Electrophysiologic Detection of the Neurotoxic Effects of Acrylamide and 2,5-Hexanedione in Rats

by

Todd L. Towell, DVM

Thesis submitted to the Faculty of the Virginia Polytechnic Institute and State University in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

in

Veterinary Medical Sciences

APPROVED:

Linda G. Shell
(Chair)

Michael S. Leib

Marion F. Ehrich

Karen R. Dyer

Bernard S. Jortner

April 18, 1994
Blacksburg, Virginia

2

Electrophysiologic Detection of the Neurotoxic Effects of Acrylamide and 2,5-Hexanedione in Rats

by

Todd L. Towell, DVM
Committee Chair: Linda Shell
Veterinary Medical Sciences

(ABSTRACT)

Brain stem auditory evoked potentials (BAEP) and somatosensory evoked potentials (SEP), recorded from subcutaneously placed electrodes in anesthetized rats, were used to detect the neurotoxic effects of acrylamide and 2,5-hexanedione. Sixty adult male rats were equally divided into four groups: acrylamide (20 mg/kg/day), 2,5-hexanedione (350 mg/kg/day), food restricted and control. Brain stem auditory evoked potentials and somatosensory evoked potentials were recorded on weeks 0,1,2 and 3 of treatment.

SEP waveforms were considerably more variable than BAEP results. Mean latencies in the control, food restricted and acrylamide groups were similar for the entire three weeks. A nonsignificant increase was seen in the mean latencies in the 2,5-hexanedione group. Brain stem auditory evoked potential latencies recorded on the pretreatment week were compared to each successive week within a treatment group. The control group had small but statistically significant prolongations in the latencies of wave II on weeks 2 and 3, and latencies III and IV on week 3. Results from the food restricted group were not statistically different at any time. The acrylamide group had prolongations in latency II and IV by week 3 of treatment. Latencies of all waveforms from the 2,5-hexanedione group were significantly longer than pretreatment values on weeks 2 and 3. Mean latencies of the two consistently identified somatosensory evoked potential waves (P1, N1) recorded from the contralateral cortex on the pretreatment week were comparable to values reported in the literature. Brain stem auditory evoked potentials recorded from subcutaneously placed electrodes in anesthetized rats can detect the neurotoxic effects of acrylamide and 2,5-hexanedione. Lack of significant differences in the food restricted group indicates the observed effects in the groups receiving neurotoxicant were not related to weight loss. Differences in the control group were of small magnitude and variance and therefore may be statistically but not biologically significant.

ACKNOWLEDGMENTS

To my husband, Tim: I am eternally grateful for his unfaltering love, understanding and sense of humor which has enabled me to complete this project and many others.

To my brother, Russell: I am thankful for his special insights and unique ability to help me maintain a realistic perspective when academic pressures threatened to overwhelm me.

To my parents of both families; Jim and Gretchen Howerton and Beth and Arnold Towell for their support during these years.

To my graduate committee: for providing guidance, encouragement and support throughout this project and my residency training.

To the faculty and staff of the College of Veterinary Medicine: for providing an intellectually challenging atmosphere which has allowed me to grow both individually and academically.

To the patients and clients I have had the pleasure of meeting: I am reminded each day how precious the bonds of love and trust are and how fleeting the time we have to share them.

Table of Contents

Figures and Tables

Purpose

The purpose of this study was threefold. First to develop a protocol for recording evoked potentials (brain stem auditory evoked potentials and somatosensory evoked potentials) in anesthetized rats from subcutaneously placed electrodes. Secondly, the repeatability of this method of recording was assessed. Third, the ability of these tests to detect deleterious effects of two prototype neurotoxicants (acrylamide and 2,5-hexanedione) on the central nervous system was evaluated.

