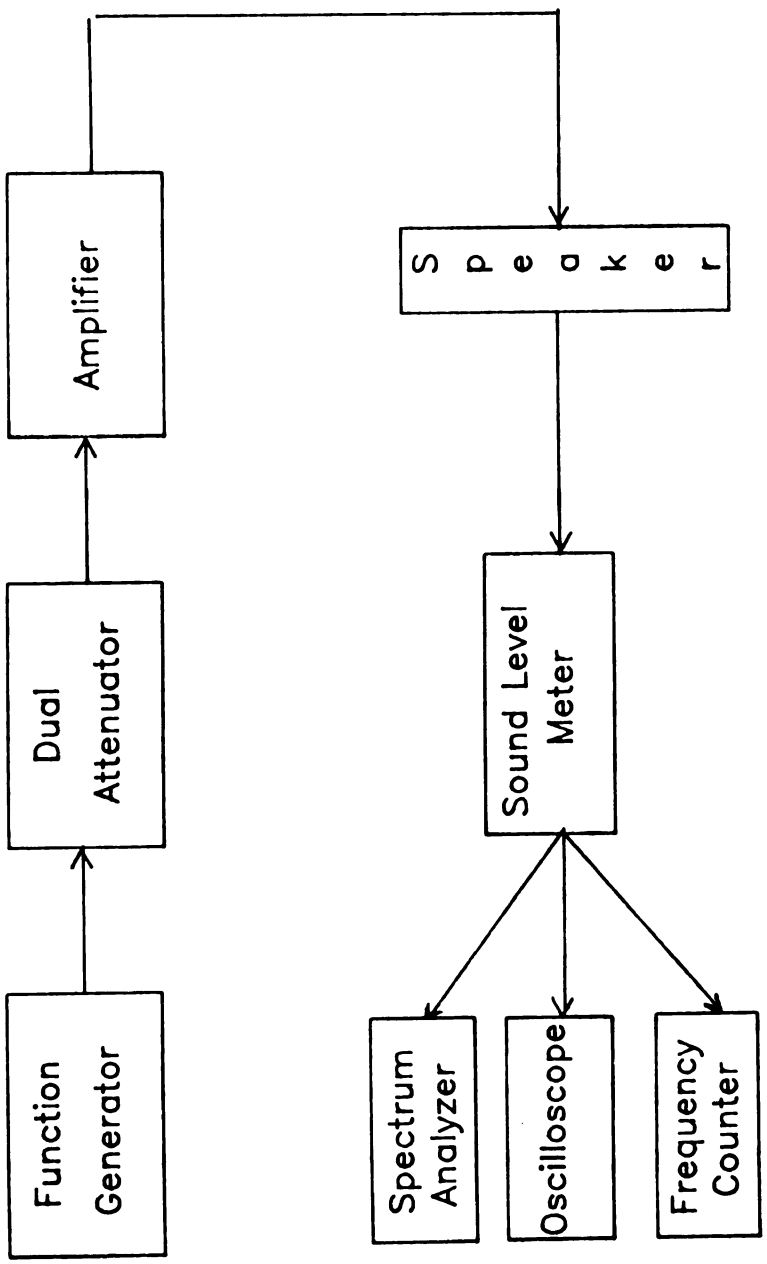
Equipment used:

- .Function generator (MI^2 208)
- .Dual attenuator (MI^2 108)
- .Amplifier (Technics power amplifier SE-9060)
- .Sound level meter (Larson & Davis)
- .Speaker (Realistic Minimus-3.5)
- .Oscilloscope (Textronix D15)
- .Spectrum analyzer (Hewlett Packard 3582A)
- .Frequency counter (Hewlett Packard 5314A)

Figure II-2: Block diagram of the calibration system

FIGURE III-2

CALIBRATION SYSTEM



Calibration Procedure

The sound level meter (SLM) was calibrated using a pistonphone. 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 routed through the attenuator, amplifier and speaker to the SLM. The intensity of the sine wave at the function generator was adjusted so that it read 100 dB SPL at a distance of 5 cm from the speaker. Tone bursts were generated with an amplitude reading of 100 mV on the oscilloscope. This level was referred to as 100 dB P.E. SPL of the tone burst.

The phase, duration and rise/decay time of the signal were measured and monitored on the oscilloscope which was connected to the output of the dual attenuator. The output from the dual attenuator was also led to a frequency counter which monitored the repetition rate of the signal. The frequency content of the signal was measured using a spectrum analyzer. The noise used for simultaneous masking was measured for its frequency spectrum using the spectrum analyzer, and was found to have a frequency spectrum of 1-6000 Hz.

Below is an overview of stimulus conditions used in this study

Number of Crickets	Stimulus Parameters Manipulated
1	Control
2	Response similarities between 2 crickets
1	polarity
5	Intensity in dB P.E. SPL
1	Intensity in 1 dB steps
3	Frequency
3	Repetition rate
3	Rise-decay time
2	Masking
3	Tetrodotoxin and kynurenic acid
1	Tetrodotoxin alone

Materials and Procedures

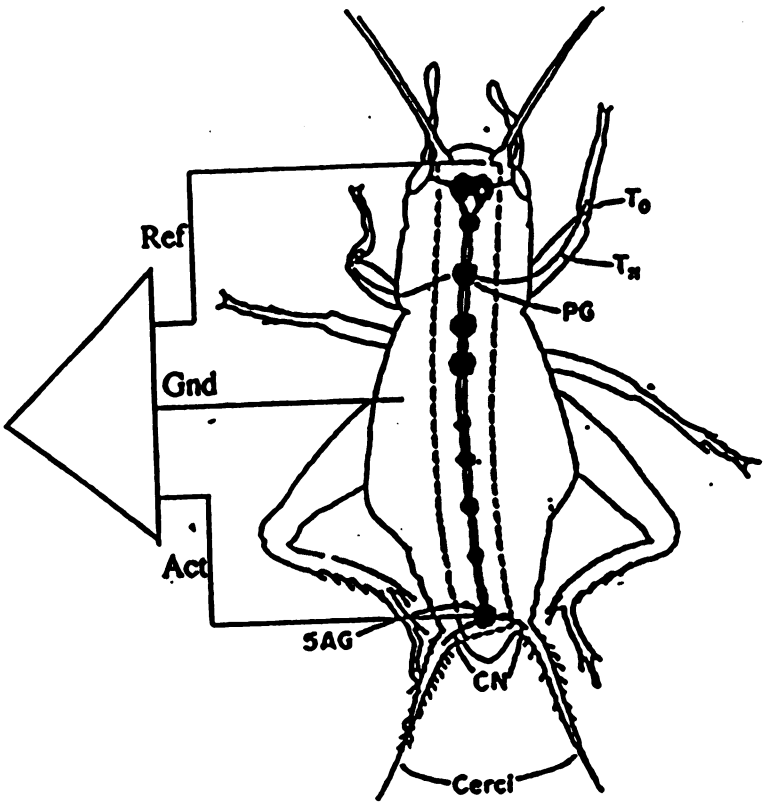
The experimental animals were healthy adult male and female crickets (Acheta domesticus), which were obtained from a local pet shop. Crickets were used instead of cockroaches because review of literature has indicated that the cercal hearing system of crickets are much more sensitive than that of the cockroaches (Edwards and Palka, 1974; Counter, 1976a). The higher hearing threshold seen in the cockroach are attributed to one or more of the following factors: the

sensilla on the cockroach cerci are more rigidly fixed and not freely articulated as in crickets, the sensilla in the cockroach are shorter and greater in mass, there are less number of sensilla on the cockroach cerci. For experimentation purposes, the cricket was immobilized by placing it on ice for about 10 minutes (control studies indicated that the optimum time period for the cricket to be on ice was 10-11 minutes). The cricket was removed from the ice and its two pairs of wings and three pairs of legs were removed and the cut ends were covered with petroleum jelly to prevent the escape of any bodily fluids. The cricket was then pinned on to bee's wax and opened dorsally, making certain not to damage any of the cercal parts or the ventral cord. Experiments were conducted in a sound-treated room on a vibration-free table.

Three electrodes were used for recording responses (see figure III-3). The electrodes were silver-silver chloride wire with a diameter of 270 μM, bared at the tip and secured to the nerve via a microdrive and a micromanipulator. The active electrode was placed on the fifth abdominal ganglion, the reference in the head, and the ground electrode in the abdomen. A saline-soaked cotton pellet was placed on the tip of the electrodes to keep the recording site moist, which also assisted in maintaining the preparation for several hours. The temperature in the room was maintained at 25^{o}C.

Figure III-3: Diagram representing typical electrode placements on the crickets (modified from Counter, 1976a). Act = Active electrode, Ref = Reference electrode, Gnd = Ground electrode, To = Tympanal organ, T_m = Tympanal membrane, PG = Prothoracic ganglion, 5AG = Fifth abdominal ganglion, and CN = Cercal nerve

Figure III-3



The acoustic stimuli generated by the function generator were delivered via a condensor speaker placed 5 cm behind the cerci, at an angle of 90° to the longitudinal axis of the cerci. The frequency and repetition rates of the tone bursts were changed when they were made the independent variables. Stimulus intensity ranged from 115 to 85 dB P.E. SPL in 10.0, 5.0 or 1.0 dB steps. The stimulus polarity was either rarefaction, condensation, or alternating. The rise-decay time, frequency, repetition rate and the duration of the tone burst were changed as necessary to achieve the desired experimental conditions. A trigger delay of -2.0 ms was used in all of the recordings to enhance the clarity of the earlier waves. The 2.0 ms recording prior to stimulus presentation provided an estimate of the noise background of the experimental instrumentation.

Initially, compound action potentials were obtained for various stimulus dependent parameters with no pharmacologic treatment. This was followed by application of TTX (concentration of 0.1 mM) on the ventral cord anterior to the fifth abdominal ganglion. Recordings were made until the N1 potential diminished below visual detection level (VDL). TTX was applied into a plastic barrel (inner diameter = 2.0 mm) which was placed just anterior to the fifth abdominal ganglion. Petroleum jelly was applied on the outer surface of the barrel to ensure that the TTX applied into the barrel did not spread beyond the point of application. Further, kynurenic acid was applied on the

fifth abdominal ganglion, and recordings were made until the No-Po/EPSP-like potential diminished below VDL.

Data Analysis

The threshold for the most prominent peak, N1, was defined as the lowest intensity required for visual detection and was measured as a 0.1 μV difference between any two consecutive peaks (as used by Moller and Blegvad, 1976; Jerger and Mauldin, 1978; Schweitzer, 1987; Amedofu, 1989). Peak-to-peak amplitude was measured from the peak to the following trough for a positive wave and from the trough to the following peak for a negative wave. Latency was measured from the onset of the stimulus to the peak of interest.

The mean and standard deviation for latency and amplitude of peaks No, Po and N1 were calculated. Composite data was computed for amplitude and latency of waves No, Po and N1 as a function of stimulus intensity. Composite data was also computed for size (relative amplitude) of waves No, Po and N1 as a function of the time following administration of various pharmacologic agents. Data were displayed, graphed and plotted using a microcomputer workstation (Dell-325, 80386).

Chapter IV

RESULTS

The research questions posed in this investigation are:

1. Is it possible to identify an EPSP-like potential in gross potential recordings of the ventral cord of Acheta domesticus, whose different components can be designated as No-Po/EPSP-like potentials? (EPSP-like potential is the gross generator potential which, if large enough, leads to the generation of action potentials. No and Po potentials are the negative and positive potentials preceding the EPSP-like potential. All of these potentials No, Po, EPSP-like and N1 are known as post-synaptic potentials).
2. Following identification of the No-Po/EPSP-like potentials, what stimulus parameters such as frequency, intensity and time best characterize the post-synaptic potentials using latency and amplitude functions?
3. Using pharmacologic agents such as tetrodotoxin and kynurenic acid, how do they assist in the identification of the No-Po/EPSP-like potentials?

In order to answer these questions, several experiments were conducted on 110 crickets. Among the 110 crickets, some were used for control studies, some did not yield good results due to either bad preparation or faulty equipment. Results from 25 crickets were chosen to constitute a final sample. Where applicable, descriptive and inferential statistics were used.

Identification of EPSP-like potential in gross potential recordings of the ventral cord of crickets, and designation of its various components as No-Po/EPSP-like potential

Subjects: Adult male and female crickets were used as the experimental preparation. All appeared to be active and moved around normally prior to being placed on ice for about 10 minutes. Cercal sensitivity was screened by presenting a 400 Hz tone at 110 dB P.E. SPL. The responses obtained were found not to be very different from each other in terms of dynamic range, amplitude, onset latency and morphological characteristics.

Procedure: The stimulus consisted of 1500 Hz tone bursts with a nominal duration of 3.3 ms, a repetition rate of 3.1/sec and an intensity of 110 dB P.E. SPL. Control recordings without acoustic stimulation were obtained (see trace 1, figure IV-1). The electrophysiologic activity was averaged 256 times using an analysis time of 20 ms. A trigger delay of -2.0 ms was used so as to obtain pre-stimulus background activity. A rest period of 1.5 minutes was provided between experimental runs so as not to cause neural fatigue. Three silver-silver chlorided wire electrodes with a diameter of 270 uM were used, with the active electrode placed on the fifth abdominal ganglion, the reference electrode placed in the head between the two eyes and the ground electrode in the abdomen.

Responses: Figure IV-1 shows the responses obtained from two animals. The first recording is a control recording with no acoustic stimulus. Following this are recordings obtained when the crickets were acoustically stimulated. The stimuli used for the second and third recordings consisted of a 1500 Hz tone burst, 110 dB P.E. SPL, 3.1/sec repetition rate, 3.3 ms duration, 20 ms analysis time and 256 averages.

The responses consist of a series of negative and positive waves. The waves from both crickets exihibit somewhat similar morphologic characteristics. The first prominent and stable negative deflection was designated N1. The positive peak following N1 was designated P1. At times there was a negative peak following P1 and it was designated as N2. Having identified the N1/P1/N2 complex, careful observations were made to identify any consistent positive and/or negative waves prior to N1. Interestingly, it was possible to identify a positive peak, Po, which occurred prior to N1. Although not as obvious as Po, there was, at times, a negative peak preceding Po and it was termed No. It is difficult, however, to identify an EPSP-like potential, i.e., a slow negative wave following Po. The probable reason is that at such a high intensity level the EPSP-like potential is masked by N1. Hence, in the study to follow, the stimulus intensity was decreased systematically until it was insufficient to trigger N1. Thus an EPSP-like potential was identified and labeled. Since there is no direct evidence that the slow negative potential which

Figure IV-1: Electrophysiologic recordings of one control condition and two stimulus conditions. The first recording depicts the background activity of a cricket to no acoustic stimuluation. Recordings two and three are responses from two crickets to alternate tone bursts of 400 Hz, 110 dB P.E. SPL, a repetition rate of 3.1/sec, a duration of 3.3 ms, and averaged 256 times. STIM = stimulus.

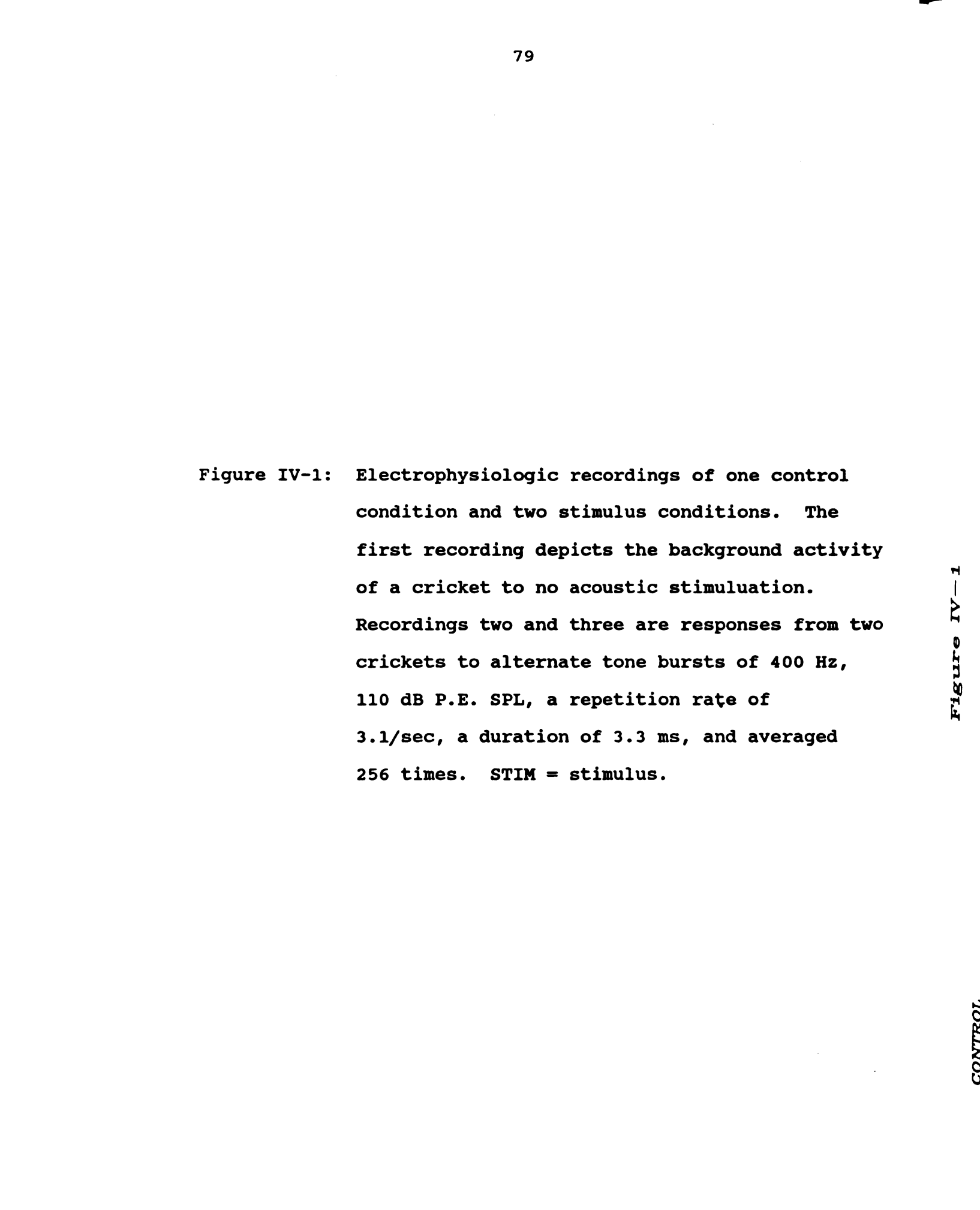
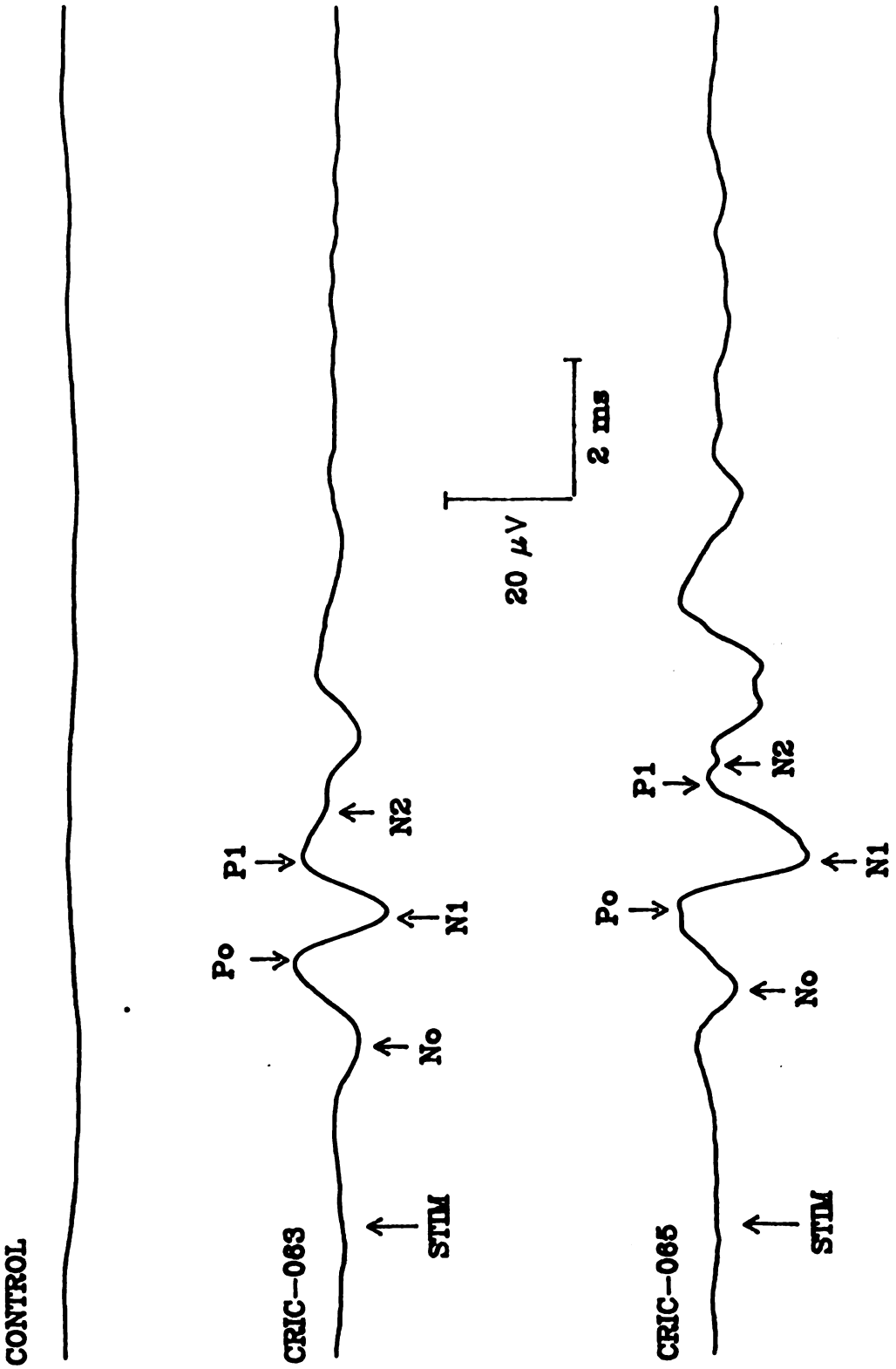


Figure IV-1


CONTROL
CRIC-063
STIM
No
Po
P1
N1
N2
20 μV
2 ms
CRIC-065
STIM
No
Po
P1
N1
N2

remains is a true EPSP, the term "EPSP-like" potential is being used.

Summary: So far we have been successful in recording the action potential complex, namely, the N1/P1/N2 and also its two preceding potentials, the No and Po potentials. However, the EPSP-like potential was still obscure, masked by the large action potential.

Stimulus parameters characterizing the No-Po/EPSP-like potential

In order to characterize the No-Po/EPSP-like potentials, the intensity, frequency and temporal parameters of the stimulus were varied. This experimental paradigm was used in order to determine stimulus dependent characteristics for these responses.