LITERATURE REVIEW

Introduction

Neurotoxicological investigations must allow for screening of potential neurotoxicants, characterization of the affected systems, elucidation of the mechanisms of neurotoxicity and evaluation of potential effects of exposure in humans.[1] No single test will fulfill all these criteria. Currently most neurotoxicological investigations rely on a combination of histochemical, pathological, behavioral and electrophysiological testing to adequately characterize neurotoxicants.[2]

Routine histopathologic examination of tissues has been and remains a mainstay of toxicologic investigations. Pathological examination of tissues from animals exposed to neurotoxicants contributes information regarding the anatomic location of lesions and the spatial-temporal evolution of changes.[3] Limitations of this technique include requirements for widespread sampling and proper sample handling in order to demonstrate lesions.[4] In addition, morphologic changes do not invariably correlate with functional changes.[4] The most obvious morphologic changes may not be responsible for the observed neurotoxic effects.

Histochemical and immunohistochemical staining of biological specimens can provide information on the location, distribution and quantity of chemical substances in a tissue specimen. Substances such as endogenous and exogenous ions and molecular fragments can be demonstrated by these techniques. In toxicological studies this technique has been used to assess mechanisms of injury at the molecular level and to correlate the presence or absence of the deleterious effects of a toxicant to the location of a lesion.[5]

Behavioral indices of neurotoxicity have become an important means of screening for neurotoxicity.[6] In some cases behavioral tests may detect central nervous system toxicity at lower doses or shorter exposure intervals than pathological, electrophysiological or neurochemical methods.[4] Behavioral tests may be the only means of evaluating toxicities that manifest as subtle functional disturbances.[2] The need for a standardized and validated battery of behavioral tests has been identified and addressed by many investigators.[2,4,6-9] The objective of this standardization and validation is to create a battery of behavioral tests which can be used to screen large numbers of potential toxicants.

However, one criticism of behavioral tests is the inability to separate the affects of a toxicant on non-neural tissues from nervous system toxicity. Most neurotoxicants have some effect on body systems other than the nervous system or will alter food consumption through either neurologic or non-neurologic disease. Several investigators have postulated that both food restriction and non-neurotoxic agents can cause changes in behavioral indices and electrophysiological parameters which mimic the effects produced by neurotoxicants.[10,11] These effects are a major confounding factor in the interpretation

of both behavioral and electrophysiological testing modalities.

Since results obtained from electrophysiologic testing are quantitative, these tests have the advantage of being more objective than behavioral tests. As a result they present fewer interpretive problems during the screening period than behavioral tests. The characteristics of electrophysiologic tests which make them useful in neurotoxicology are fourfold: 1) the ability to directly reflect the function of neuronal tissue sampled by the electrodes, 2) the consistency of most tests not only within and between individuals but also across species, 3) the ability to objectively quantify data and 4) the opportunity to extensively evaluate the nervous system in a relatively non-invasive manner.[12] For these reasons electrophysiological testing has gained favor as a screening tool for potential neurotoxicants.[13] However, there is some subjectivity involved in wave-form recognition and parameter measurements.

Evoked Potential Monitoring

Evoked potentials are a group of electrophysiologic tests which have proven useful in evaluating neurotoxicants.[1] The sensory systems are those parts of the nervous system which relay information from the external environment. Once this information has been processed, the motor systems convey output to appropriate organ systems. Both sensory and motor systems can be evaluated in neurotoxicological testing. Motor systems are best evaluated with a combination of behavioral testing and motor nerve conduction or action potential studies. The sensory system can be evaluated by using specific electrophysiologic tests (evoked potentials).

Electrophysiologic testing of sensory systems is performed by evaluating responses to a stimulus which can be strictly controlled. The use of precisely controlled input signals reduces variations in the results from normal subjects. This congruity in baseline data will allow for detection of subtle deviations from normal. An additional benefit is that identical testing procedures can be performed in a variety of species. This does not inevitably allow for extrapolation of results between species, but does permit more convincing comparisons to be made.[14] Sensory systems are particularly susceptible to neurotoxicants. There are relatively few incidences where nonsensory systems are affected without concomitant sensory system involvement.[1] However, toxicants can have severe effects on one sensory system and only mild effects on another.[12] Therefore, in the context of a screening test it is important to evaluate a variety of sensory systems.