Polarity: Figure IV-2 (Cric-075) shows the effects of stimulus phase on No, Po and N1 to rarefaction, condensation and alternating tone bursts. All other stimulus parameters were held constant, i.e., intensity was 110 dB P.E. SPL, repetition rate was 3.1/sec and frequency was 400 Hz. These recordings indicate that for rarefaction and condensation phases, there were earlier responses which changed in polarity with phase, but were absent for the alternating phase. The earlier potentials were perhaps stimulus artifacts or some other condition in which the responses are

Figure IV-2: Electrophysiologic recordings of No, Po and N1 potentials as a function of polarity of the stimulus. Frequency = 400 Hz, repetition rate = 3.1/sec and intensity = 110 dB P.E. SPL. STIM = stimulus

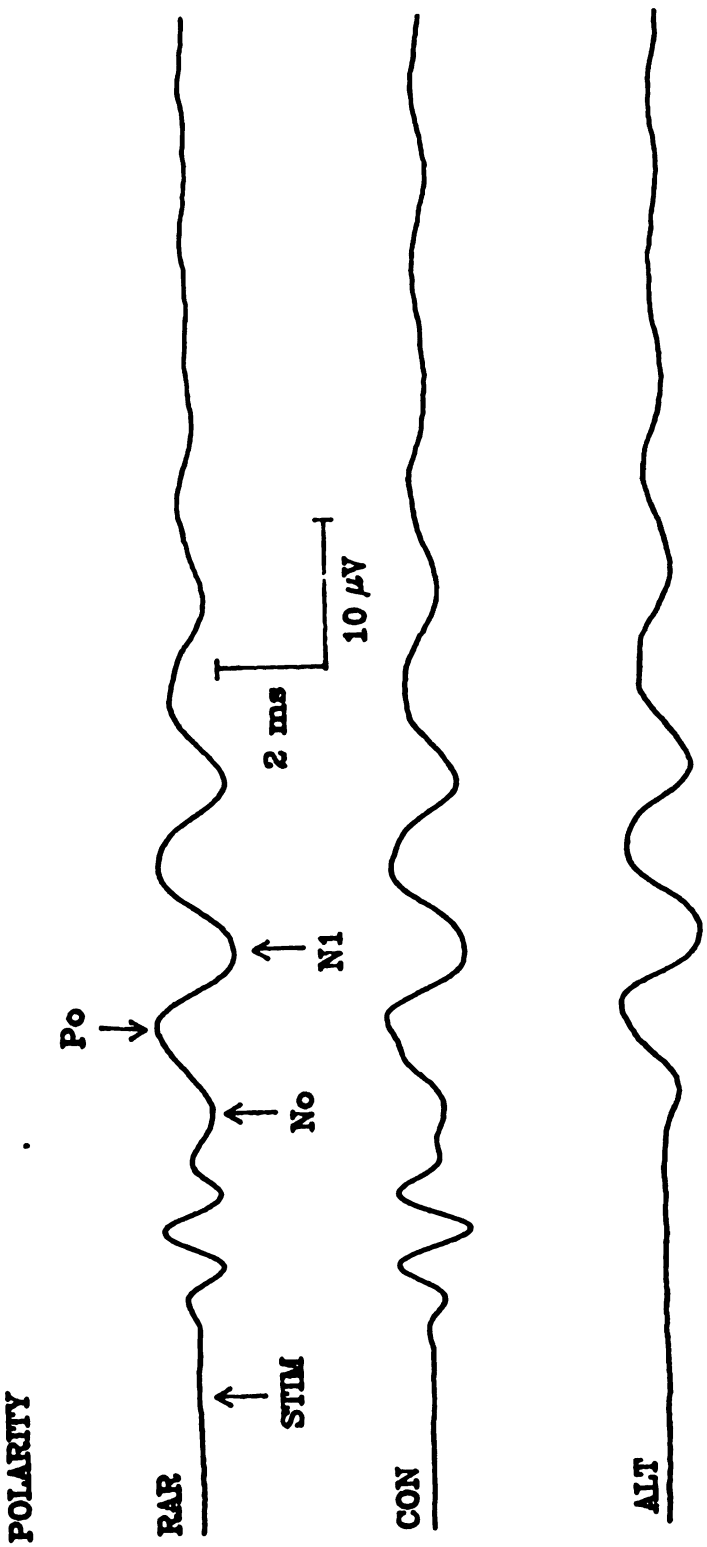


FIGURE IV-2

CRIC-076

stimulus dependent, but cancel during the alternating phase. However, the potentials of interest, i.e., No, Po and N1 show no reversals, strongly suggesting that these are neural responses. It is well known that neural responses have no opposing polarity and do not average out for alternating stimuli.

Intensity: Figure IV-3 (Cric-063) shows the effects of intensity on the potentials. Results from four other crickets (Cric-071, Cric-072, Cric-076 and Cric-092) are presented in appendix A. It can be seen that the responses are quite systematic with variations in intensity. The amplitude of No, Po and N1 decreased, and latency increased as the stimulus level was decreased. At high intensity levels the waves were readily identifiable, but they became less distinguishable as intensity of stimulation decreased. This trend was consistent in all the crickets tested (Table IV-1A depicts the numerical values of latencies in five crickets, Cric-063, Cric-071, Cric-072, Cric-076 and Cric-092, and table IV-1B depicts the numerical values for amplitudes in these crickets). At 100 dB P.E. SPL level, N1 could no longer be identified but in its place the EPSP-like potential could be seen. This perhaps indicates that the EPSP-like potential can be identified only at low intensity levels when N1 is not present. That is, when the intensity is increased the EPSP-like potential is perhaps masked by N1, and is no longer observable. At low intensity levels in which the threshold of N1 is not reached, it is possible to

Figure IV-3: Electrophysiologic recordings of No, Po and N1 potentials as a function of varying the intensity in 5.0 dB steps. Frequency = 1500 Hz, repetition rate = 3.1/sec, and phase = alternating. STIM = stimulus

FIGURE IV-3

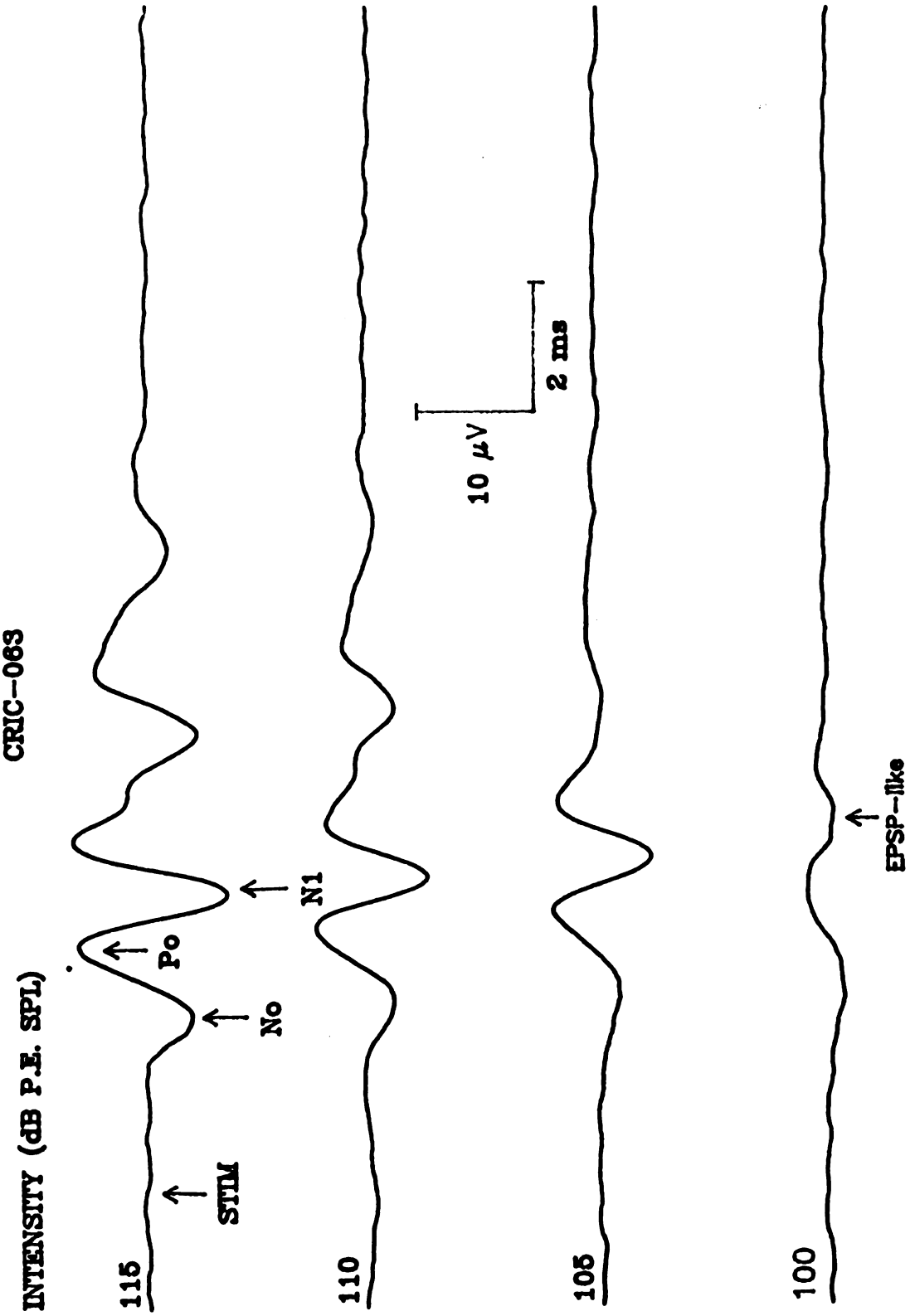

CRIC-063
INTENSITY (dB P.E. SPL)
115
110
105
100
STIM
No
Po
N1
10 μV
2 ms
EPSP-like

Table IV.1A: Latency values of No, Po and N1 potentials in Cric-063, Cric-067, Cric-071, Cric-072 and Cric-076 as a function of intensity level (dB P.E. SPL) of the stimulus.

TABLE IV.1A

Latency (ms) of No, Po and N1 potentials in Crickets 063, 067, 071, 072 and 076.

	100 dB			105 dB			110 dB			115 dB		
	No	Po	N1	No	Po	N1	No	Po	N1	No	Po	N1
063	2.84	4.40	5.48	2.80	4.08	4.88	2.68	3.84	4.60	2.48	3.56	4.32
067	1.76	2.48	3.08	1.64	2.36	2.96	1.16	2.32	2.80	1.20	2.20	2.68
071	1.48	1.84	3.08	1.44	1.80	2.80	1.40	1.72	2.76	1.36	1.68	2.68
072	1.64	1.92	3.52	1.44	1.72	3.32	1.36	1.66	2.60	1.30	1 .60	2.56
076	3.00	3.20	5.28	2.64	2.92	4.12	1.28	1.76	2.29	1.04	1.64	2.28
Mean	2.14	2.77	4.09	1.99	2.58	3.62	1.58	2.26	3.01	1.48	2.14	2.90
S.D.	0.71	1.06	1.19	0.67	0.96	0.87	0.62	0.92	0.91	0.57	0.83	0.81

Table IV.1B: Amplitude values of No, Po and N1 potentials in Cric-063, Cric-067, Cric-071, Cric-072 and Cric-076 as a function of intensity level (dB P.E. SPL) of the stimulus.

TABLE IV.1B

Amplitude (μV) of No, Po and N1 potentials in Crickets 063, 067, 071, 072 and 076.

	100 dB			105 dB			110 dB			115 dB		
	No	Po	N1	No	Po	N1	No	Po	N1	No	Po	N1
063	6.14	4.62	2.80	11.62	17.36	16.42	13.70	19.72	17.78	19.67	25.81	26.56
067	9.30	28.12	27.22	11.34	33.40	32.20	13.28	38.56	36.12	15.02	42.96	37.12
071	0.41	1.65	0.41	0.62	4.58	2.89	1.03	17.73	11.13	5.57	60.60	51.80
072	0.13	0.37	1.07	0.14	1.07	1.15	0.16	3.40	2.42	0.21	3.42	3.38
076	0.21	0.10	0.82	0.52	1.50	1.57	1.13	2.16	2.37	1.65	5.67	6.29
Mean	3.24	6.97	6.46	4.85	11.57	10.84	5.86	16.32	13.97	8.42	27.68	25.03
S.D.	4.24	11.95	11.63	6.06	13.88	13.57	6.98	14.79	12.49	8.54	24.46	20.52

identify the EPSP-like potential.

In order to examine more closely the transition from N1 to the EPSP-like potential, 1.0 dB steps were incorporated in our next study. The effect of varying the intensity in 1.0 dB steps is depicted in figure IV-4 (Cric-067). It is seen that 1.0 dB steps were more effective in depicting the N1 to EPSP-like transition.

Frequency: Tone bursts at frequencies ranging from 400 Hz to 2500 Hz were used. The results indicated that the responses for lower frequencies appeard to elicit more distinct waveforms. Tone bursts of frequencies of 2000 Hz and above elicited responses of which the potentials were prolonged in latency and decreased in amplitude. Figure IV-5 (Cric-067) depicts the responses to 400, 600 and 1500 Hz. Responses from two other crickets (Cric-054 and Cric-075) are presented in appendix B. The results obtained in this study agree favorably with the results of literature which show that the lower frequencies tend to elicit more distinct responses than higher frequencies (Edwards and Palka, 1974; Counter, 1976a).

Rise-decay time: Figure IV-6 (Cric-094) indicates that with an increase in the rise-decay time of the stimuli, the potentials increased in latency and decreased in amplitude. It is well known from previous studies that evoked responses are influenced by various rise-decay times of the input signal (Moore, 1983). A point is usually reached wherein

Figure IV-4: Electrophysiologic recordings of No, Po and N1 potentials as a function of 1.0 dB increments in intensity. Frequency = 400 Hz, repetition rate = 3.1/sec, and duration = 3.3 ms. STIM = stimulus.

FIGURE IV-4

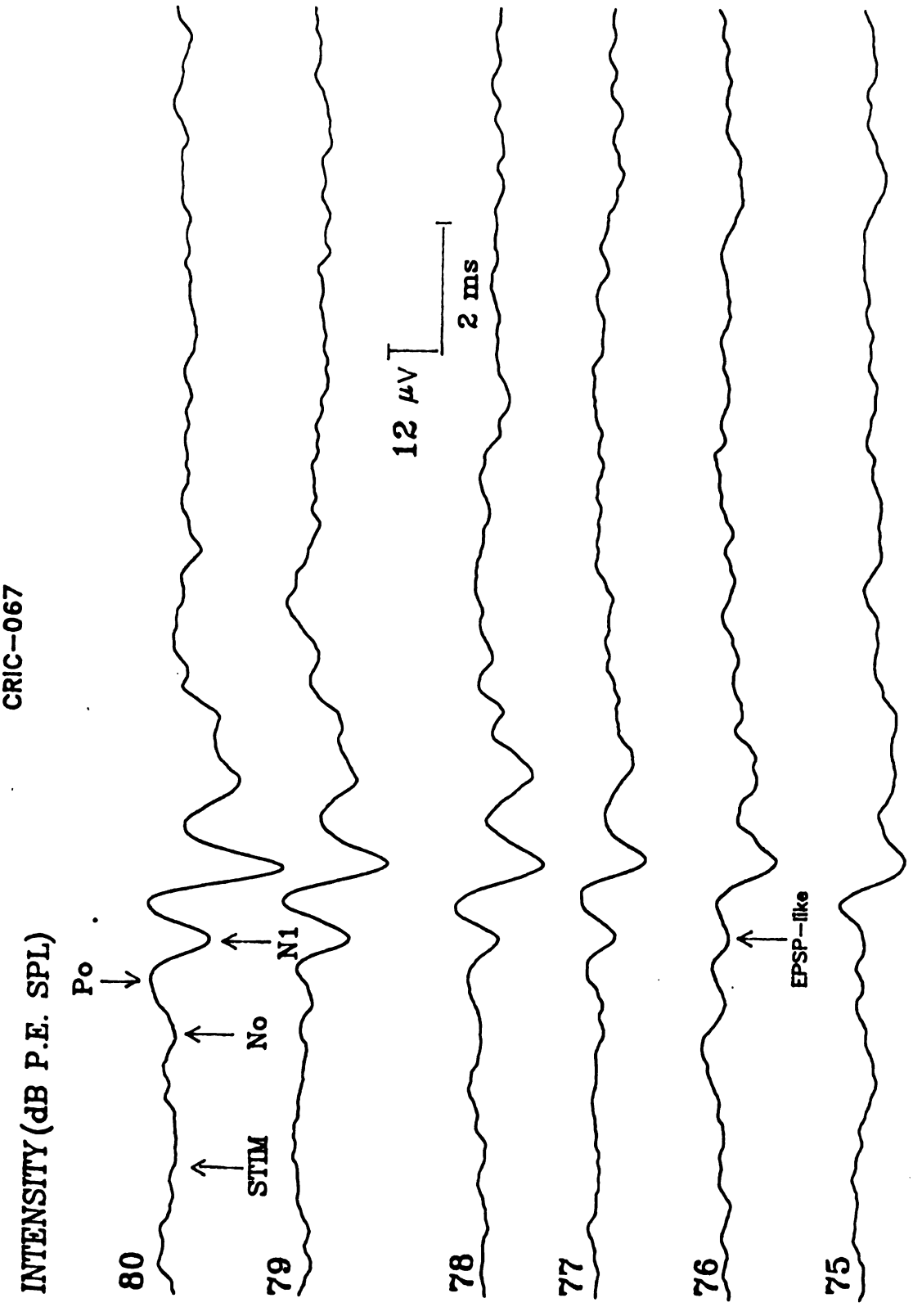

CRIC-067
INTENSITY (dB P.E. SPL)
Po
No
N1
STIM
80
79
78
77
76
75
12 μV
2 ms
EPSP-like

Figure IV-5: Electrophysiologic recordings of No, Po and N1 potentials as a function of frequency. Intensity = 110 dB, repetition rate = 3.1/sec, phase = alternating. STIM = stimulus.

FIGURE IV-5

CRIC-067

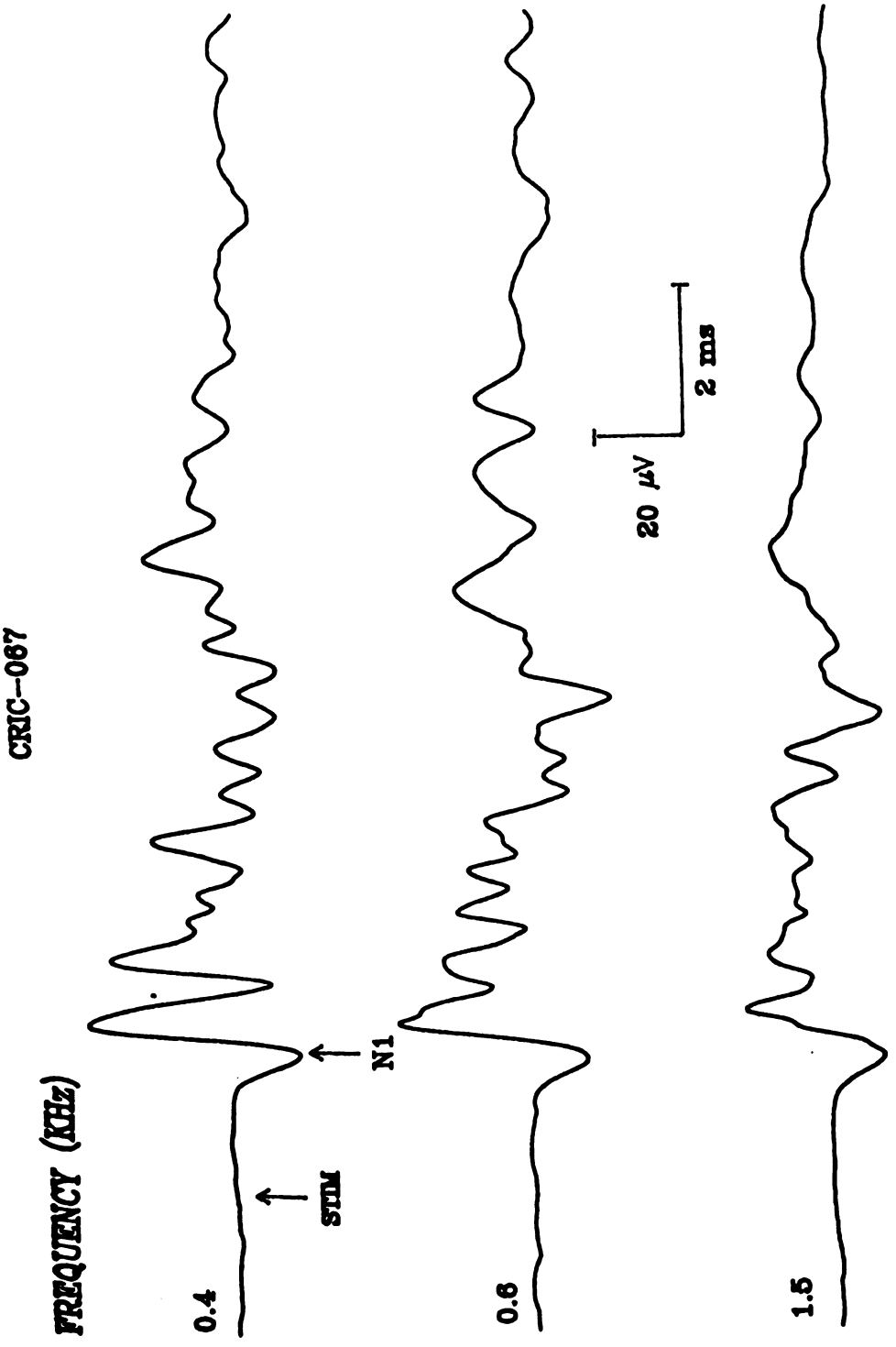
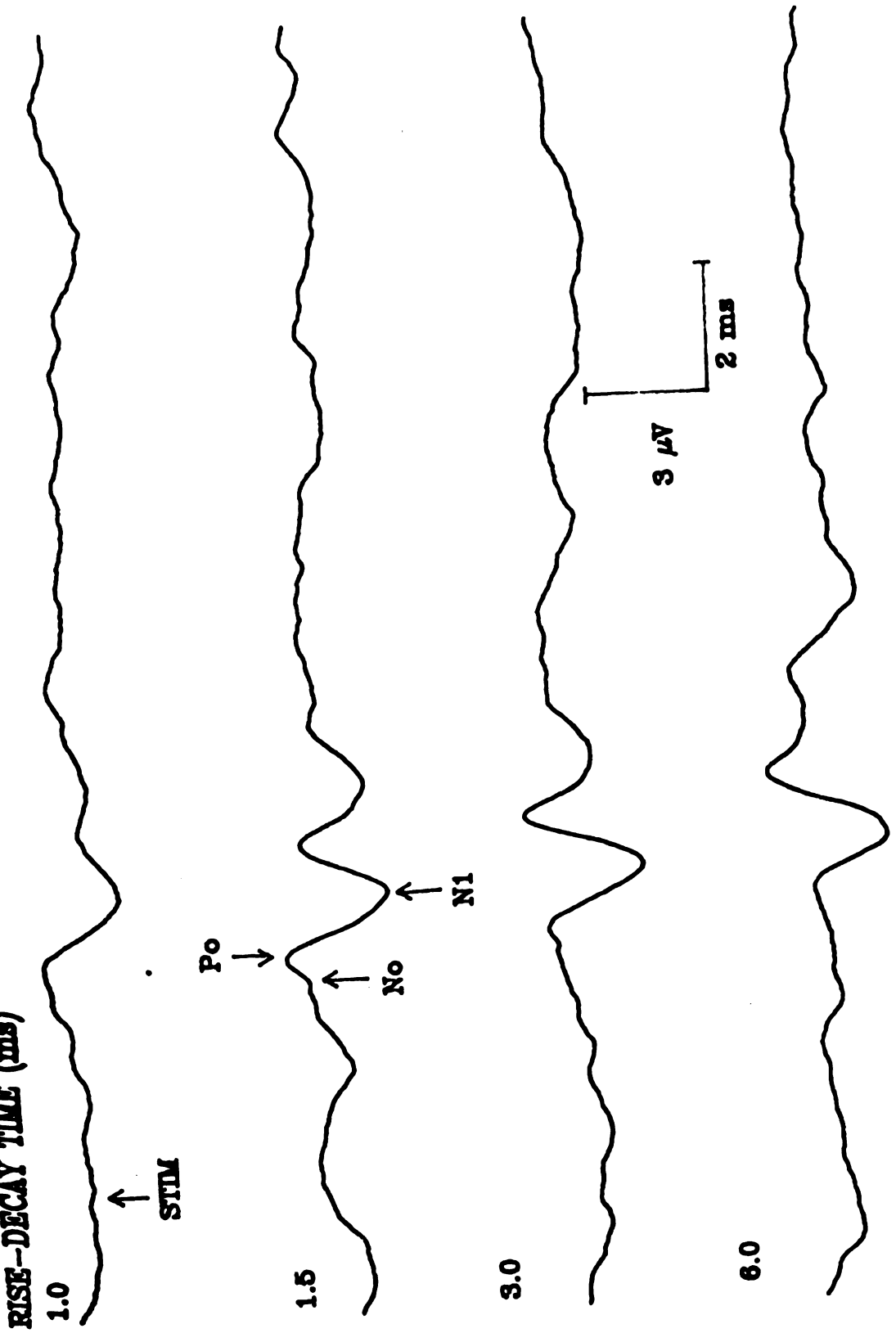


Figure IV-6: Electrophysiologic recordings of No, Po and N1 potentials as a function of rise/decay time. Intensity = 110 dB, repetition rate = 3.1/sec, phase = alternating.
STIM = stimulus.

FIGURE IV-8
CRIC-094



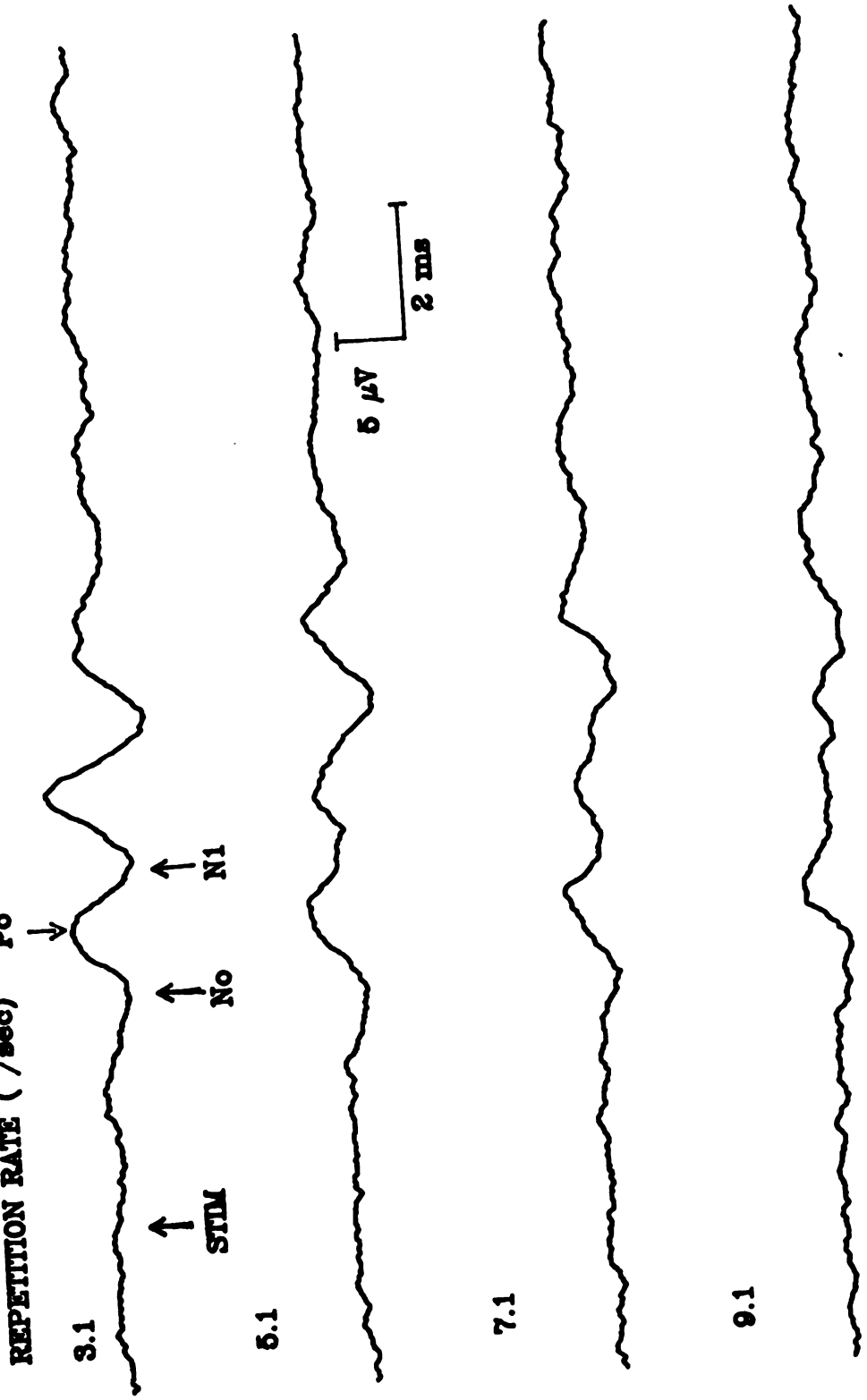
with any further increase in the rise-decay time, response identification became extremely difficult. This effect is typical of neural responses, since neural impulses are best excited by fast-rising stimuli. Results from two other crickets (Cric-065 and Cric-076) are presented in appendix C. From the results obtained it would appear that response identification improves when the rise-decay time is increased from 1.0 ms to 1.5 ms. Rise-decay times greater than 6.0 ms causes the waves to diminish in amplitude, prolong in latency and become less distinct in their morphological characteristics. This supports the view that the responses depend on the synchrony of the nerve discharges, and faster rise-decay stimuli elicit better synchrony in the nerve fibers.

Repetition rate: Figure IV-7 (Cric-075) depicts the effects of repetition rate on the potentials (see appendix D for Cric-076 and Cric-107). As is typical of neural responses, the potentials decreased in amplitude and increased in latency as repetition rate was increased, which, in essence, is also a reflection of decreasing the inter-stimulus interval.

The morphology of the responses changed also as repetition rate was increased. In fact, as the repetition rate was increased from 1.1/sec to 3.1/sec (as seen in responses from Cric-076 in appendix E) the potentials increased in amplitude. However, when the repetition rate was increased to a rate greater than 3.1/sec, the potentials decreased in

Figure IV-7: Electrophysiologic recordings of No, Po and N1 potentials as a function of repetition rate. Frequency = 400 Hz, intensity = 110 dB, phase = alternating. It is noted that on these data, there is high frequency energy superimposed on the responses. STIM = stimulus.

FIGURE IV-7
CRIC-075



amplitude and the morphology became less distinct in most of the recordings. It can be seen that the data obtained in this study are not significantly different from previous studies of auditory brain stem recordings in humans (Don, Allen and Starr, 1977; Moore, 1971) which indicated that as repetition rates were increased beyond 10/sec the responses became less distinct, but the effect did not go unnoticed at rates below 10/sec.

Simultaneous masking: One purpose of masking experiments is to differentiate between mechanical and neural responses (Abbas, 1984). Figure IV-8 (Cric-096) depicts the effect of simultaneous masking on the potentials. The first recording was obtained to 1.0 KHz tone bursts at 100 dB P.E. SPL with no masking, while the second recording was obtained to the same stimulus presented simultaneously with a wide-band (1-6000 Hz, -3 dB), 100 dB P.E. SPL random masking noise. The third recording depicts the response when the masking noise was increased to 104 dB P.E. SPL, while keeping the intensity of the tone burst constant. In the first recording, N1 and the earlier part of the EPSP-like response can be readily seen. In the second recording, however, in the presence of noise, the potentials were masked considerably, resulting in a decrease in the amplitude of N1 and a shift in latency.

Figure IV-8: Electrophysiologic responses as a function of simultaneous masking. Stimulus frequency = 1000 Hz, repetition rate = 3.1/sec, phase = alternating. Masking noise was 0 dB P.E. SPL for the first recording, 100 dB P.E. SPL for the second recording and 104 dB P.E. SPL for the third recording. STIM = stimulus.

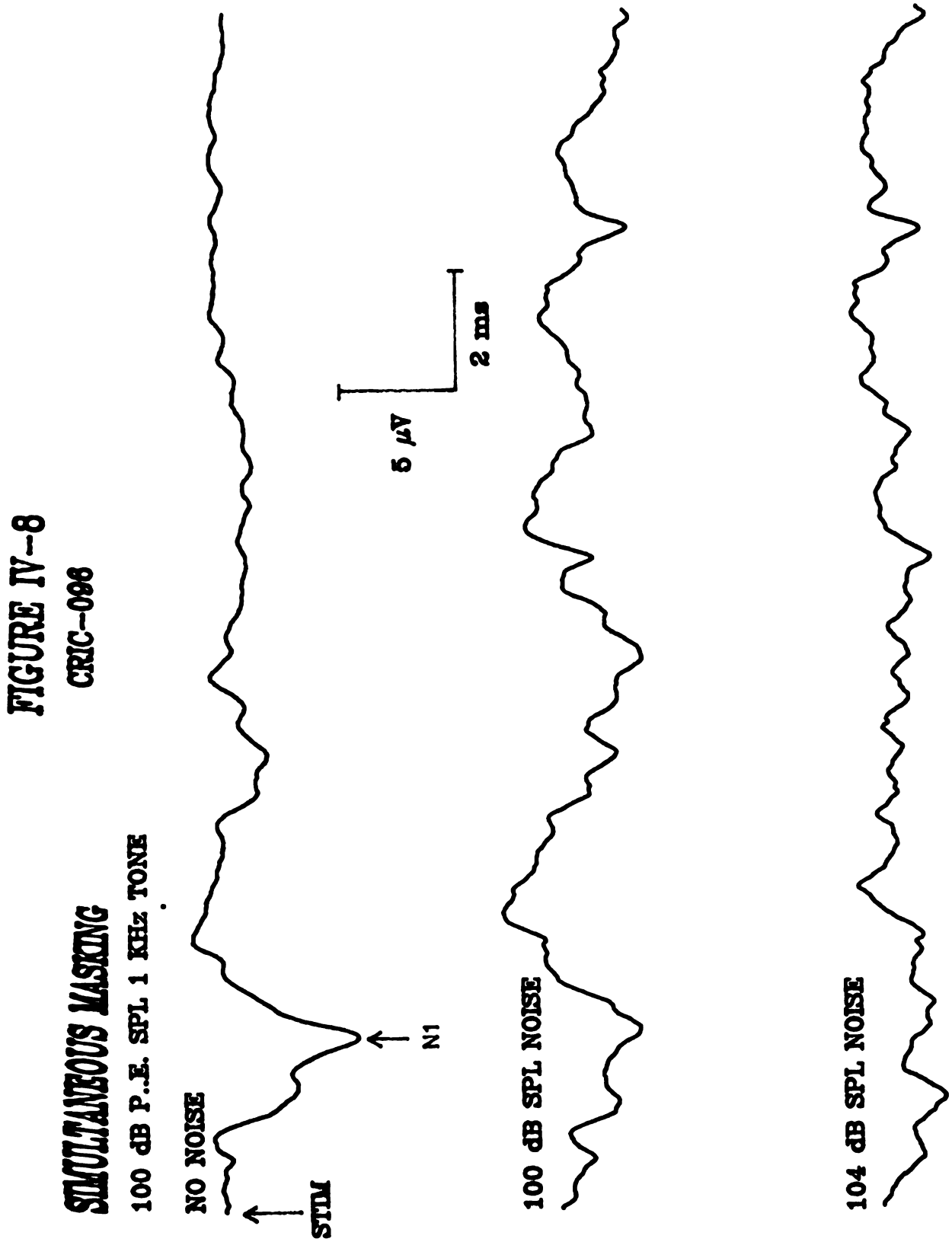


FIGURE IV-8
CRIC-096
SIMULTANEOUS MASKING
100 dB P.E. SPL 1 KHz TONE
NO NOISE
STIM
N1
5 μV
2 ms
100 dB SPL NOISE
104 dB SPL NOISE

This effect became even more obvious when the masking noise was increased to 104 dB P.E. SPL, again, an effect typical of neural responses. The EPSP-like potential which is co-incidental with N1 is not seen in the recordings at high stimulus intensity levels. With masking noise, however, the N1 potential reduced in amplitude and prolonged in latency, enhancing the appearance of the EPSP-like potential. It is to be noted here that the tone burst was initiated at the beginning of the trace, rather than 2.0 ms after the computer was initiated.

Latency and Amplitude functions

The mean latency and amplitude (size) data from five crickets for tone bursts at 100, 105, 110 and 115 dB P.E. SPL are shown in table IV.2. Figures IV-9A and IV-9B depict the input-output functions of the waveforms. From the input-output functions it can readily be seen that as the intensity of the stimulus is increased, all waves show a systematic decrease in latency (Fig IV-9A) and an increase in size (Fig IV-9B). Input-output latency functions for each potential of interest, as seen in figure IV-9A, indicate that as intensity is increased, the standard deviation of the waves decreased indicating less variability in the responses. A gradual sloping configuration of the input-output plot is seen for all waves. Variability is not represented in the size/intensity function since, a relative measure was used to represent the size on the y-axis.

Table IV.2: Mean and standard deviation values of latency and size of No, Po and N1 potentials in Cric-067, Cric-071, Cric-072, Cric-76 and Cric-92.

TABLE IV.2

Mean and standard deviation of latency and size of No, Po and N1 potentials in crickets 067, 071, 072, 076 and 092.

	100 dB			105 dB			110 dB			115 dB		
	No	Po	N1	No	Po	N1	No	Po	N1	No	Po	N1
LATENCY (ms)												
Mean	1.97	2.34	3.64	1.74	2.14	3.21	1.33	1.84	2.63	1.15	1.64	2.51
S.D.	0.63	0.55	0.93	0.51	0.50	0.55	0.11	0.27	0.21	0.21	0.40	0.18
SIZE (%)												
Mean	40.4	19.33	24.57	51.28	37.72	32.54	68.18	67.16	59.88	100	100	100
S.D.	28.03	26.22	29.53	28.63	26.23	24.86	29.14	31.60	30.11	0	0	0

Figure IV-9A: Input-output mean latency vs intensity functions for No, Po and N1 potentials from five crickets are shown (for better clarity only negative standard deviation bars are plotted).

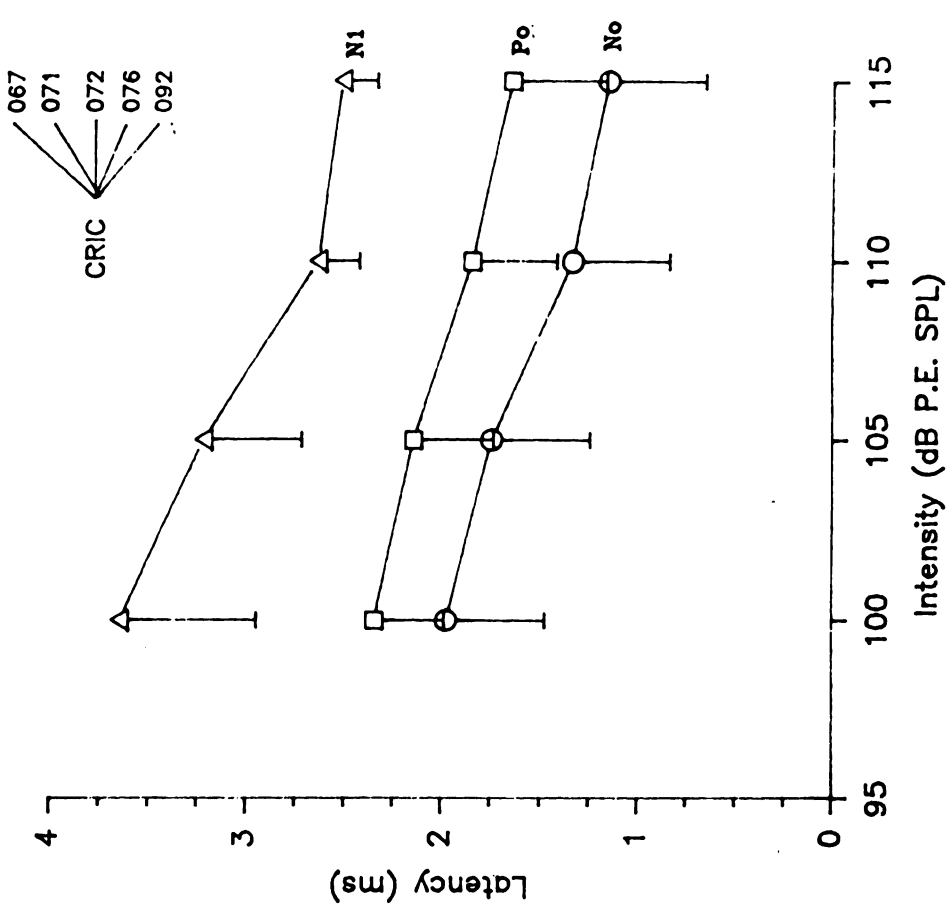
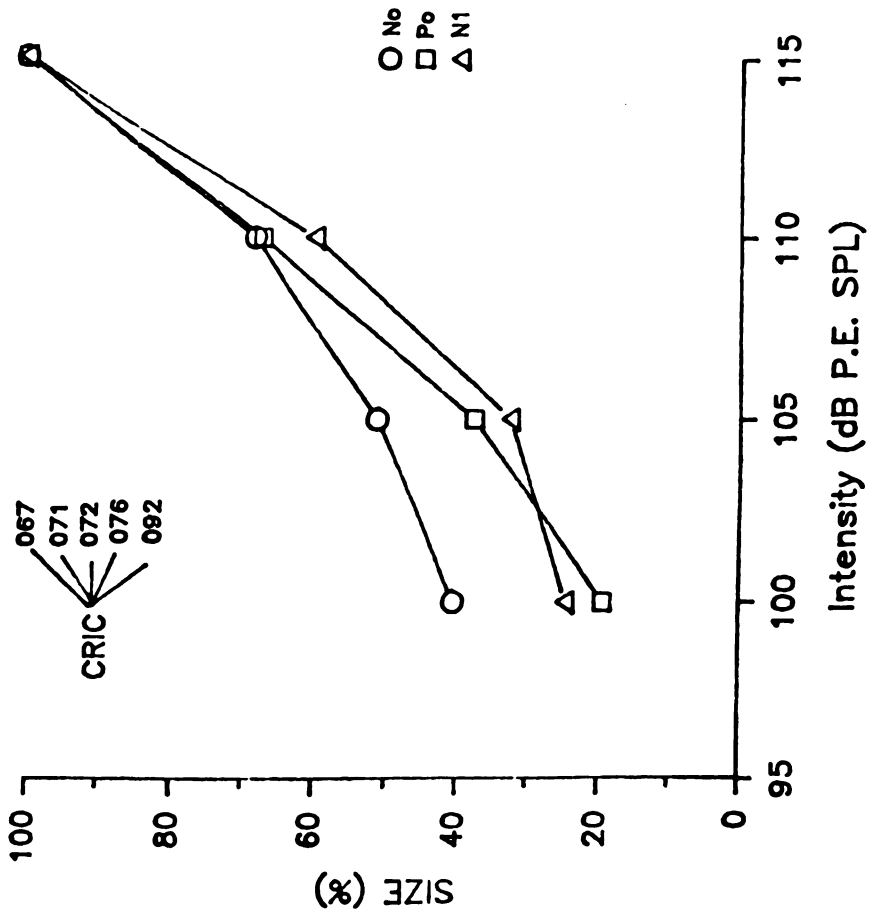

067
071
072
076
092
CRIC
N1
Po
No
Latency (ms)
0
1
2
3
4
95
100
105
110
115
Intensity (dB P.E. SPL)

FIGURE VI-9A

Figure IV-9B: **Input-output mean size (amplitude) vs intensity curves for No, Po and N1 potentials from five crickets.**

FIGURE IV-9B


No
Po
N1
067
071
072
076
092
CRIC
SIZE (%)
100
80
60
40
20
0
95
100
105
110
115
Intensity (dB P.E. SPL)

Figures IV-10A and IV-10B depict the input-output latency/intensity and size/intensity functions when the intensity was varied in 1.0 dB steps. Numerical data of latency and size values for No, Po and N1 potentials are represented in table IV.3. Here again the potentials No, Po and N1 decreased in latency and increased in amplitude when the intensity was increased.

Table IV-4 shows the slope and intercept of the three potentials, No, Po and N1, represented in figures IV-9A, IV-9B, IV-10A and IV-10B. It is seen that the slopes for No, Po and N1 are not very different from each other. The intercepts however, are different for each of the potentials, indicating that their origins are different (these waves are temporal characteristics of spatial representation of the potentials). The correlation co-efficients for the No, Po and N1 potentials for the latency/intensity function for the 5.0 dB steps were -0.989, -0.997 and -0.974 respectively. For the 1.0 dB steps data, the correlation co-efficients were -0.910, -0.920 and -0.938. For the size/intensity function, the co-efficients of correlation for No, Po and N1 for the 5.0 dB steps were 0.964, 0.993 and 0.962, and for the 1.0 dB steps they were 0.905, 0.982 and 0.967 respectively. The negative correlation values for the latency/intensity functions were indicative of decreasing latency as a function of intensity. The positive correlation values for the amplitude (size)/ intensity functions were indicative of increasing amplitude as a function of intensity. Similar functions investigated

Figure IV-10A: Input-output latency vs intensity (in 1.0 dB steps) curves for No, Po and N1 potentials.

Figure IV-10B: Input-output size vs intensity (in 1.0 dB steps) curves for No, Po and N1 potentials.

FIGURE IV–10A & 10B

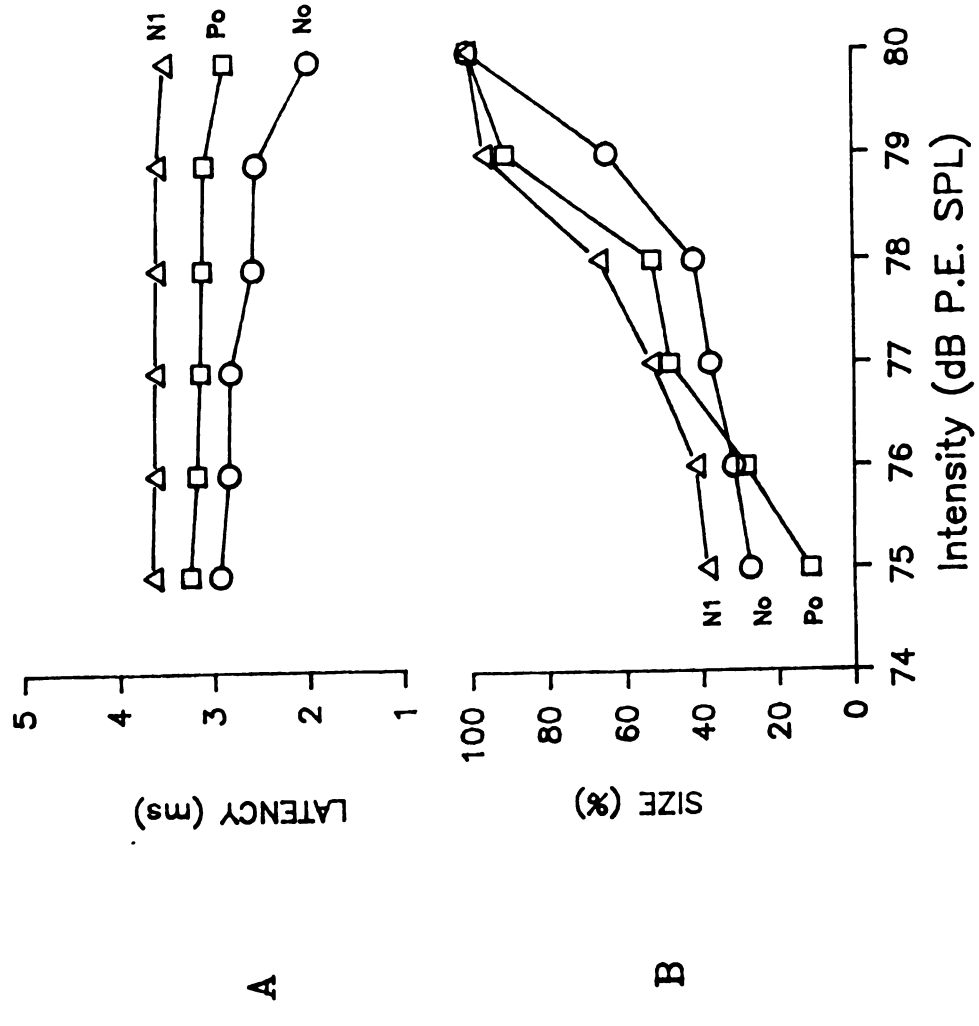


Table IV.3: Latency and size values for potentials No, Po and N1 in Cric-067 as a function of intensity (1.0 dB step).