Evoked potentials commonly used in neurotoxicologic evaluations include visual evoked potentials, somatosensory evoked potentials, and brain stem auditory evoked potentials.[12] All of these techniques involve recording voltage alterations from a particular brain area that are temporally related to an evoking stimulus. The voltage alterations reflect postsynaptic potentials integrated over a large

population of neurons in the vicinity of the recording electrode.[15] Evoked potentials are of very low amplitude and are difficult to distinguish from spontaneous neuronal activity without signal averaging. During the averaging process the evoked potential will summate and random background activity will tend to be negated.[16] The individual contributions of a given neuronal cell type to the averaged potential depends on the extent of active input to that cell type and the number of functional cells in the vicinity of the recording electrode.[1] Consequently the size of the recorded response is an indirect reflection of the number and size of neuronal cells in a specific area of the nervous system responding to the stimulus and the distance between the neuronal generator and recording electrodes.

One or more electrical potentials (waves) may be produced after a repeated stimulus. Evaluation of the underlying sensory structures and their interconnections is possible if each wave is thought to be generated by a specific anatomic structure or region.[17] Detection of alterations in the evoked potentials is based on the presence or absence of appropriate waves and the latencies and amplitudes of the existing waves. In general, changes in latency are related to conduction abnormalities as would be observed with myelin damage while amplitude changes are a result of decreased voltage. Voltage loss occurs when there is a reduction in the number of firing units (cells or axons) or loss of synchronous firing. Since most toxicants produce pathologic changes in neurons, axons and myelin to varying degrees, waveforms will often have alterations in both latencies and amplitudes.[13]

Brain Stem Auditory Evoked Potentials

Introduction

Brain stem auditory evoked potentials (BAEP) have been used extensively in human medicine for diagnosis of nonspecific demyelinating diseases, surgical monitoring, audiometry, and localization of brain stem lesions.[18-21] Within the last decade, there has been substantial interest in applying this technique to the study of neurotoxicity in laboratory animals. The BAEP is one of the most easily and consistently recorded waveforms of the commonly studied rat auditory potentials.[16]

Auditory evoked potential waveforms reflect far-field volume conducted signals which are generated in the auditory nerve and the ascending brain stem auditory pathway.[22] Far-field recordings are defined as voltage changes recorded at a considerable distance from their generators. This implies that for a standard recording protocol the exact position of the recording electrodes is not a crucial determinant of the characteristics of the waveforms. However, this assertion has been challenged by experiments documenting alterations in the latency and configuration of rat BAEP waveforms with different electrode positions.[23] The size limitations dictated by the rat cranial vault may preclude recording of true far-field potentials. This would imply that the inaccuracy inherent in repeated

positioning of subcutaneously placed electrodes might result in large variations in latencies between testing days. This increased variability would decrease the ability of this testing method to detect subtle differences in latencies. This study was designed, in part, to determine the repeatability of the BAEP using subcutaneously placed electrodes in an effort to determine the sensitivity of this testing protocol.

Waveform Origins

The rat BAEP response generally consists of four to five vertex positive principal waveforms recorded within the first 10 msec after the stimulus onset. By convention the positive apex of each wave has been designated with a Roman numeral (I - V) (Figure 1). The exact origins of each BAEP waveform in rats continue to be debated. There is general agreement that the waveforms arise from sequential activation of the acoustic nerve and various nuclei and tracts within the brain stem. Wave I has been attributed to the compound action potential of the acoustic nerve.[24] Although auditory information is transmitted by both crossed and uncrossed projections within the pontine tegmentum, it is agreed that waves I and II are generated ipsilateral to the stimulated ear before any decussation occurs.[25] Original studies in rats indicated waves II, III and IV corresponded to activity in or near the cochlear nucleus, the superior olivary complex and the inferior colliculus, respectively (Figure 2).[16] However, the origin of wave IV was questioned when experimental destruction of the inferior colliculus failed to alter wave IV in rats.[26] Experimental work in the guinea pig suggests the origin of wave IV is in the vicinity of the ipsilateral superior olivary complex.[15] Several investigators have suggested all components after wave I reflect activity from multiple overlapping generators within the brain stem pathways and specific origins cannot be determined.[27,28] This hypothesis has been brought into question by additional investigations which suggested concurrent activity at sites other than the principal neural generator of a BAEP wave do not necessarily contribute to the recording.[29,30] There is agreement that the origin of wave IV is no more proximal than the lateral lemniscus and that the midbrain makes no substantial contribution to the four principle waveforms.[16]

In addition to controversy about anatomic localization of the neural generators, there is debate over the relative contributions of axonal and synaptic activity in producing waves II through IV. One theory proposes each component represents the summation of action potentials along fiber tracts connecting the brain stem nuclei.[31,32] Alternatively the potentials, after wave I, could represent synaptic or postsynaptic events in the nuclei.[19] Recent work which supports the theory of multiple brain structures (auditory cortex, hippocampal and cingulate cortices, and possibly the thalamus) as generators of the rat auditory evoked potential makes the former theory appear more likely.[33,34] It seems reasonable that both axonal and synaptic activity may be involved. Wave II likely reflects synaptic activity of the cochlear

nucleus, while other components may reflect axonal discharges.[16,32,35]

Detection of most central nervous system disorders that also affect auditory pathways relies on determining variations in the latencies of the primary waveforms. This is best achieved by comparing baseline normal data to suspected abnormal data. Unfortunately, baseline data is not always available in the clinical setting. Since the latencies of the waveforms are remarkably similar both between subjects of the same species and between species, normal values have been determined.[36] Brain stem auditory evoked potential latencies recorded from normal individuals of different species (human, monkey, cat and rat) have been compared after adjusting for relative brain stem size and weight. [37] Calculations of estimated latencies were based on a percentage of the total brain stem transmission time (BSTT = IPL I-IV). The predicted values were within 0.18 msec of the measured latencies.[37] This indicates there is considerable homology between species even though the exact identity of the neural generators may not be known or identical in every species.

Waveform Characteristics

Several studies have documented variations in the morphology of the BAEP waveforms in response to altering stimulus parameters such as intensity and repetition rate.[37-42] One potential mechanism of negating the changes in absolute latencies with variations in stimulus parameters is the calculation of interpeak latencies (IPL) or brain stem transmission time (BSTT). BSTT is defined as the mathematical difference of the latencies of wave IV and wave I. BSTT is considered to represent conduction time from the eighth cranial nerve across the subarachnoid space, through the cochlear nucleus and into the lower pons.[43] Whereas alterations in latency or amplitude of BAEP wave I would indicate a peripheral lesion, alterations in BSTT (IPL I-IV) imply the presence of central lesions.[44] This has become the most commonly interpreted BAEP parameter in clinical medicine.[43]

Original studies describing BSTT documented no alteration when stimulus intensity or frequency were varied.[16,45-48] This lack of variation was assumed to be a result of a uniform decrease in the absolute latencies of all waveforms.[49] Subsequent studies in experimental animals and humans have documented differential effects on early and late waveform latencies with variations in stimulus intensity and frequency. These are reflected by significant alterations in interpeak latencies.[38, 50-52] Increased intensity decreased the time required for sound waves to cause maximal deflection of the basilar membrane towards the base of the cochlea.[53] This preferentially shortens the latencies of early waves. Synaptic delays and axonal conduction times, which are responsible for latter waveforms, are independent of stimulus intensity.[53] Increasing stimulus rate (8 - 120/sec) increases all waveform latencies. This effect is greater in each successive wave, resulting in an alteration of the interpeak latencies.[38] This rate

dependent latency increase is likely a result of synaptic thresholds being reached more slowly.[54] These successive changes represent a cumulative central adaptation process rather than fatigue or habituation.[54] Adaptation is defined as the change in response of a neural pathway to successive stimuli. Adaptation occurs when the input signal to a sensory pathway is re-coded at various sites along the neuraxis.[54] This re-coding can occur at synaptic junctions or as a result of alterations in the firing rates or numbers of active nerve fibers.[54] Most investigators agree that central adaptation is a synaptic process similar to peripheral adaptation.[55]