TABLE IV.3

Latency and size of potentials No, Po and N1 in Cric–067 as a funtion of intensity varied in 1.0 dB steps

Intensity in dB P.E.SPL	Latency			Size		
	No	Po	N1	No	Po	N1
75	2.92	3.24	3.64	27.10	11.46	38.46
76	2.82	3.16	3.61	31.25	28.17	41.21
77	2.80	3.12	3.60	37.50	47.99	52.75
78	2.56	3.09	3.58	41.67	52.32	66.21
79	2.52	3.06	3.56	63.89	90.40	96.00
80	1.96	2.84	3.48	100	100	100

Table IV.4: Slopes and intercepts for No, Po and N1 potentials for latency/intensity and size/intensity functions.

TABLE IV.4

Slopes and intercepts for No, Po and N1 potentials for various functions

	Five dB steps						One dB steps					
	Latency vs intensity function			Size vs intenity function			Latency vs intensity function			Size vs intenity function		
	No	Po	N1	No	Po	N1	No	Po	N1	No	Po	N1
Slope	−0.06	−0.05	−0.08	3.91	5.43	5.07	−0.17	−0.07	−0.03	13.33	18.11	13.87
Intercept	7.72	7.15	11.53	−355.52	−527.57	−491.0	15.76	8.25	5.73	−982	−1348	−1009.3

in humans and cats have indicated that latency is inversely proportional to intensity, and amplitude is directly proportional to intensity (Moore and Semela, 1985; Moore et al 1988a and 1988b, 1989).

Summary: By varying the stimulus parameters, it was observed that the No-Po/EPSP-like potentials can best be seen at lower intensities just below the threshold for N1. Alternating tone bursts of frequencies below 2000 Hz, with a rise-decay time between 1.5 ms and 6.0 ms, and a repetition rate of 3.1/sec enhance the post-synaptic potentials, No, Po and N1.

Experiments designed to investigate the latency/intensity and amplitude/intensity functions on crickets have indicated that No, Po and N1 exhibit similar trends i.e., as intensity was increased the latencies of all the potentials decreased and the amplitude (size) increased. The slopes of their functions were not very different from each other, but their intercepts were different, indicating different sites of origin of these waves. The correlation co-efficients were also similar for No, Po and N1 potentials. This trend seen in the No, Po and N1 potentials in crickets is similar to the trend seen in the EPSP-like and N1 potentials in humans and cats.

Characterization of the EPSP-like potential using Pharmacologic agents

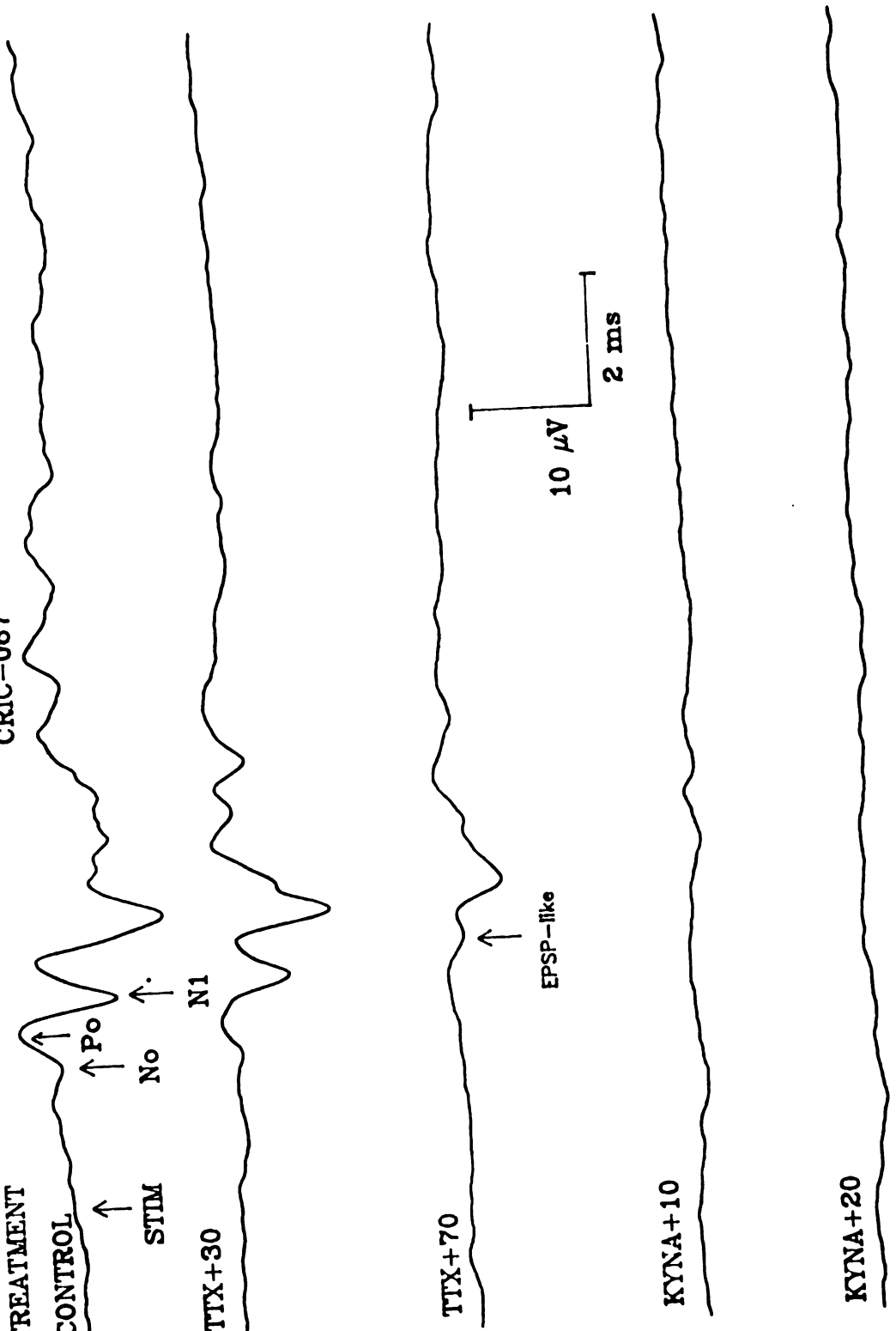
In order to identify the EPSP potential, the classic approach is to minimize the effects of the action potential which is usually superimposed on the EPSP. In neuro-pharmacology, tetrodotoxin (TTX) is an agent which acts selectively on fast sodium channels, thus inhibiting the propagation of action potentials. TTX does not interfere with the post-synaptic potentials generated by the dendrites of the post-synaptic fibers.

Figure IV-11 (Cric-067) depicts the effect of pharmacologic agents on the potentials. Recordings from Cric-058 and Cric-063 are presented in appendix E. In figure IV-11 the first recording is a control record which was obtained prior to any treatment. A prominent N1 can be seen, along with the No and Po potentials. Following this, 1.0 µl of TTX at a concentration of 0.1 mM was applied anterior to the fifth abdominal ganglion, and recordings were made at various intervals of time. Thirty minutes after application of TTX (depicted as TTX + 30), the N1 amplitude decreased, and at 70 minutes after application of TTX (TTX + 70) N1 disappeared, but in its place a broad, low frequency wave could be identified which was termed "EPSP-like" potential.

Kynurenic acid (KYNA), which is a broad spectrum excitatory amino acid antagonist was applied on the 5AG. Following the application of 1.0 µl of KYNA at a concentration of 1.0 mM,

Figure IV-11: **Electrophysiologic recordings of No, Po, N1 and the EPSP-like potential as a function of tetrodotoxin and kynurenic acid.**
STIM = stimulus.

FIGURE IV-11


CRIC-067
TREATMENT
CONTROL
STIM
Po
No
N1
TTX+30
TTX+70
EPSP-like
10 μV
2 ms
KYNA+10
KYNA+20

recordings were made at various intervals of time. Recordings made ten minutes after application of KYNA (Kyna + 10) indicated that the EPSP-like potential and the No/Po potentials diminished in amplitude, and at twenty minutes post application (Kyna + 20), no major peaks were identified.

The same data are represented as input-output functions in Figure IV-12 (Cric-067). The input-output functions for Cric-058 and Cric-063 are presented in appendix F. The graph in figure IV-12 depicts the size (amplitude in μV) of potentials as a function of time. The first vertical line next to the y-axis represents the time at which TTX was applied, and the second vertical line represents the time at which KYNA was applied. Following the application of TTX the potentials decreased in amplitude; however, they did not reach a zero percent level until KYNA was applied. Following application of KYNA all the potentials decreased in size. The diamonds representing the EPSP-like potential which was present (size = 100%) prior to application of KYNA, decreased in size immediately after KYNA was applied. The dashed lines connecting the function of the EPSP-like potentials (diamonds) indicate that these data were extrapolated, since it is not possible to identify the EPSP-like potential in the presence of N1. In the next experiment the behavior of the potentials subjected to TTX alone was investigated.

Figure IV.12: Input-output functions of No, Po, N1 and EPSP-like potentials as a function of tetrodotoxin and kynurenic acid.

FIGURE IV-12

CRIC-067

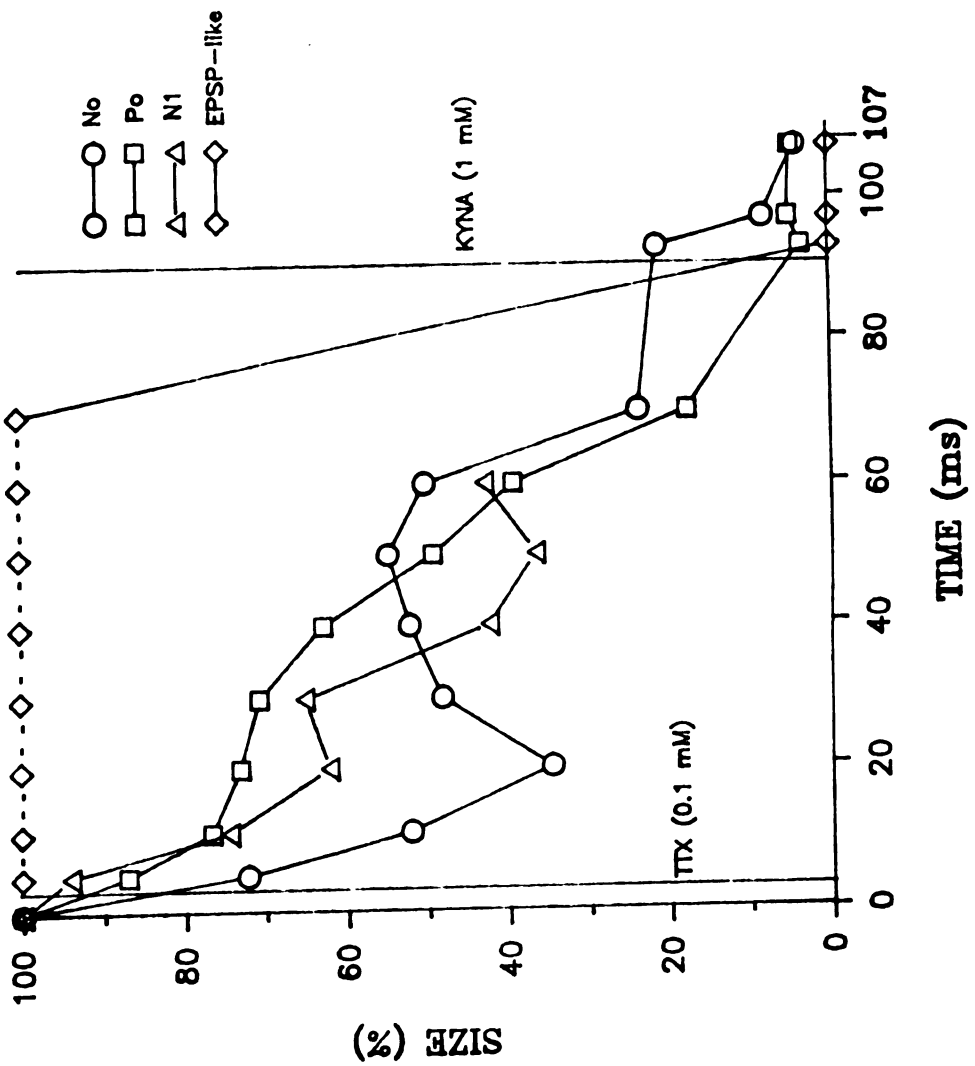


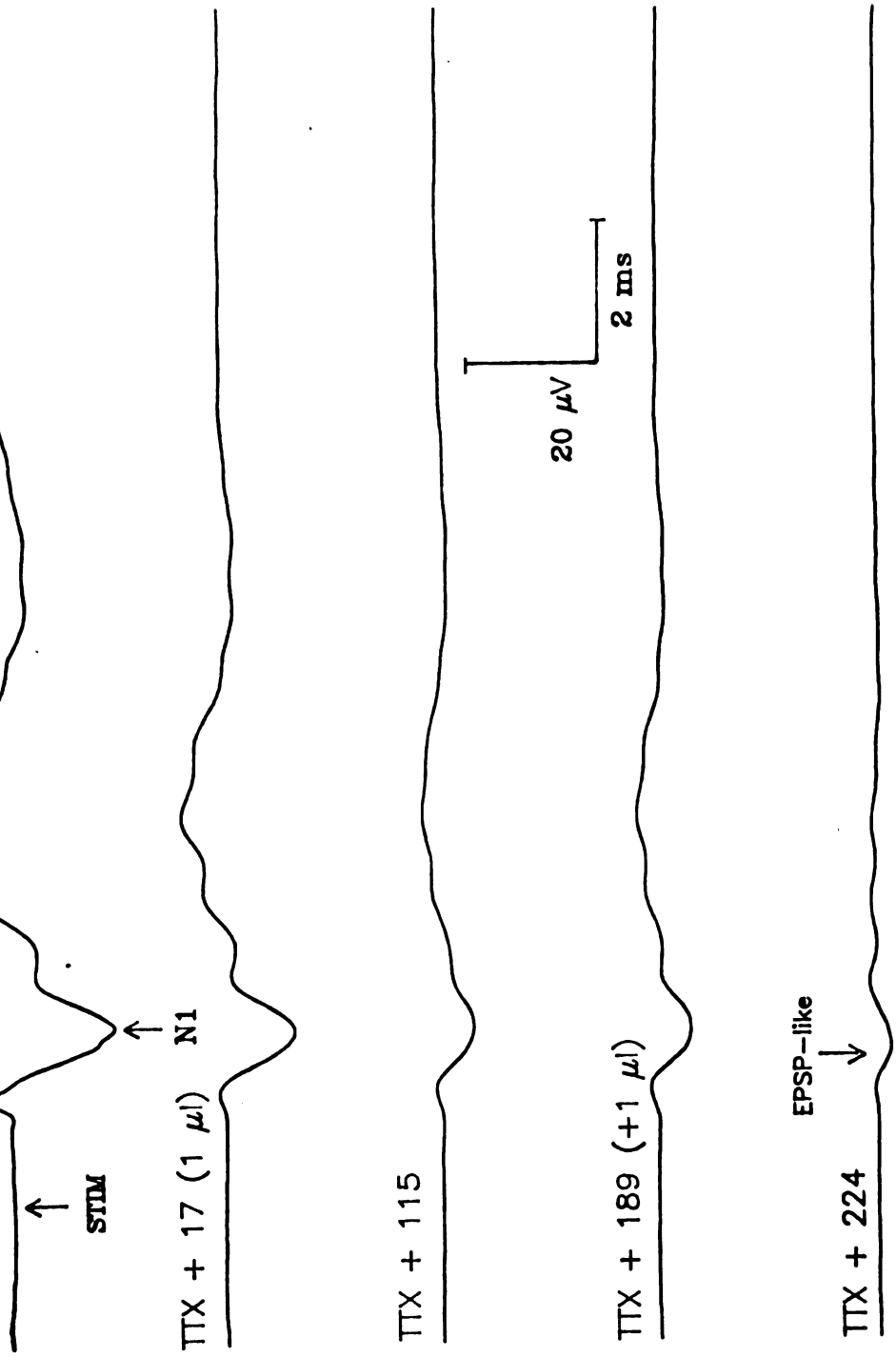
Figure IV-13 depicts the effect of TTX on the potentials over a period of 224 minutes (3 hours, 44 minutes). N1 decreased in amplitude; however, even at 224 minutes the EPSP-like potential remained, indicating perhaps that it is resistant to TTX. This study suggests that the decrease of potentials to almost a zero percent amplitude (Figure IV-12) is most probably due to the KYNA, and is not due to the long-term effects of TTX.

Summary: The experiments designed to investigate the effect of TTX and Kyna indicate that TTX has a strong effect on the N1 potential but not on the EPSP-like potential. However, within a few minutes after application of kyna the EPSP-like potential reduced drastically.

From previous studies we know that the cercal hearing system, which is one of the two main hearing systems in crickets, is comprised of hundreds of freely articulated sensilla that are innervated by sensory neurons whose axons combine to form the cercal nerve. The cercal nerves synapse on to the dendrites of the giant interneurons in the terminal abdominal ganglion. It is the EPSP-like potentials generated by the dendrites in the terminal abdominal ganglion that we set out to record. This is of interest since the cricket does not possess a cochlea. If we are successful in recording the EPSP-like potential in such a model it may strengthen the earlier studies which have indicated that the potential Po appearing prior to the N1 potential in animals (Moore et al 1988, 1989) and wave I' in

Figure IV-13: Electrophysiologic recordings of No, Po, N1 and EPSP-like potentials as a function of tetrodotoxin over a period of 224 minutes. STIM = stimulus.

FIGURE IV-13
CRIC-071



humans is perhaps an EPSP potential (Hughes and Fino, 1980; 1985 and Hughes et al 1981), and is not a cochlear receptor potential, i.e., a summating potential.

Our results indicate that with appropriate techniques it is possible to record the EPSP-like potential in the cricket's cercal hearing system. Experiments conducted in which frequency of the tone bursts was the independent variable, indicated that frequencies below 2000 Hz evoked greater responses. Intensity was found to have an important role in the identification of the EPSP-like potential. Higher intensities elicited larger APs but masked the EPSP. Hence, lower intensities that were unable to evoke APs were necessary to prevent enough depolarization of the axons, thus, allowing the EPSP-like potential to emerge. As typical of neural responses the potentials recorded showed variations with changes in repetition rate and rise-decay time of the stimuli. With an increase in repetition rate and rise-decay time No, Po and N1 amplitude decreased, latency increased and morphology changed. Simultaneous broad-band masking noise effectively masked the N1 response and the masking effect increased with an increase in the intensity of the masking noise.

Another successful method resulting in identification of the EPSP-like potential was to use pharmacologic agents. With the application of TTX (a neurotoxin which blocks fast Na^+ channels) anterior to the terminal ganglion, we were able to decrease the magnitude of the N1 potential and in its place

could be seen the low frequency negative EPSP-like potential. Further, with application of a broad spectrum excitatory amino acid antagonist KYNA on the terminal ganglion, No, Po and the EPSP-like potential diminished below visual detection level.

In this study, we have seen that with appropriate stimulus parameters, instrumentation and pharmacologic agents it is possible to identify and characterize the EPSP-like potential even in the cricket, which does not possess a cochlea. This supports the view that EPSPs can be recorded successfully in a variety of animals from different phylogenetic stages, e.g., crickets, gerbils, cats and humans (Moore et al, 1991).

CHAPTER V

DISCUSSION

In the mammalian inner ear, it is well known that the hair cell is an active transducer utilizing the incoming vibratory energy to modulate a local energy pool that is maintained by metabolic processes. It is also well accepted that due to the shearing motion of the basilar and tectorial membranes the hair cell stereocilia bend, resulting in the generation of receptor potentials (Davis, 1961; Dallos, 1984). The electrical changes in the hair cell result in the release of neuro-transmitter substance(s) glutamate and/or aspartate (Bobbin and Thompson, 1978; Guth and Melamed, 1982; Klinke, 1986), on to afferent nerve dendrites of the eighth cranial nerve.

The neuro-transmitter(s) depolarize the post-synaptic membrane, resulting in the production of post-synaptic potentials. These post-synaptic potentials are associated with the occurrence of transmissional activity at junctions between hair cells and the dendrites of the afferent nerve fibers. The post-synaptic potentials are neurally evoked potentials produced at the excitable electrogenic membrane of the post-synaptic afferent nerve dendrites. If the post-synaptic potentials are excitatory they are referred to as

excitatory post-synaptic potentials (EPSP). If the EPSPs are adequate in magnitude, they will lead to the generation of action potentials (AP). The production of the EPSP is thus essential for the generation of APs and its importance cannot be overlooked. In fact, if the EPSP is well characterized, it may be a more sensitive indicator of synaptic activity, rather than the all-or-none AP. The high sensitivity of the post-synaptic potential to the synaptic activity, which, in turn, is sensitive to many varieties of drugs, makes the post-synaptic potential an useful tool for investigating synaptic activity (Callec et al., 1971).

Potentials conjectured to be EPSPs of the auditory system have been recorded in humans (Hughes and Fino, 1980, 1985; Hughes, Fino and Gagnon, 1981; Moore and Semela, 1985) and in a variety of laboratory animals, e.g., gold fish, burbot, turtle, bullfrog, gerbil, cat and guinea pig (Furukawa and Ishii, 1967; Flock and Russell, 1976; Crawford and Fettiplace, 1980; Furukawa and Matsuura, 1985; Palmer and Russell, 1986; Siegel and Dallos, 1986; Moore, Caird, Löwenheim and Klinke, 1988a, 1988b, 1989; Klinke, Caird, Löwenheim and Moore, 1988; Sewell, 1990). It has been suggested that the EPSP may be the generator potential of the auditory nerve, having as its origin auditory nerve dendrites (Dallos and Cheatham, 1984; Siegel and Dallos, 1986). From the recordings of auditory evoked potentials in humans and laboratory animals, wave I in humans and wave N1

in animals have been shown to originate from the axonal portion of the auditory nerve (Antoneli-Candela and Kiang, 1978; Møller and Janetta, 1985). A few investigators have shown that the Po/EPSP-like potential precedes wave N1 in cat (Moore et al., 1988a, 1988b, 1989; Klinke et al., 1988) and guinea pig (Dolan et al., 1989; Xi et al., 1989).

In order to suppress the afferent activity of the axonal part of the auditory nerve, Moore et al., (1988a and 1988b) applied tetrodotoxin (TTX), which blocks the influx of Na^+ of nerve membranes. This led to the elimination of N1 and N2 responses leaving intact the EPSP-like potential. Further, with application of Kynurenic acid (KYNA) or L-Glutamic acid diethyl ester (GDEE, broad-spectrum excitatory amino acid receptor antagonists), which have been previously shown to suppress auditory nerve activity (Bledsoe, Bobbin and Chihal, 1981; Bobbin, Bledsoe and Chihal, 1981; Bobbin and Ceasar, 1987), the EPSP-like potential diminished. Xi, Dolan and Nuttall (1989) and Dolan, Xi and Nuttall (1989) have demonstrated a Po/EPSP-like potential in the guinea pig, which was resistant to TTX, but was eliminated with the application of kainic acid.

There exists a controversy as to whether the recorded EPSP-like potentials are truly generator potentials leading to APs, or receptor potentials from the cochlea. In order to clarify this argument, an animal which does not possess a

cochlea was used, i.e., the house cricket (Acheta domesticus), an invertebrate model. The aim of this study was to identify and characterize the EPSP-like potential in the cercal hearing system of the cricket at its first synapse, which is at the level of the fifth abdominal ganglion.

In order to identify and characterize the EPSP-like potentials in the cricket cercal hearing system, two sets of experiments were conducted. The objective of the first set of experiments was to vary a number of stimulus parameters and to specify a set of optimum stimulus parameters as related to intensity, frequency and time, which will best characterize the EPSP-like potential. So far little is known about the effects of acoustic stimulus paramaters on the cercal hearing system of crickets. Some of the acoustic parameters relevant to fundamental neuro-physiological processes are: Sound pressure level (SPL) and rise-decay time, which apply to the spatio-temporal configuration of activity in neural aggregrates; repetition rate, as it refers to the pulses within a signal envelope, applies to the integration of acoustic energy; interstimulus interval as it bears upon successive characteristics at varying levels of the auditory system, applies to the relative distribution of excitatory and inhibitory mechanisms; and frequency which applies to the locus of stimulation of the afferent pathways (Eisenberg, 1965). The above parameters

were manipulated in the first set of experiments. In the second set of experiments pharmacologic agents such as TTX and KYNA were used to characterize the EPSP-like potential.

We observed that the responses consisted of a series of negative and positive waves. The waveform morphology, thresholds, latencies and amplitudes were not similar in all the crickets tested. However, there were some general features that were commonly seen in all the waveforms and were designated as follows: The first prominent and stable negative deflection was designated N1, the positive peak following N1 was designated P1. The positive peak preceding N1 was designated Po, and the negative wave preceding Po was termed No. This method of designating the positive and negative waves was earlier adopted by a number of investigators during the process of identifying cochlear action potentials and auditory brain-stem responses (Sohmer and Feinmesser, 1967: Jewett and Williston, 1971; Moore, 1971).

In order to ascertain that these responses were purely neural responses, the polarity of the tone bursts was varied. The No, Po and N1 potentials appeared regardless of the polarity. In addition to the No, Po and N1 potentials, there appeared several early responses for condensation and rarefaction stimuli which reversed in polarity, but were cancelled with the alternating stimuli. The absence of the

earlier responses for the alternating stimuli suggests that these responses are phase dependent or stimulus artifacts, which then cancel during the alternating phase. The insensitivity of No, Po and N1 potentials to stimulus polarity suggests that these potentials are neural rather than electro-mechanical in nature.

At high intensity levels peaks No, Po and N1 could be easily identified, however, the EPSP-like potential could be identified only at lower intensity levels, i.e., at levels in which the N1 response was not triggered. When the intensity was increased the EPSP-like potential was masked by the larger N1 response. The transition phase from N1 to the EPSP-like potential was better seen when 1.0 dB intensity increments were incorporated. This agrees with the results from the study by Callec and Sattelle (1973) where they stimulated the cercal receptors in the cockroach with mechanical and electrical stimulation, and made recordings from the terminal ganglion. Stimulation of the receptors resulted in the depolarization of the neurons and production of EPSPs, as the stimulation was made stronger it resulted in greater depolarization, reaching the threshold for spike generation.

The auditory system in mammals is capable of performing excellent temporal resolutions and frequency analysis (Keidel, Kallert, Korth and Humes, 1983). Experiments

conducted with the frequency of the tone burst as the independent variable suggest that the responses for lower frequencies (most often lower than 1500 Hz) elicited more distinct waveforms. The responses for frequencies above 2000 Hz were found to be less distinct in all our recordings. This agrees well with earlier studies, which have indicated that the cercal hearing system in the cricket is more sensitive to lower frequencies than high frequencies (Edwards and Palka, 1974; Counter, 1976a). Although there is disagreement in the specific adaptive functions assigned to the auditory sensitivity of the cerci, it is not unreasonable to say that the cerci perhaps play an important role in the detection of low frequency rustling noises of predators as well as other similar biologically significant sounds (Counter, 1976a). Earlier, katsuki and Suga (1960) had suggested that the cercal sensilla of many orthoptera were used in conjunction with the tympanal organs to provide a mechanism of sound discrimination. The cerci were found to respond to low and middle frequencies and the tympanal organs to the middle and high frequencies. Other than the conspecific chirps of the crickets, most biologically significant sounds in the cricket's environment are complex low frequency signals detected by the cerci.

Typical neural responses are influenced by the rise-decay times of the input signal. Distinct waveforms depend upon a synchronous discharge of the nerve fibers. Longer rise-

decay times cause a delayed neural discharge, hence affecting the morphology, latency and amplitude of the waveforms. Responses to tone bursts at various rise-decay times indicate that rise-decay times less than 1.5 ms, but greater than 3.0 ms caused an increase in latency and a decrease in the amplitude of the waveforms. This indicates that there is an optimum range for rise-decay time in which response identification is best, beyond a 6.0 ms rise-decay time the response identification becomes difficult. The underlying reason for this observation may be that beyond the optimal levels, there could be less synchrony of neural responses, as well as delayed neural discharges. In studying the effect of various rise-decay times on the ABR in humans, Moore (1983) noted that as rise-decay time increased, the response identification became more difficult to a point where peak identification was not possible.

The stimulus repetition rate or better, the inter-stimulus interval is of high significance for the amplitude of the evoked potentials (Keidel et al., 1983). With an increase in repetition rate, as is typical of neural responses, the potentials decreased in amplitude and increased in latency. We know from previous studies on humans and laboratory animals that increasing the repetition rate of a signal increases latency but decreases the amplitude of the waveforms (Moore, 1971; Don, Allen and Starr, 1977; Campbell, Picton and Wolfe, 1981; Amedofu, 1989). However,

in humans and laboratory animals such an effect is more pronounced for repetition rates greater than 11.1/sec. In crickets the effect is seen much earlier, i.e., around 9.1/sec, although we are not certain as to why this occurs, we would surmise that the unmyelinated nerve fibers in the crickets are less capable of transducing these more rapid stimuli. What is really happening with increase in repetition rate is that the inter-stimulus interval is being shortened which in effect leaves less time for the nerve fibers to recover, thus the system gets overloaded. Earlier studies mentioned above have also indicated that the effect of repetition rate is restricted only to neural responses, and other mechanical responses including cochlear microphonics do not decrease with increase in repetition rate.

Simultaneous masking experiments indicated that the N1 potential can be successfully masked with adequate broad-band masking noise. The N1 potential diminished in magnitude with an increase in the masking noise. The EPSP-like potential which is co-incident with N1 is not seen in the recordings at high intensity levels, however, with masking noise N1 reduced in amplitude and prolonged in latency, enhancing the appearance of the EPSP-like potential.

Latency/intensity functions for No, Po and N1 exhibited a

strong negative correlation between latency and intensity for 5 dB increments (r= -0.989, -0.997 and -0.974 respectively), and also for 1 dB increments (r= -0.910, -0.920 and -0.938 respectively). Size (amplitude)/intensity functions for No, Po and N1 exhibited a strong positive correlation between size and intensity for 5 dB increments (r= 0.964, 0.993 and 0.962 respectively), and also for 1 dB increments (0.905, 0.982 and 0.967 respectively). The slopes were similar, but the intercepts were different for No, Po and N1 potentials (slopes for latency vs intensity functions were -0.06, -0.05 and -0.08 for 5 dB increments, and -0.17, -0.07 and -0.03 for 1 dB increments. Slopes for size vs intensity functions were 3.91, 5.43 and 5.07 for 5 dB increments and 13.33, 18.11 and 13.87 for 1 dB increments. Intercepts for latency vs intensity functions were 7.72, 7.15 and 11.53 for 5 dB increments, and 15.76, 8.25 and 5.73 for 1 dB increments. Intercepts for size vs intensity functions were -355.52, -527.57 and -491.0 for 5 dB increments, and -982, -1348 and -1009.2 for 1 dB increments), indicating possibly different anatomical sites of origin of the three waves. Similar functions investigated in humans and cats have indicated that latency is inversely proportional to intensity, and amplitude is directly proportional to intensity. In humans Moore and Semela (1985) have noted that the I' potential (an EPSP-like potential in humans) closely paralleled the latency intensity function of waves I and II (equivalent to N1 and

N2 potentials in animals). Experiments conducted by Moore et al (1988a, 1988b, 1989) have also yielded similar trends. When intensity was increased Po (an EPSP-like potential in animals), N1 and N2 potentials increased in amplitude and decreased in latency. Po closely paralleled the input/ output function of N1 and N2. The results of these investigations indicate that the No, Po and N1 potentials in crickets have similar latency/intensity and amplitude/ intensity functions and that they are comparable to the Po and N1 potentials in mammals.

TTX is among the deadliest poisons known to man. It is found in the largest quantities in the gonads of some fish. TTX interferes with the early transient conductance increase that is associated with downhill movement of Na^+ in most common excitable cells (Kao, 1972). Application of TTX anterior to the terminal ganglion brought about a significant change in N1 amplitude and latency over time. Similar effects have been seen in mammalian studies (Moore et al., 1988a, 1988b, 1989; Dolan et al., 1989; and Xi et al., 1989). In the mammalian studies, as well as in the present study, in order to identify the EPSP-like potential, the AP was reduced in size with the application of TTX. Recordings made at regular intervals after application of TTX indicated that the N1 potential started to diminish in amplitude, and eventually what remained was the broad low frequency EPSP-like potential. In order to determine if the

EPSP-like potential disappeared over time, in one of the control experiments post-TTX recordings were made for 3 hours and 44 minutes. Responses indicated that even after 224 minutes after application of TTX (where the experiment ended) the EPSP-like potential remained.

These experiments suggest that the EPSP-like potential has characteristics different from the N1 potential and is not generated by the fast conductivity of axonal sodium channels. In the previous studies (Moore et al., 1988a, 1988b, 1989; Dolan et al., 1989; Xi et al., 1989, 1989) a similar paradigm was used. Intracochlear infusion of TTX in the mammalian inner ear brought about little change in the CM or electromechanical component. However, the compound APs N1 and N2, declined to zero over a period of about 30 - 60 minutes, resulting in the appearance of a slow negative wave. This wave which was resistent to TTX was identified as an "EPSP-like" potential (Dolan et al., 1989; Xi et al., 1989).

It is significant that the slow wave remnant normally masked by the much larger AP emerges after application of TTX, and is therefore a candidate for the remainder of the EPSP (Moore et al., 1988a 1988b, 1989; klinke et al., 1988; Xi et al., 1989; Dolan et al., 1989). Further, it is of interest to know that the No-Po/EPSP-like potential can be abolished by transmitter blockers, which suggests that these

potentials are post-synaptic potentials, yet are different from APs.

In the experiments conducted in this study, after application of TTX the N1 potential diminished and in its place the EPSP-like potential could be identified. Following this, KYNA, a quinoline derivative and a broad spectrum excitatory amino acid antagonist was applied on the fifth abdominal ganglion. Antagonist is a substance that tends to nullify the action of another drug that binds to a cell receptor, and does not elicit a biological response (Kao, 1972). The reason of our choice of KYNA over other antagonists was because previous studies have demonstrated that KYNA did not significantly interact with other neuro-transmitters, did not have nonspecific actions on cellular excitability, and did not alter the input resistance significantly (Gribkoff and Dudek, 1988, 1990). The mechanism of action of KYNA as synaptic blocker is probably via a post-synaptic block of transmitter receptors because of its antagonist properties (Ganong, Lanthorn and Cotman, 1983).

The EPSP-like potential that was resistant to TTX began to diminish in size with time after kYNA was applied, and finally disappeared. The similarities in the results obtained in mammalian studies and this study with crickets, indicate that the EPSP-like responses can be recorded by

reducing the magnitude of the AP, and the EPSP-like potentials are perhaps post-synaptic potentials, and the presence of a cochlea is not necessary for their production.

It is also interesting to note that KYNA applied on the terminal ganglion reduced the amplitude of No, Po and N1 potentials to almost zero in a short time (20 - 50 minutes). This strongly suggests that excitatory amino-acids are likely transmitters at the first synapse in the cricket's cercal hearing system. Earlier studies (Callec and Sattelle, 1973; Meyer and Reddy, 1985) have shown the possibility of acetylcholine receptors in the giant neurons, but no mention has been made of amino-acid receptors. KYNA is a broad spectrum excitatory amino-acid antagonist, but it does not clearly distinguish between different excitatory amino acid receptors. So, in order to characterize amino-acid sub-receptor types, studies with more specific agonists and antagonists will need to be conducted.

There is also a need to attempt to block the EPSP-like potential with a potassium blocker, such as tetraethyl-ammonium, in order to rule out the possibility that the EPSP-like potential is the potassium generated negative after potential. Is the EPSP-like potential a membrane potential? This is an important question, and should be tested by using a membrane blocker such as Joro spiker toxin.

Although the role of EPSPs in auditory nerve functioning is not well established, it may not be erroneous to conclude that the No-Po/EPSP-like potentials are the generator potentials that initiate the action potential. The generator potential has its origin auditory nerve dendrites, while action potentials have as their origin the axons of the auditory nerve. Hence, the No-Po/EPSP-like potential is an integral part of the afferent auditory mechanism, and this study suggests that crickets can be used as a suitable model for the study of synaptic and post-synaptic activities.

CHAPTER VI

SUMMARY AND CONCLUSIONS

In the mammalian auditory system the hair cells, which are the receptors of sound stimuli in the organ of Corti, release neuro-transmitters to activate the peripheral dendrites of the spiral ganglion fibers (Dallos, 1984). The neuro-transmitters in turn excite the dendrites of the spiral ganglion fibers, leading to generation of post-synaptic potentials which, if excitatory in nature, are called excitatory post-synaptic potentials (EPSP). If and only when the EPSP reaches threshold will it lead to the generation of action potentials (AP). The EPSP, which is the first potential among the post-synaptic potentials, is a good indicator of synaptic activity, and, if well characterized, could be used as a sensitive index of synaptic activity.

Although the importance of the EPSP is generally accepted, very little is known about the auditory EPSP. There are only a few studies that have investigated the auditory EPSP. In human beings the EPSP is recorded as the "I'" potential, the potential preceding wave I of the auditory brain stem

response (Hughes and Fino, 1980; Hughes, Fino and Gagnon, 1981; Benito et al 1984, Hughes and Fino, 1985; Moore and Semela, 1985). In laboratory animals the EPSP was recorded and designated as the "Po" potential (Moore et al., 1988a and 1988b; Klinke et al., 1988; Moore et al., 1989). Xi et al., (1989) and Dolan et al., (1989) recorded the EPSP potential and named it "EPSP-like" potential. However, among the investigators a controversy arose as to whether the recorded potential was truly an EPSP or the summating potential (a receptor potential from the cochlea). This study was undertaken in which the house cricket (Acheta domesticus) was used as a model to study the EPSP potentials in their cercal hearing system. The cricket basically has two important hearing systems, the tympanal hearing system and the cercal hearing system. The receptors for the cercal hearing system are freely articulated hairs called sensilla, which are innervated by sensory neurons. The axons of the sensory neurons combine to form the cercal nerve and synapse on the giant interneurons in the fifth abdominal ganglion (also called the terminal ganglion).

The EPSP are studied at the first synaptic level, i.e., in the terminal ganglion where the cercal nerve fibers synapse on to the dendrites of the giant interneurons. The potentials recorded from the terminal ganglion are gross potentials and not single unit recordings, hence the term EPSP-like potential is used rather than the term EPSP.

Twenty five crickets were used in this study to answer the following questions:

1. Is it possible to identify an EPSP-like potential in gross potential recordings of the ventral cord of Acheta domesticus, whose different components can be designated as No-Po/EPSP-like potentials?
2. Following identification of the No-Po/EPSP-like potentials, what stimulus parameters such as frequency, intensity and time best characterize the post-synaptic potentials using latency and amplitude functions?
3. Using pharmacologic agents such as tetrodotoxin and kynurenic acid, how do they assist in the identification of the No-Po/EPSP-like potentials?

Adult male and female crickets serving as subjects were immobilized in ice. Three pairs of legs and two pairs of wings were removed and petroleum jelly was applied on the cut ends so as prevent escape of bodily fluids. The cricket was pinned on to bee's wax glued to a vibration-free table in a sound-treated room.

Three 270-micron silver-silver chlorided wires were used as electrodes. The active electrode was placed on the fifth abdominal ganglion, the reference electrode in the head and the ground electrode in the abdomen. Acoustic stimulation was provided by tone bursts from a speaker which was located

at a distance of 5 cm, at an angle of 90^{o} to the longitudinal axis of the cerci.

In order to answer the questions posed, two sets of experiments were conducted. In the first set, various stimulus parameters such as stimulus intensity, frequency, repetition rate, polarity, masking noise and rise-decay time were varied. Compound action potentials were recorded using various experimental conditions.

The results of the experiments indicated that an EPSP-like potential can be identified and characterized using appropriate stimulus parameters. The No, Po and N1 potentials were easily identified at high intensity levels; however, the intensity had to be lowered in order to identify the EPSP-like potential since, EPSP-like potential was obscured in the presence of N1. At low intensity levels the stimuli were insufficient to trigger N1, and in its place was seen the EPSP-like potential. The input/output intensity/latency curves and intensity/amplitude curves indicate that No, Po and N1 potentials decreased in latency and increased in amplitude with an increase in intensity.

The No, Po and N1 responses were better seen at lower frequencies, i.e. below 2000 Hz. As the repetition rate was increased from 1.1/sec to 3.1/sec, the potentials increased in amplitude and decreased in latency. With further

increase in repetition rate the latencies increased and amplitude decreased. Similarly, with an increase in rise-decay time, as is typical of neural responses, the potentials decreased in amplitude and increased in latency. With an increase in simulataneous masking of the tone burst with broad band noise, the N1 potential gradually decreased in amplitude.

The latency/intensity and size (amplitude)/intensity functions indicated similar trends in No, Po and N1 potentials. The trend seen in this study was similar to the trend seen in other human and cat studies (Moore and Semela, 1985; Moore et al 1988a and 1988b; Klinke et al, 1988; Moore et al 1989). There exists a negative correlation between latency and intensity and a positive correlation between size and intensity for all the potentials tested, namely, No, Po and N1.

The second set of experiments was conducted so as to characterize the EPSP-like potentials using pharmacologic agents. In these experiments the stimulus parameters were held constant. In order to identify the EPSP-like potential the N1 potential was decreased in amplitude by applying tetrodotoxin (TTX, which is a neurotoxin known to affect Na^+ channels) into a 2.0 mm plastic barrel placed anterior to the fifth abdominal ganglion. Recordings made at different intervals of time after application of TTX indicated that

with time, the N1 potential gradually decreased in amplitude. In its place we could see a low frequency EPSP-like potential which was highly resistant to TTX. We were able to show that even 224 minutes after application of TTX, the EPSP-like potential remained. Application of kynurenic acid (a broad-spectrum excitatory amino-acid antagonist) on the fifth abdominal ganglion brought about a gradual decrease in the EPSP-like potential, and within 25-30 minutes there were no potentials that were recognizable.

The potentials recorded from various crickets indicated that there is variability in the morphology, latency, amplitude and threshold measurements. Although we are not certain of the underlying reasons for the variability, we can speculate that it could be due to the differences in age, size and the genetic make up of the crickets.

Suggestions for Further Research

Based on the results obtained in this study, the cricket can be used as a model for investigating synaptic activity and post-synaptic potentials. The following recommendations are suggested for additional research:

1. An investigation similar to the present investigation should be conducted using crickets whose age, size and genetic make up are known. Such crickets can be obtained from known suppliers such as Fluckers Cricket Farm, P.O. Box

378, Baton Rouge, LA 70821.

2. A similar investigation should be conducted at the level of the prothoracic ganglion, which is the first synapse in the tympanal hearing system in crickets.

3. A similar investigation with electrical stimulation should be conducted in order to determine whether electrical stimulations evoke similar responses to those of acoustic stimuli.

4. Single unit recordings of dendrites and giant interneurons in the ventral cord of the cricket nervous system should be made so as to obtain more discrete recordings of synaptic activity.

5. Patch-clamp studies need to be conducted on the giant interneuron dendrites so as to investigate individual currents responsible for depolarization and generation of the EPSP.

6. A mechanical system should be developed which will be capable of stimulating an individual sensillum, while making single-unit recordings, so as to investigate contributions from more discrete portions of the cercus.

7. There is a need to use more specific NMDA and non-NMDA antagonists in order to identify possible receptor sub-types of the post-synaptic membranes.

8. Studies using excitatory amino acid agonists are needed in order to determine excitatory effects of these compounds.

9. There is a need to use nicotinic and muscarinic antagonists in an attempt to identify possible sub-

populations of receptors of this class.

10. Investigations need to be conducted which utilize tetraethylammonium (TEA) to block K^+ efflux, and Joro spider toxin to block the membrane response.

REFERENCES

REFERENCES

Abbas, P. (1984). Recovery from long-term and short-term adaptation of the whole nerve action potential. Journal of Acoustical Society of America, 75, 1541-1547.

Antoli-Candela, F., & Kiang, N. (1978). Unit activity underlying the N1 potential. In R. Nauton & G. Fernandez (Eds.), Evoked Electrical Activity in the Auditory System. New York: Academic press.

Amedofu, G. K. P. (1989). Effects of the gene Wh on the hearing of hamsters using auditory brain-stem evoked responses. Unpublished Ph.D. dissertation, Michigan State University, East Lansing, Michigan.

Bacon, J. P., & Murphey, R. K. (1984). Receptive fields of cricket giant interneurones are related to their dendritic structure. Journal of Physiology, 352, 601-623.

Barnard, E. A., & Henley, J. M. (1990). The non-NMDA receptors: types, protein structure and molecular biology. Trends in Pharmacological Sciences, 11, 500-507.

Benito, M., Falco, P., & Lauro, A. (1984). Brain-stem auditory evoked potentials: Description of the "O" wave of possible electrocochlear origin. Electroencephalography and Clinical Neurophysiology, 58, 100p.

Blagburn, J. M., Beadle, D. J., & Sattelle, D. B. (1984). Synapses between an identified giant interneurone and a filiform hair sensory neurone in the terminal ganglion of first instar cockroaches (Periplaneta americana). Journal of Experimental Biology, 113, 477-481.

Bobbin, R. P. (1979). Glutamate and aspartate mimic the afferent transmitter in the cochlea. Experimental Brain Research, 34, 389-393.

Bobbin, R. P., Bledsoe, Jr. S., & Chihal, D. (1981). Effects of various excitatory amino acid antagonists on guinea pig cochlea potentials. Association for Research in Otolaryngology Abstracts, IV, 27.

Bobbin, R. P., & Ceasar, G. (1987). Kynurenic acid and r-D-glutamylaminomethylsulfonic acid suppress the compound action potential of the auitory nerve. Hearing Research, 25, 77-81.

Bobbin, R. P., & Thompson, M. H. (1978). Effects of putative transmitters on afferent cochlear transmission. Annals of Otology, Rhinology, and Laryngology, 87, 185-190.

Boyan, G. S., & Ball, E. E. (1989). Parallel inputs shape the response of a giant interneurone in the cercal system of the locust. Journal of Insect Physiology, 35(4), 305-312.

Callec, J. J., Guillet, J. C., Pichon, Y., & Boistel, J. (1971). Further studies on synaptic transmission in insects. II. Relationships between sensory information and its synaptic integration at the level of a single giant axon in the cockroach. Journal of Experimental Biology, 55, 123-149.

Callec, J. J., & Sattelle, D. B. (1973). A simple technique for monitoring the synaptic actions of pharmacological agents. Journal of Experimental Biology, 59, 725-738.

Campbell, K. B., Picton, T. W., & Wolfe, R. G. (1983). Auditory Potentials. In E. J. Moore (Ed.), Bases of Auditory Brain-Stem Potentials. New York: Grune and Stratton Inc.

Comis, S. D., & Leng, G. (1979). Action of putative neurotransmitters in the guinea pig cochlea. Experimental Brain Research, 36, 119-128.

Cotman, C., & Iversen, L. (1987). Excitatory amino acids in the brain-focus on NMDA receptors. Trends in Neuroscience, 10, 263-265.

Counter, S. A. (1976a). An electrophysiological study of sound sensitive neurons in the "primitive ear" of Acheta domesticus. Journal of Insect Physiology, 22, 1-8.

Counter, S. A. (1976b). Fourier and electrophysiological analyses of acoustic communication in Acheta domesticus. Journal of Insect Physiology, 22, 589-593.

Crawford, A. C., & Fettiplace, R. (1980). The frequency selectivity of auditory nerve fibres and hair cells in the cochlea of the turtle. Journal of Physiology, 306, 79-125.

Dallos, P., & Cheatham, M. (1974). Generator Potentials: are they identifiable from gross cochlear recordings? Journal of Acoustical Society of America, 66, S10.

Dallos, P. (1984). Peripheral Mechanisms of Hearing. In: I. Darian-Smith (Ed.), The Nervous System: Sensory Processes, vol III, part 2, 595-637.

Davis, H. (1961). Some principles of sensory receptor action. Physiology Review, 41, 391-416.

Dolan, D. F., Xi, L., & Nuttall, A. L. (1989). Characterization of an EPSP-like potential recorded remotely from the round window. Journal of Acoustical Society of America, 86(6), 2167-2171.

Don, M., Allen, A. R., & Starr, A. (1977). Effects of click rate on the latency of auditory brain stem responses in humans. Annals of Otology Rhinology and Laryngology, 86, 186-196.

Drejer, J., & Honore, T. (1988). New quinoxalinediones show potent antagonism of quisqualate responses in cultured mouse cortical neurons. Neuroscience Letters, 87, 104-108.

Dumpert, K., & Gnatzy, W. (1977). Cricket combined mechanoreceptors and kicking response. Journal of Comparative Physiology, 122, 9-25.

Eccles, J. C. (1959). Neuron physiology. In: J. Field, H. W. Magoun, and V. E. Halt (Eds.), Handbook of Physiology, Section 1: Neurophysiology, vol I. Washington D. C. American physiological society.

Edwards, J. S., & Palka, J. (1974). The cerci and abdominal giant fibers of the house cricket, Acheta domesticus-I. Anatomy and physiology of normal adults. Proceedings of Royal Society of London (B), 185, 83-103.

Eisenberg, R. B. (1965). Auditory behavior in the human neonate: I. Methodologic problems and the logical design of research procedures. Journal of Auditory Research, 5, 159-177.

Erler, G. (1983). Reduction of mechanical sensitivity in an insect mechanoreceptor correlated with destruction of its tubular body. Cell Tissue Research, 234, 451-461.

Evans, E. F., & Klinke, R. (1982). The effects of intracochlear cyanide and tetrodotoxin on the properties of

single cochlear nerve fibres in the cat. Physiology, 331, 385-406.

Fex, J., & Martin, M. R. (1980). Lack of effect of DL-a-Aminoadipate, an excitatory amino acid antagonist, on cat auditory nerve responses to sound. Neuropharmacology, 19, 809-811.

Flock, A., & Russell, I. J. (1973). The post-synaptic action of efferent fibres in the lateral line organ of the burbot Lota lota. Journal of Physiology, 235, 591-605.

Flock, A., & Russell, I. J. (1976). Inhibition by efferent nerve fibers: Action on hair cells and afferent synaptic transmission in the lateral line canal organ of the burbot Lota lota. Journal of Physiology, 257, 45-62.

Furukawa, T., & Ishii, Y. (1967). Neurophysiological studies on hearing in goldfish. Journal of Neurophysiology, 30(2), 1377-1403.

Furukawa, T. (1978). Sites of termination on the saccular macula of auditory nerve fibers in the goldfish as determined by intracellular injection of procion yellow. Journal of Comparitive Neurology, 180, 807-814.

Furukawa, T., & Matsuura, S. (1978). Adaptive rundown of excitatory post-synaptic potentials at synapses between hair cells and eighth nerve fibers in the goldfish.

Journal of Physiology, 276, 193-209.

Furukawa, T., Hayashida, Y., & Matsuura, S. (1978). Quantal analysis of the size of excitatory post-synaptic potentials at synapses between hair cells and afferent nerve fibers in goldfish. Journal of Physiology, 276, 211-226

Furukawa, T., Kuno, M., & Matsuura, S. (1982). Quantal analysis of a decremental response at hair cell-afferent fibre synapses in the goldfish sacculus. Journal of Physiology, 322, 181-195.

Furukawa, T., & Matsuura, S. (1985). Mode of operation of afferent synapses between hair cells and auditory fibers. In D. Dresher (Ed.), Auditory Biochemistry. Springfield, IL: Charles C. Thomas, Publisher.

Ganong, A. H., Lanthorn, T. H., & Cotman, C. W. (1983). Kynurenic acid inhibits synaptic and acidic amino-acid-induced responses in the rat hippocampus and spinal cord. Brain Research, 273, 170-174.

Gnatzy, W. (1976). The ultrastructure of the thread-hair on the cerci of the cockroach Periplaneta americana: The intermoult phase. Journal of Ultrastructure Research, 54, 124-134.

Gnatzy, W. (1980). Ultrastructure and mechanical

properties of an insect mechanoreceptor: Stimulus-transmitting structures and sensory apparatus of the cercal filiform hairs of Gryllus. Cell Tissue Research, 213, 441-463.

Gnatzy, W., & Tautz, J. (1980). Ultrastructure and mechanical properties of an insect mechanoreceptor: Stimulus-transmitting structures and sensory apparatus of the cercal filiform hairs of Gryllus. Cell and Tissue Research, 213, 441-463.

Goode, J. (1984). Ultrasound elicits tonic responses and diminishes the phasic responses to adequate stimuli in thread-hair mechanoreceptors of Acheta domesticus. Biophysics of Strucuture and Mechanism, 10, 275-296.

Gribkoff, V. K., & Dudek, E. F. (1988). The effects of the excitatory amino acid antagonist kynurenic acid on synaptic transmission to supraoptic neuroendocrine cells. Brain Research, 442, 152-156.

Gribkoff, V. K., & Dudek, E. F. (1990). Effects of excitatory amino acid antagonists on synaptic response of supraoptic neurons in slices of rat hypothalamus. Journal of Neurophysiology, 63(1), 60-71.

Guth, P., & Melamed, B. (1982). Neurotransmission in the auditory system: A primer for pharmacologists. Pharmacology and Toxicology, 22, 383-412.

Gymer, A., & Edward, J. S. (1967). The development of the insect nervous system. 1. An analysis of postembryonic growth in the termimal ganglion of Acheta domesticus. Journal of Morphology, 123, 191-198.

Honore, T., Davies, S., Drejer, J., Fletcher, E., Jacobsen, P., Lodge, D., & Nielsen, F. (1988). Quinoxalinediones: Potent competitive non-NMDA glutamate receptor anatagonists. Science, 241, 701-703 (1988).

Huber, F., Moore, T. E., & Loher, W. (Eds.). (1989). Cricket Behavior and Neurobiology. New York: Cornell university press.

Hughes, J., & Fino, J. (1980). Usefulness of piezoelectric earphones in recording the brain stem auditory evoked potentials: A new early deflection. Electroencephalography and Clinical Neurophysiology, 48, 357-360.

Hughes, J., Fino, J., & Gagnon, L. (1981). The importance of phase of stimulus and the reference recording electrode in brain stem auditory evoked potentials. Electroencephalography and Clinical Neurophysiology, 50, 611-623.

Hughes, J., & Fino, J. (1985). A review of generators of the brainstem auditory evoked potential: Contribution of an experimental study. Clinical Neurophysiology, 2(4), 355-381.

Hudspeth, A. J., & Corey, D. P. (1977). Sensitivity, polarity and conductance change in the response of vertebrate hair cells to controlled mechanical stimuli. Proceedings of the National Acadamy of Science, USA, 74, 2407 - 2411.

Insecta. In: The New Encyclopedia Britannica, 9, (1984). 608-621. Chicago: Encyclopedia Britannica Inc.

Jenison, G. L., Bobbin, R. P., & Thalmann, R. (1985). Potassium-induced release of endogenous amino acids in the guinea pig cochlea. Journal of Neurochemistry, 44, 1845-1853.

Jenison, G. L., Winbery, S., & Bobbin, R. P. (1986). Comparative actions of quisqualate and N-methyl-D-asparate, excitatory amino acid agonists, on guinea pig cochlear potentials. Comparative Biochemistry Physiology, 84, 385-389.

Jerger, J. (1978). Prediction of sensorineural hearing level from the Brain stem evoked response. Archives of Otolaryngology, 104, 456-461.

Kandel, E., & Siegelbaum, S. (1985). Principles Underlying Electrical and Chemical Synaptic Transmission. In: E. Kandel, and J. Schwartz, (Eds.), Principles of Neural Sciences, New York: Elsevier Inc.

Kao, C. Y. (1972). Pharmacology of tetrodotoxin and saxitoxin. Federation Proceedings, 31(3), 1117-1123.

Katsuki, Y., & Suga, N. (1960). Neural mechanisms of hearing in insects. Journal of Experimental Biology, 37, 279-290.

Keidel, W. D., Kallert, S., & Korth. (1983). The Physiological Basis of Hearing. New York: Threme-Stratton Inc.

Koester, J. (1985). Resting membrane potential and action potential; Nongated channels and the passive membrane properties of the neuron; Functional consequences of passive membrane properties of the neuron; Voltage-Gated channels and the generation of the action potential. In: E. Kandel., and J. Schwartz (Eds.). Principles of Neural Sciences, Elsevier, Inc., New York.

Kiang, N. Y. S., Rho, J. M., Northrop, C. C., Liberman, M. C., & Ryugo, D. K. (1982). Hair-cell innervation by spiral ganglion cells in adult cats. Science, 217, 175-177.

Klinke, R. (1986). Neurotransmission in the inner ear., Hearing Research, 22, 235-243.

Klinke, R., Caird, D., Lowenheim, H., & Moore, E. J. (1988). Beeinfluat e in intracochleares exz:tatorisches post synaptisches potential die Hirnstammpotentiale?

Archives of Oto-Rhino-Laryngology, Supp 2, 159-160.

Levine, R. B., & Murphey, R. K. (1980). Pre- and postsynaptic inhibition of identified giant interneurons in the cricket (Acheta domesticus). Journal of Comparative Physiology, 135, 269-282.

Liberman, M. C. (1980). Morphological differences among radial afferent fibers in the cat cochlea and electron-microscopic study of serial sections. Hearing Research, 3, 45-63.

Littman, T., Puel, J.L., Fallon, M., & Bobbin, R. P. (1989). The quinoxalinediones DNQX, CNQX, and two related congeners suppress hair cell-to-auditory nerve transmission. Association for Research in Otolaryngology Abstracts, XII, 149.

Mendenhall, B., & Murphey, R. K. (1974). The morphology of cricket giant interneurones. Journal of Neurobiology, 5(6), 565-580.

Meyer, M. R., & Edwards, J. S. (1982). Metabolic changes in deafferented central neurons of an insect, Acheta domesticus. Journal of Neuroscience, 2, 1651-1659.

Meyer, M. R., & Reddy, R. (1985). Muscarinic and nicotinic cholinergic binding sites in the terminal abdominal

ganglion of the cricket (Acheta domesticus). Journal of Neurochemistry, 45, 1101-1112.

Meyer, M. R., Reddy, R. G., & Edwards, J. S. (1986). Metabolic changes in deafferented central neurons of an insect, Acheta domesticus. II. Effects on cholinergic binding sites and acetylcholinestrase. The Journal of Neurochemistry, 6(6), 1676-1684.

Moller, K., & Blegvad, B. (1976). Brain stem responses in patients with sensorineural hearing loss. Scandinavian Audiology, 5, 115-127.

Moller, K., & Jannetta, P. (1985). Neural generators of the auditory brainstem response. In T. Jacobsen (Ed.), The Auditory Brainstem Response, San Deigo: College-Hill press.

Moore, E. J. (1971). Human Cochlear Microphonics and Auditory Nerve Action Potentials From Surface Electrodes. Unpublished Ph. D. dissertation, University of Wisconsin, Madison, Wisconsin.

Moore, E. J. (Ed.). (1983). Bases of Auditory Brain-Stem Responses, New York: Grune and Stratton Inc.

Moore, E. J., & Semela, J. (1985). The "BI" potential of the auditory brain stem response: A neural response or systematic artifact?. Inner Ear Biology Workshop, XXXII, Wurzburg, FRG.

Moore, E. J., Caird, D., Klinke, R., & Lowenheim, H. (1988a). Effects of intracochlear tetrodotoxin and transmitter blockers on auditory evoked potentials in the cat. Association for Research in Otolaryngology Abstracts, XI, Clearwater Beach, Florida.

Moore, E. J., Caird, M., Klinke, R., & Lowenheim, H. (1988b). Effects of Neural Antagonists on Auditory Nerve Activity. Inner Ear Biology Workshop, London, 25, 35.

Moore, E. J., Caird, D., Klinke, R., & Lowenheim, H. (1989). Effects of intracochlear infusion of TTX and GDEE on brain stem auditory evoked potentials. Association for Research in Otolaryngology Abstracts, XII, 12, St. Petersburg Beach, Florida.

Moore, E. J., Semela, J. J. M., Rakerd, B., Robb, R., & Ananthanarayan, A. K. (1991). The I' potential of the brain stem auditory evoked potential. Accepted, Scandinavian Audiology.

Moran, D. T., & Varela, F. G. (1971). Microtubules and sensory transduction. Proceedings of National Academy of science, U.S.A., 68(4), 757-760.

Nadol, J. B., Jr. (1983). Serial section reconstruction of the neural poles of hair cells in the human organ of corti, Inner hair cells. Laryngoscope, 93, 599-614.

Narahashi, T. (1972). Mechanism of action of tetrodotoxin and saxitoxin on excitable membranes. Federation Proceedings, 31(3), 1124-1132.

Oldfield, B. P. (1988). Tonotopic organization of the insect auditory pathway. Trends in Neuroscience, 11(6), 267-270.

Petrovskaya, Y. D., Rozhkova, G. I., & Tokareva, V. S. (1970). Characteristics of single receptors of the cercal auditory system of the house cricket. Biofizika, 15(6), 1112-1119.

Pichon, Y., & Callec, J. J. (1970). Further studies on synaptic transmission in insects. I. External recording of synaptic potentials in a single giant axon of the cockroach, Periplaneta americana L. Journal of Experimental Biology, 52, 257-265.

Pickles, J. O. (1982). An Introduction to the Physiology of Hearing, London: Academic press.

Puel, J. L., Bobbin, R. P., & Fallon, M. (1988). 2-Amino-4-phosphobutyric acid receptors are not involved in synaptic transmission from hair cells to auditory neurons. Hearing Research, 37, 83-87.

Pumphrey, R. J., & Rawdon-smith, A. F. (1936). Hearing in insects: The nature of the response of certain receptors to

auditory stimuli. Proceedings of Royal Society, B, London, 121, 18-29.

Pujol, R., & Lenoir, M. (1986). The four types of synapses in the organ of corti. In R. Altschuler, R. P. Bobbin, & D. W. Hoffman (Eds.), Neurobiology of Hearing: The Cochlea. New York: Raven Press.

Randell (1986). Classification of crickets. In V. R. Vickery, & D. K. Mc Kevan. The Insects and Arachnids of Canada Part 14. The Grasshoppers, Crickets and Related Insects of Canada and Adjacent regions. Canadian goverment publication centre, Ottawa, Canada.

Rozhkova, G. I., & Polishchuk, N. A. (1976). Study of the mechanism of constant representation of the position of the source of the sound signal by the neurones of the cercal system of the cricket. Biofizika, 21(4), 725-729.

Rozhkova, G. I. (1980). Comparison of the constancy mechanisms in the cercal systems of crickets (Acheta domesticus and Gryllus bimaculatus). Journal of Comparative Physiology, A, 137, 287-296.

Rozhkova, G. I., Rodionova, H. I., & Popov, A. V. (1984). Two types of information processing in cercal systems of insects: Directional sensitivity of giant interneurons. Journal of Comparative Physiology, A, 154, 805-815.

Schwartz, I., & Siegel, G. (1985). Excitation, conduction and transmission of the nerve impulse. In West, J. (Ed.), Best and Taylor's Physiological basis of medical practise. Baltimore: William and Wilkins.

Seigel, J. H., & Dallos, P. (1986). Spike activity recorded from the organ of Corti. Hearing Research, 22, 245-248.

Sewell, W. F. (1990). Synaptic potentials in afferent fibers innervating hair cells of the lateral line organ in Xenopus laevis. Hearing Research, 44, 71-82.

Sheardown, M. J., Nielson, E. O., Hansen, A. J., Jacobsen, P., & Honore, T. (1990). 2, 3-Dihydroxy-6-nitro-7-sulfamoyl-benzo (F) quinoxaline: A neuroprotectant for cerebral ischemia. Science, 247, 571-574.

Spoendlin, H., (1972). Innervation densities of the cochlea. Acta Otolaryngologica, 73, 235-248.

Sproull, N. L. (1988). Handbook of Research Methods. London: The Scarecrow Press.

Suga, N., & Katsuki, Y. (1961). Central mechanism of hearing in insects. Journal of Experimental Biology, 38, 545-558.

Thurm, U., Erler, G., Godde. J., Kastrup, H., Keil.,

Volker, W., & Vohwinkel, B. (1983). Cilia specialized for mechanoreception. Submicroscopic Cytology, 15(1), 151-155.

Tobias, M., & Murphey, R. K. (1979). The response of cercal receptors and identified interneurons in the cricket (Acheta domesticus) to airstreams. Journal of Comparative Physiology, 129, 51-59.

Watkins, J., & Evans, R. (1981). Excitatory amino acid transmitters. Pharmacology and Toxicology, 21, 165-204.

Watkins, J., & Oliverman, H. (1987). Agonists and antagonists for excitatory amino acid receptors. Trends in Neuroscience, 10, 265-272.

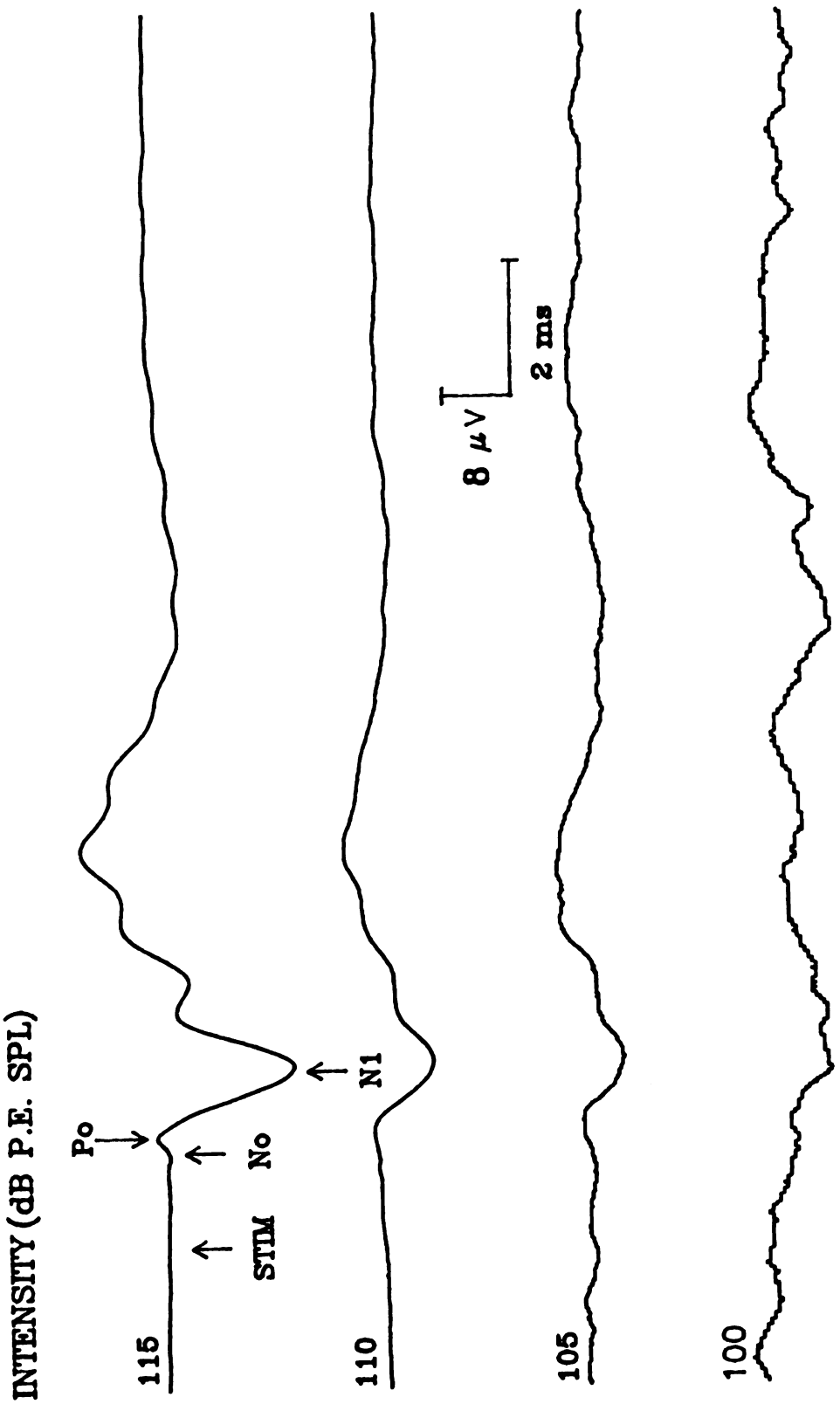
Wenthold, R. (1985). Glutamate and Aspartate as Neurotransmitters of the Auditory Nerve. In D. Dresher (Ed.). Auditory Biochemistry. Illinois: Charles C. Thomas, Pub.

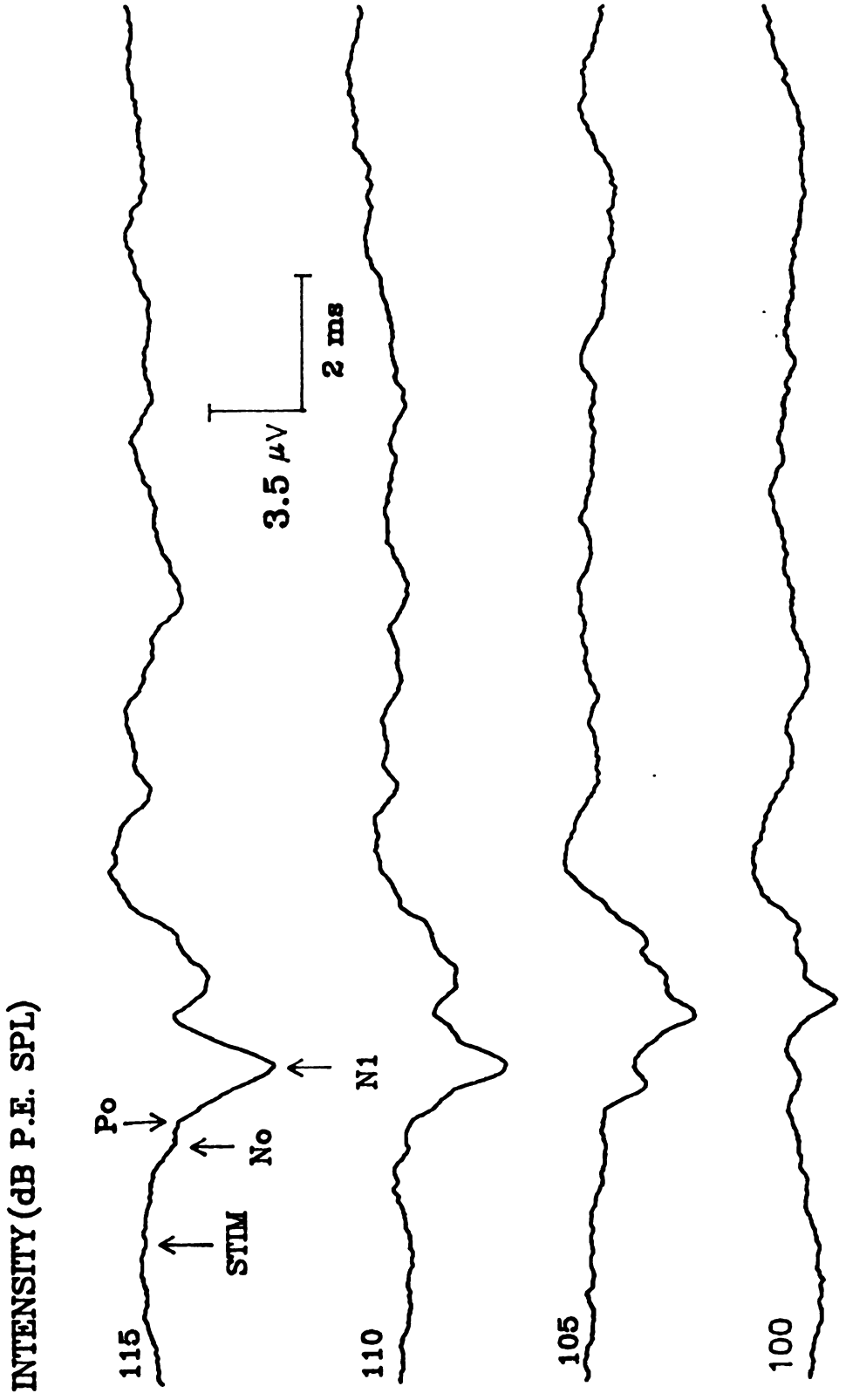
Werman, R. (1966). Criteria for identification of a central nervous system transmitter. A review. Comparative Biochemistry and Physiology, 18, 745 - 766.

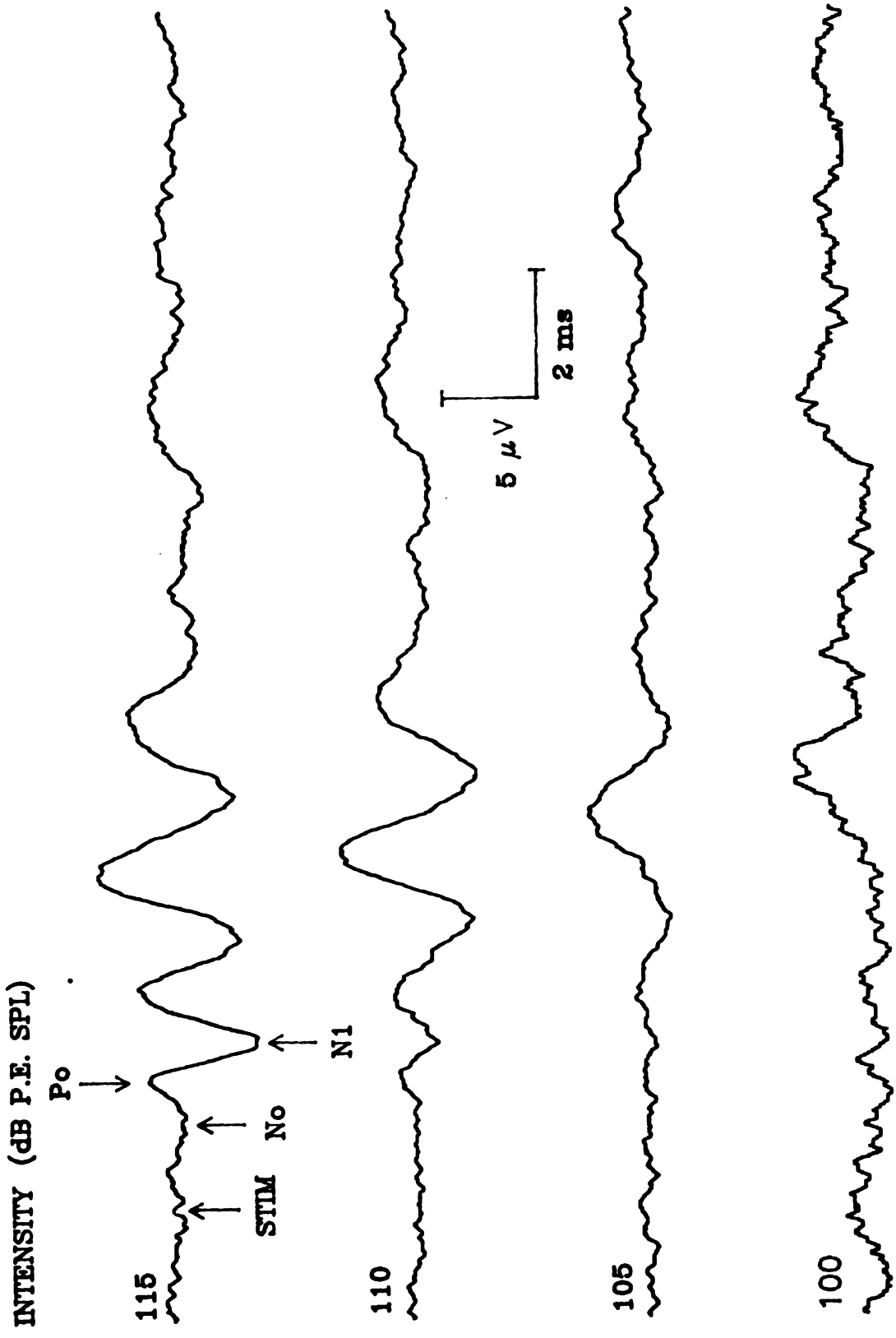
Xi, L., Dolan, D. F., & Nuttall, A. L. (1989). Characterization of the residual neural potential after application of tetrodotoxin to the round window membrane. Association for Research in Otolaryngology Abstracts, XII, St. Petersberg Beach, Florida.

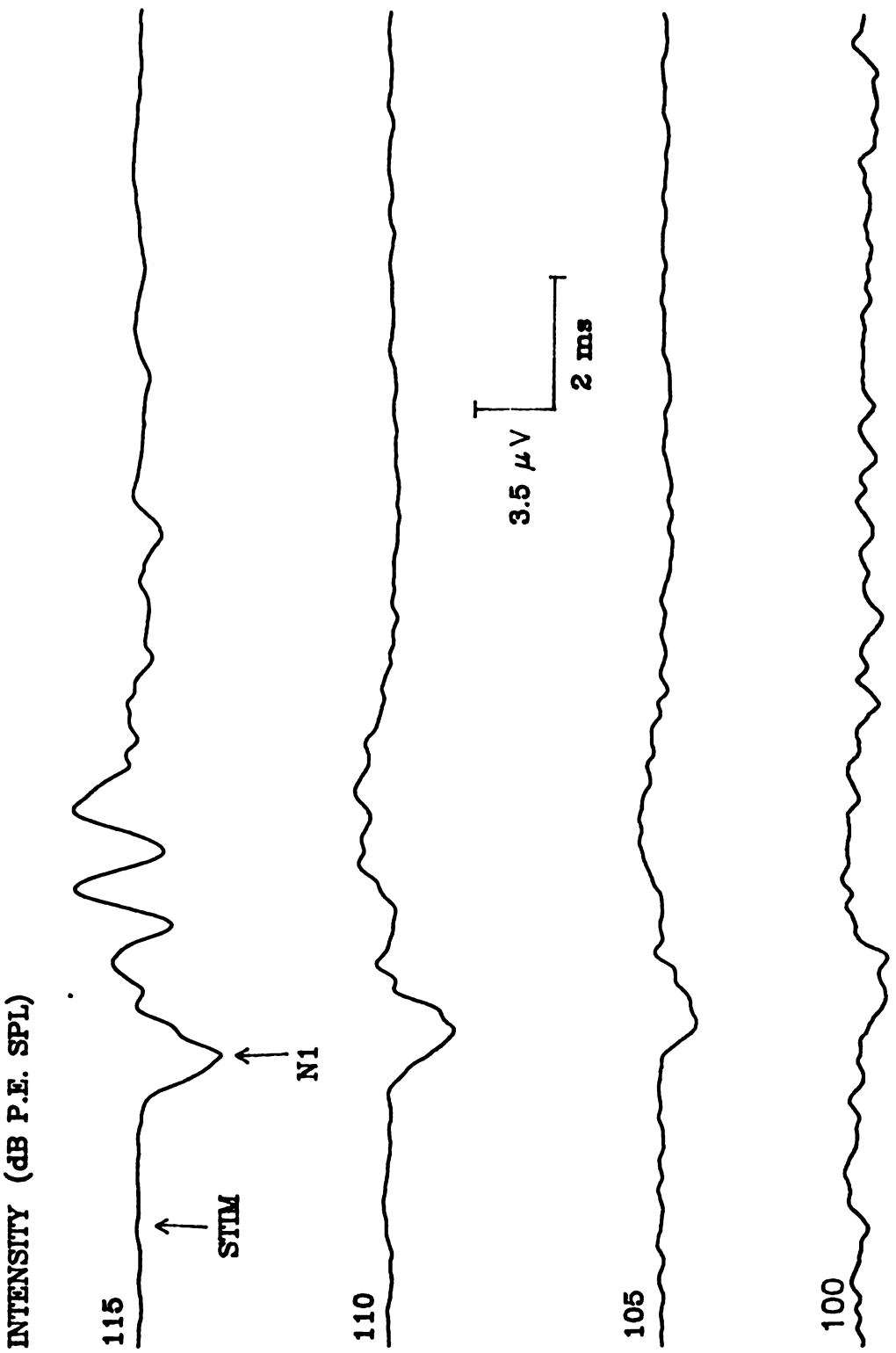
APPENDICES

Appendix A: **Analog waveforms of CAP recordings from four crickets (Cric-071, Cric-072, Cric-076 and Cric-092) depicting the effect of decreasing intensity levels.**

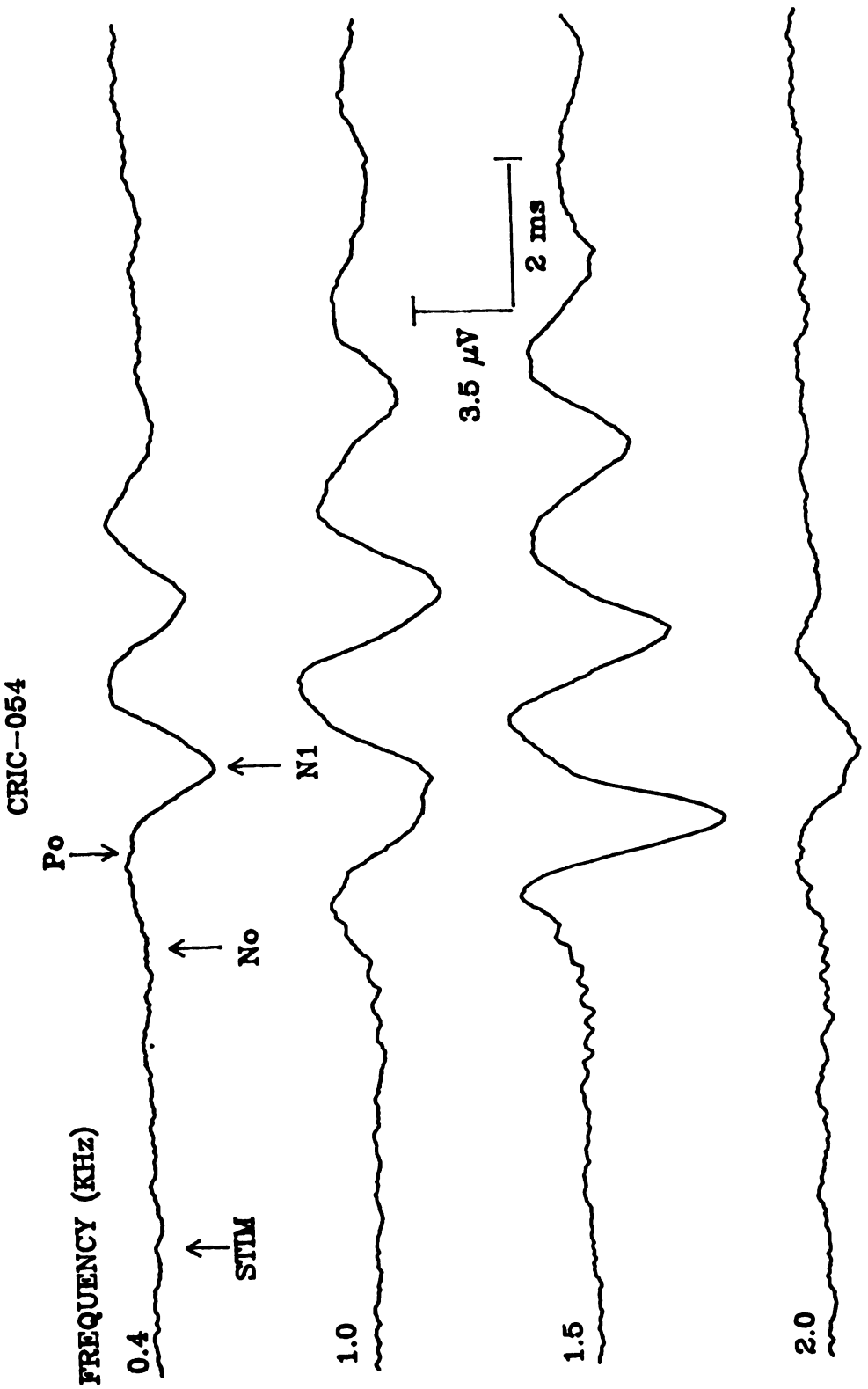

CRIC-071
INTENSITY (dB P.E. SPL)
115
110
105
100
Po
No
N1
STIM
8 μV
2 ms

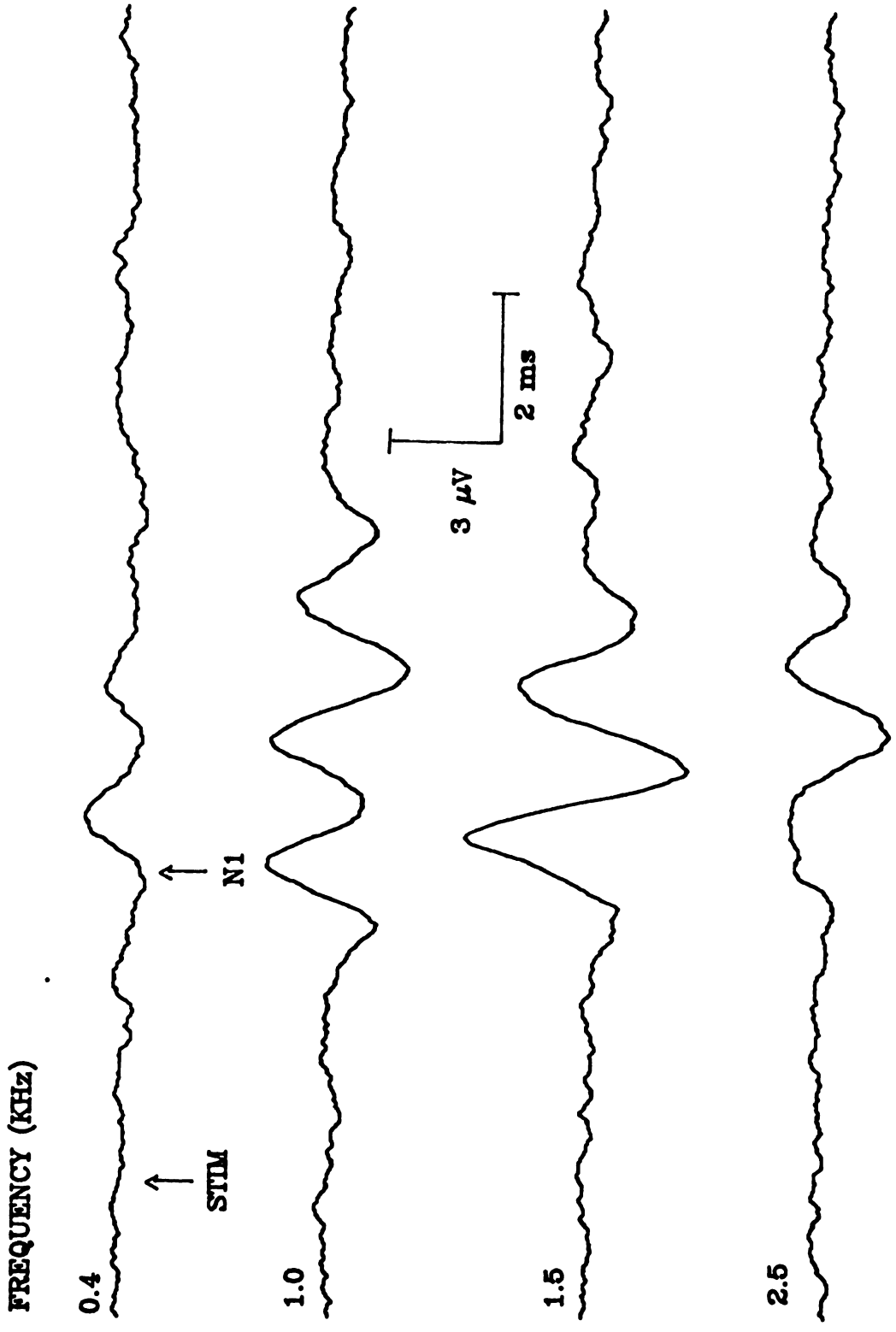

CRIC-072
INTENSITY (dB P.E. SPL)
115
110
105
100
STIM
No
Po
N1
3.5 μV
2 ms


CRIC-076
INTENSITY (dB P.E. SPL)
Po
No
N1
STIM
115
110
105
100
5 μV
2 ms

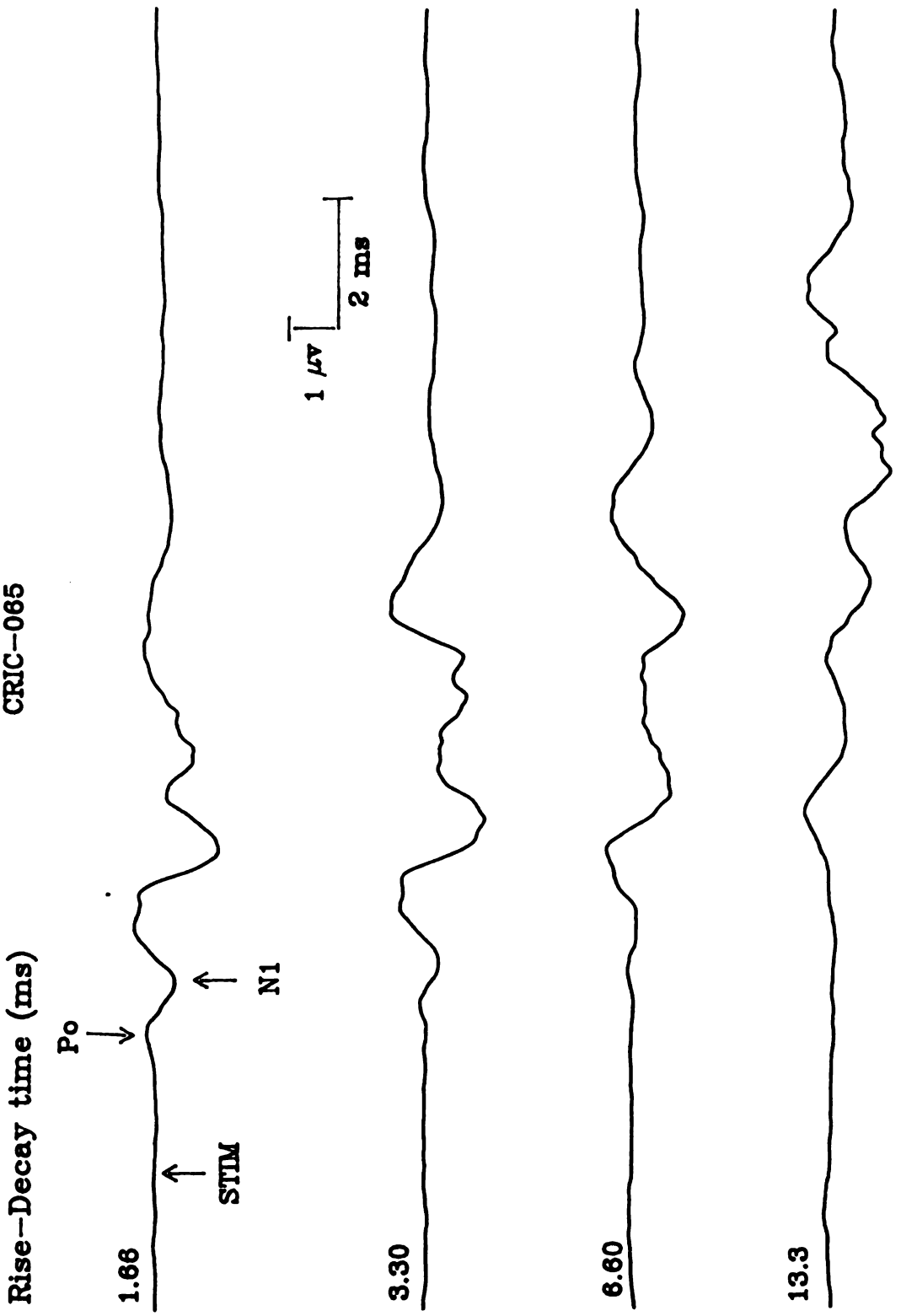

CRIC-092
INTENSITY (dB P.E. SPL)
115
STIM
N1
110
3.5 μV
2 ms
105
100

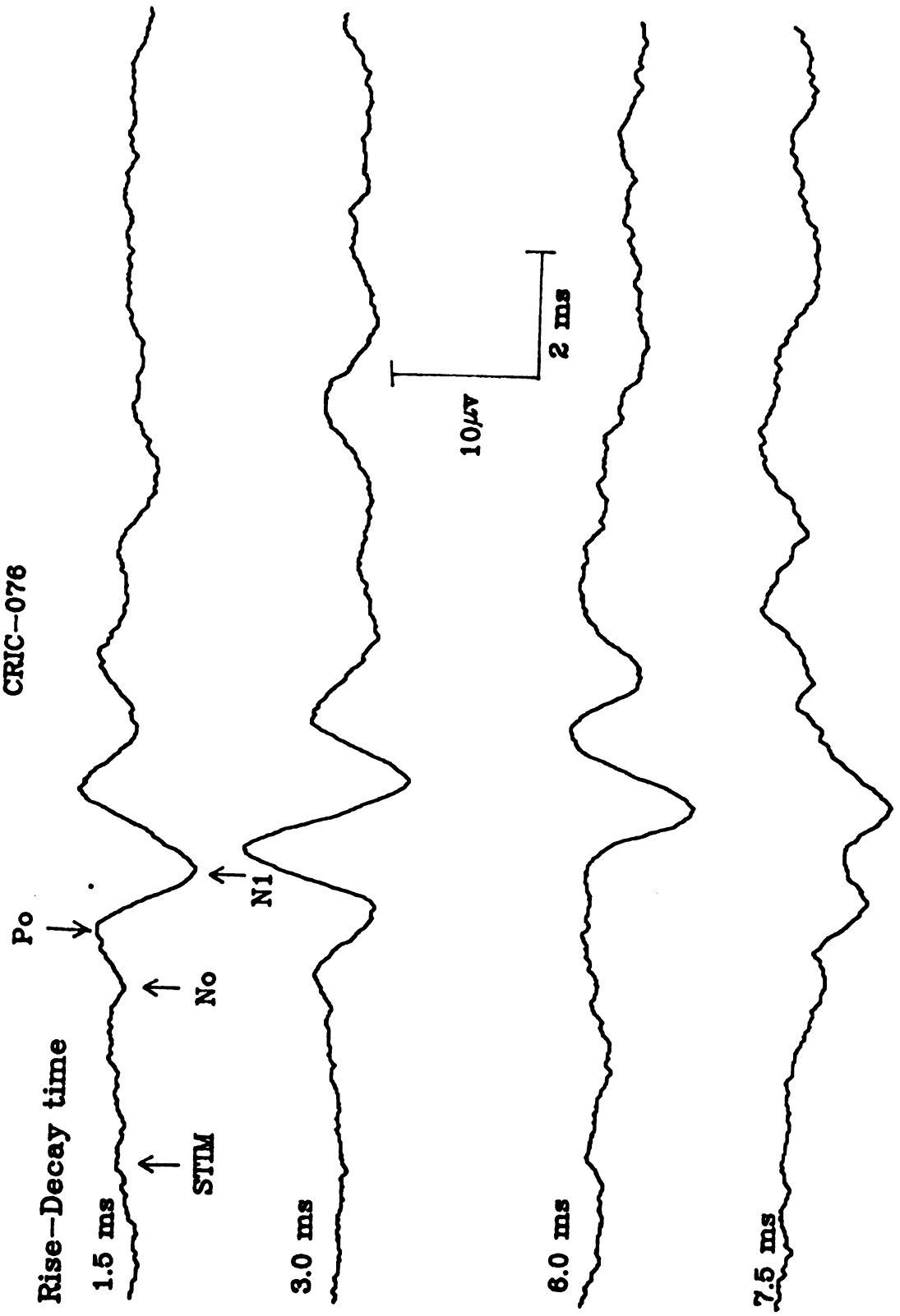
Appendix B: Analog waveforms of CAP recordings from two crickets (Cric-054 and Cric-075) depicting the effect of varying stimulus frequency.


CRIC-054
FREQUENCY (KHz)
0.4
1.0
1.5
2.0
STIM
Po
No
N1
3.5 μV
2 ms

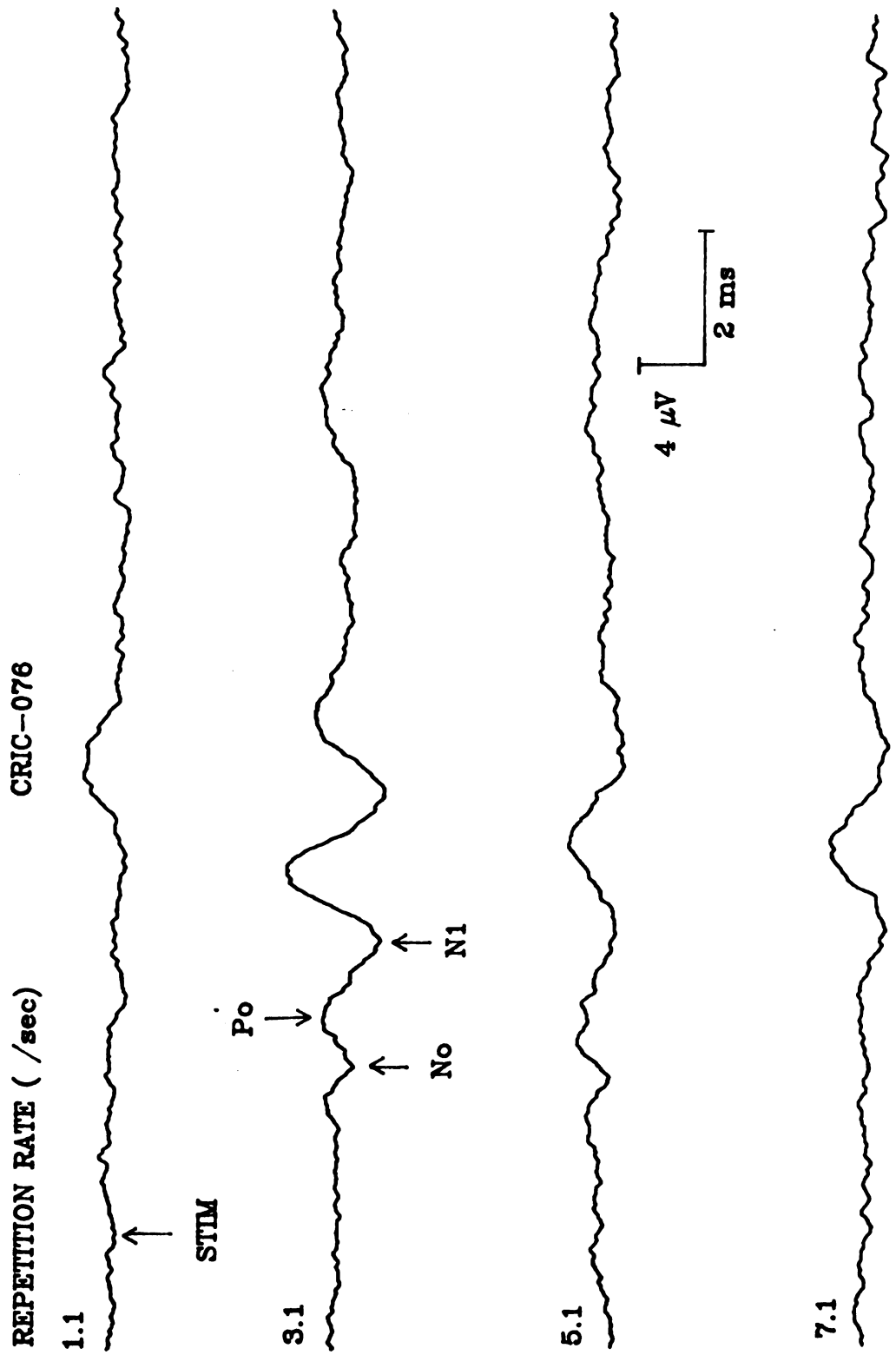

CRIC-075
FREQUENCY (KHz)
0.4
1.0
1.5
2.5
STIM
N1
3 μV
2 ms

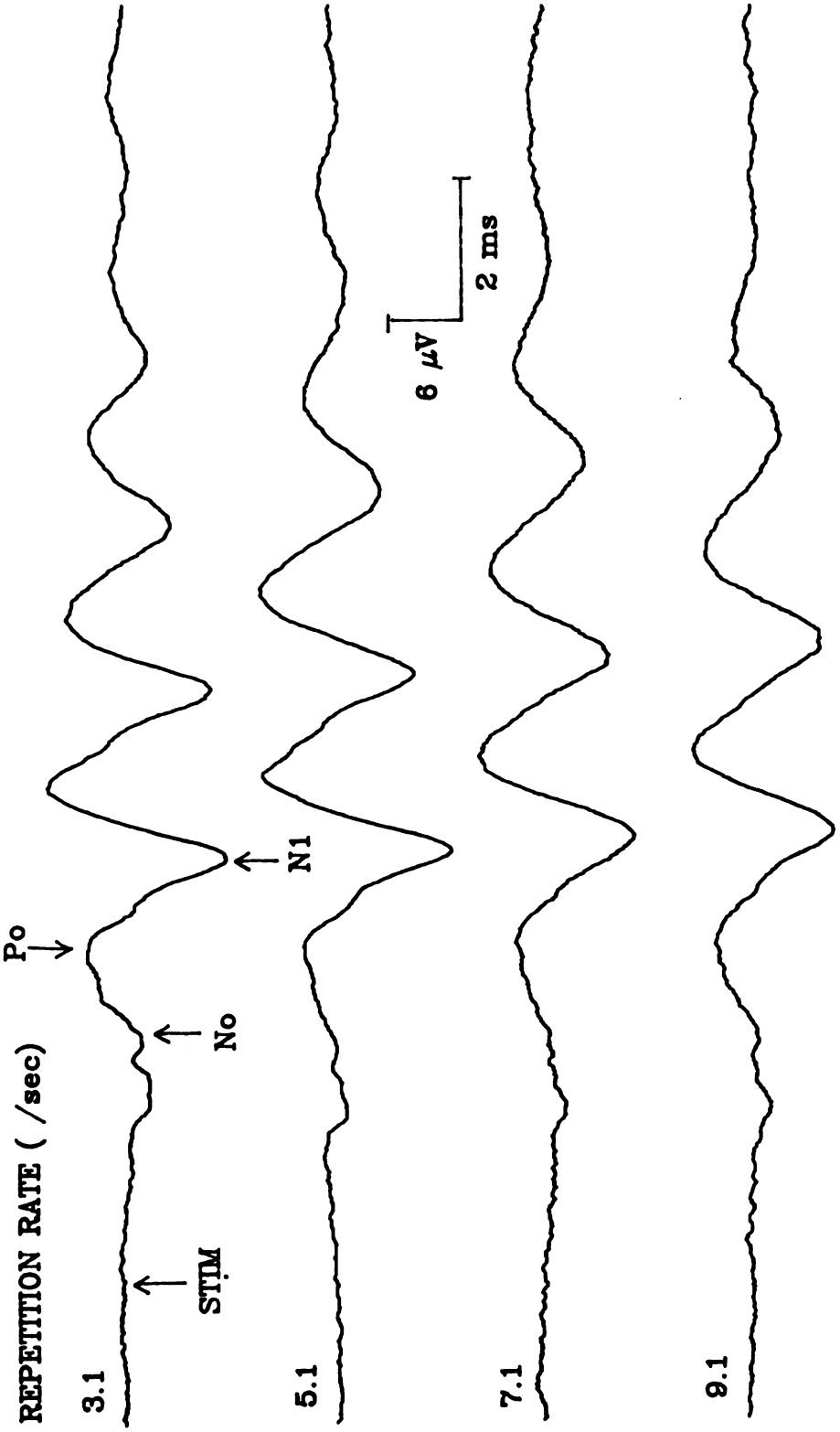
Appendix C: **Analog waveforms of CAP recordings from two crickets (Cric-065 and Cric-076) depicting the effect of varying rise-decay times of the stimulus.**


CRIC-065
Rise-Decay time (ms)
1.66
3.30
6.60
13.3
Po
N1
STIM
1 μv
2 ms

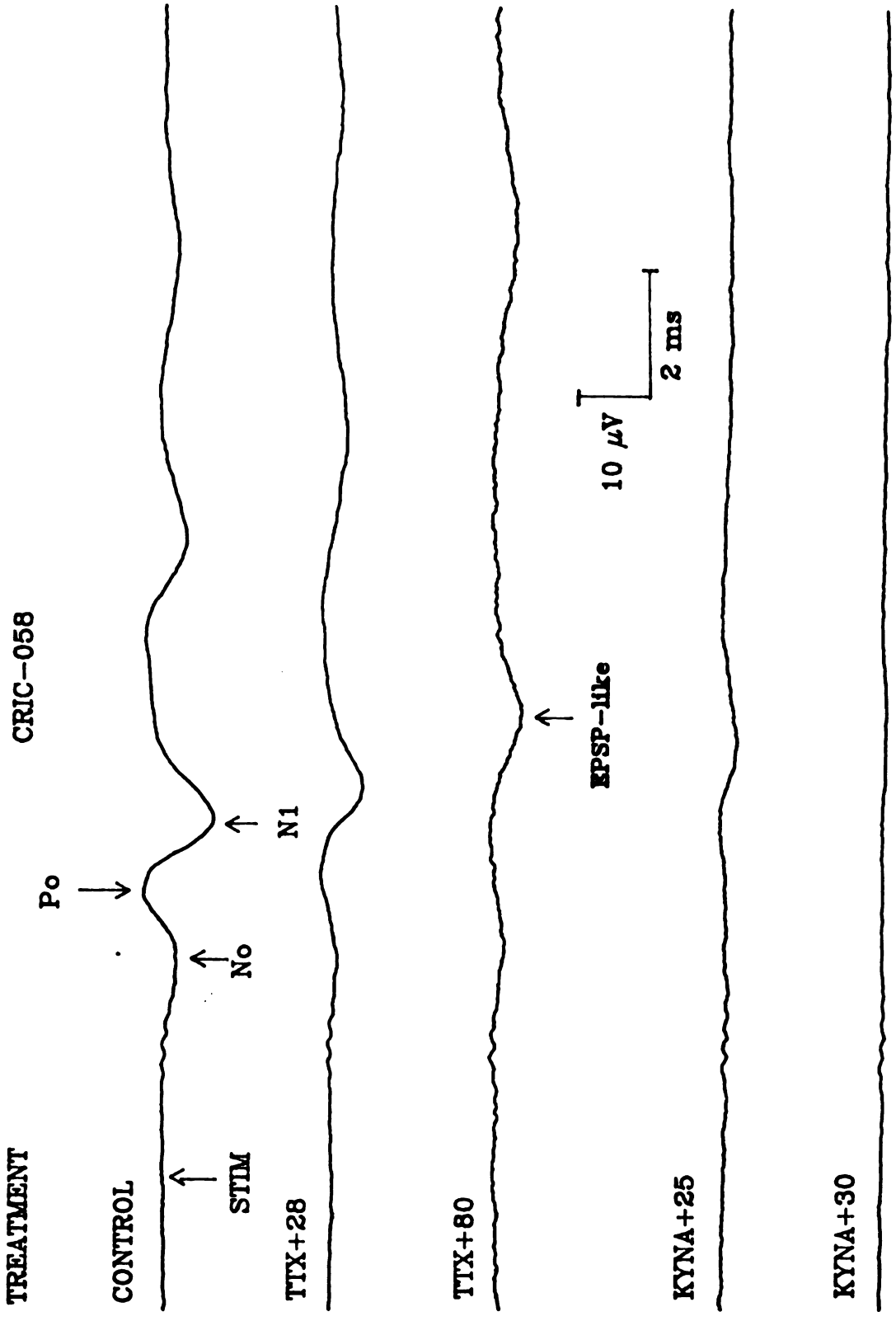

CRIC-076
Rise-Decay time
1.5 ms
3.0 ms
6.0 ms
7.5 ms
STIM
No
Po
N1
10μV
2 ms

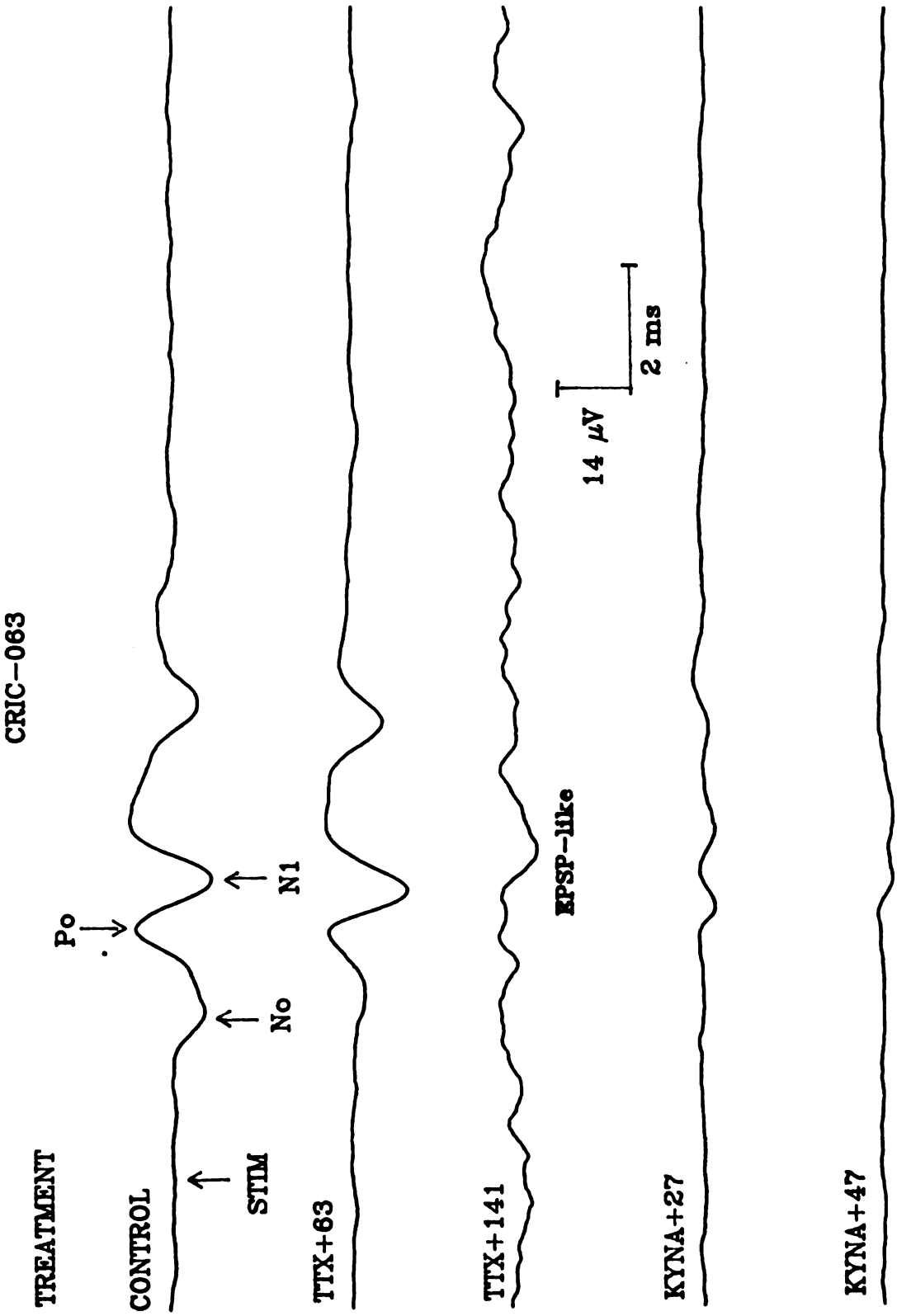
Appendix D: **Analog waveforms of CAP recordings from two crickets (Cric-076 and Cric-107) depicting the effect of varying repetition rate of the stimulus.**


REPETITION RATE (/sec)
CRIC-076
1.1
STIM
3.1
Po
No
N1
5.1
4 μV
2 ms
7.1

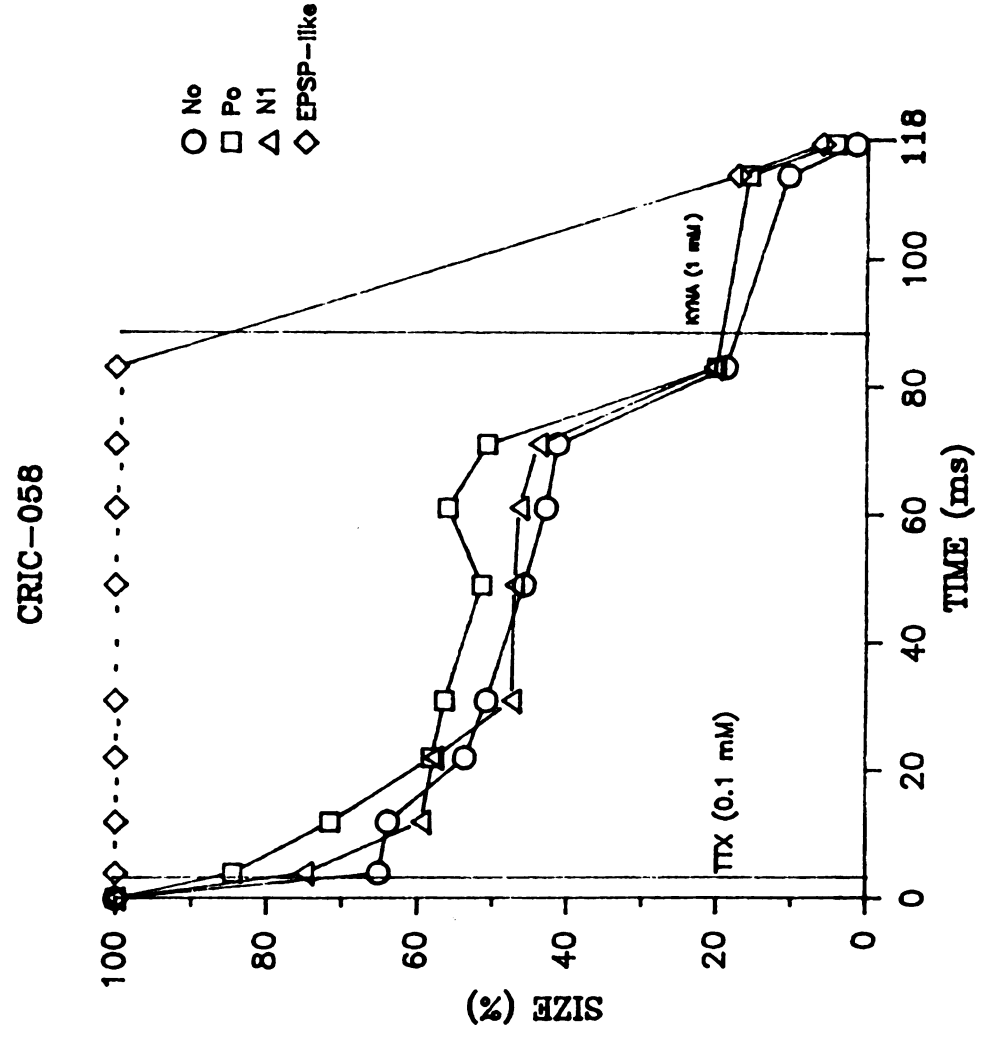

CRIC-107
REPETITION RATE (/sec)
3.1
5.1
7.1
9.1
STIM
No
Po
N1
6 μV
2 ms

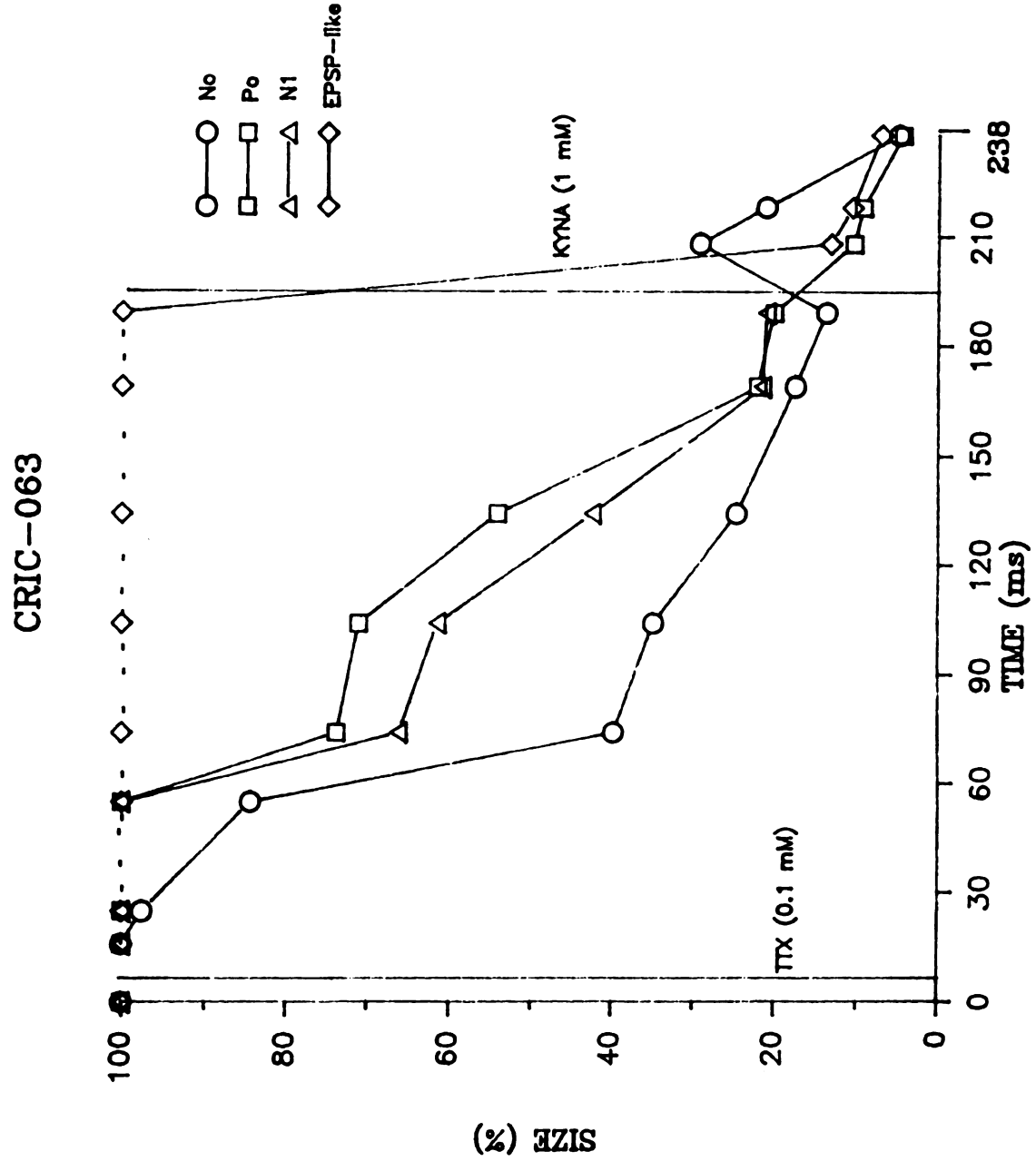
Appendix E: **Analog waveforms of CAP recordings from two crickets (Cric-058 and Cric-063) depicting the effect of pharmacologic agents (TTX and KYNA.**


CRIC-058
TREATMENT
CONTROL
STIM
Po
No
N1
TTX+28
TTX+80
EPSP-like
10 μV
2 ms
KYNA+25
KYNA+30


CRIC-063
TREATMENT
CONTROL
STIM
Po
No
N1
TTX+63
TTX+141
EPSP-like
14 μV
2 ms
KYNA+27
KYNA+47

Appendix F: Input-Output functions of No, Po, N1 and EPSP-like potentials as a function of TTX and KYNA in two crickets (Cric-058 and Cric-063).


CRIC-058
No
Po
N1
EPSP-like
KYNA (1 mM)
TTX (0.1 mM)
SIZE (%)
100
80
60
40
20
0
0
20
40
60
80
100
118
TIME (ms)


CRIC-063
No
Po
N1
EPSP-like
KYNA (1 mM)
TTX (0.1 mM)
SIZE (%)
100
80
60
40
20
0
0
30
60
90
120
150
180
210
238
TIME (ms)