Stimulus rate and intensity must be reported for each neurotoxicologic study. Comparisons of data between laboratories or experiments from the same laboratory must take these factors into consideration. Subtle differences in waveform latencies may reflect variations in stimulus parameters rather than actual effects of a toxicant. Accepted stimulus rates are between 10 and 30 presentations per second.[43] Stimulus intensity has been reported in a variety of ways: as the number of decibels of intensity compared to the threshold hearing in average normal subjects (dB NHL, dB HL or dB nHL); as sensory level (dB SL) where 0 is the point at which the individual patient can barely hear the stimulus; as peak equivalent sound pressure level (dB SPL) where 0 is approximately -32 dB HL; or as the individual machine scale.[43] Accepted levels range between 30 to 90 dB HL or SL.[43] This can be particularly confusing because not all papers report the type of decibel scale used.

Another confounding factor which complicates comparison of results between laboratories arises from discrepancies in nomenclature of analog filter settings. Originally filters were named for the frequency they allowed to pass through, i.e., low pass filters removed high frequency activity and permitted low frequency activity to pass through. Currently the same filter would be called a high filter because it removes high frequency activity.[56] The BAEP is considered a high frequency potential; therefore, it is unwise to have a high filter set at less than 3 kHz.[57,58] Studies have shown that systematic lowering of the high frequency filter will increase the latencies of the four principle BAEP waveforms.[58-60] The opposite effect occurs when the low filter (previously high pass filter) is raised: the latencies of the waveforms decrease and there can be alteration in component position.[59] Optimal settings for the low filter appear to be between 30 and 200 Hz while those for the high filter are between 3 and 8 kHz.[43] Subject variables are also a source of artifactitious changes in the latencies of the BAEP waveforms. These include body temperature, gender, strain of rodent and anesthetic agents. Body temperature is particularly important as many anesthetic agents and neurotoxicants alter this parameter.[61] Studies designed specifically to examine the capacity of hypothermia to alter BAEP waveforms revealed increased latencies with systematic lowering of body temperature.[62,63] The effect was progressive and the greatest latency shifts (2.0 msec) were seen in wave IV. Similar findings have been shown in the mouse,

cat and man.[64-67] In man, at temperatures between 34°C and 25°C, wave V increased 0.4 msec /°C and wave I increased 0.15 msec /°C.[66] Studies designed to evaluate the effects of neurotoxicants on BAEPs must strictly control or statistically adjust for changes in body temperature to prevent artifactitious discrepancies. In the rat a core body temperature of 37.5°C is normal.[68]

Both gender and strain of rodent have been shown to affect BAEP waveform latencies. Female rats tend to have shorter BAEP waveform latencies than males.[38] This parallels findings in humans which have demonstrated women to have shorter latencies than men.[42,50] The latency discrepancies in rats and humans are thought to be a result of reduced anatomical distances secondary to a gender dependent decrease in brain size.[38] Strain of rat (Long Evans, Wistar and Sprague-Dawley) has also been documented to cause subtle differences in the absolute latencies and interpeak latencies of the BAEP waveforms.[69] This further emphasizes the importance of controlling subject as well as stimulus characteristics when performing neurotoxicological studies. Variations in both subjects and stimuli may account for many of the discrepancies noted in reports from different laboratories investigating similar problems.

Perhaps the most controversial area of investigation involving the BAEP is the effect of anesthetic agents on waveform parameters. This is particularly problematic since both human and experimental BAEPs are often generated with the subject under the influence of a sedative or anesthetic agent. An alternative to the use of anesthetics in experimental settings is the use of implanted electrodes.[70] Once the recording electrodes have been surgically implanted in the skull, under general anesthesia, evoked potentials can be recorded with mild restraint. This technique requires additional expertise and expense to perform as compared to subcutaneously placed electrodes. Additionally, in human medicine, some conditions require the BAEP be recorded during anesthesia. For this reason the effects of anesthetic agents on BAEP waveforms of both normal and abnormal subjects will remain an important area of investigation.

The effects of many anesthetic agents on rat and human BAEP waveforms have been investigated. The present discussion will be limited to the effects of ketamine and xylazine since these were the agents employed in this study. Ketamine is classified as a dissociative anesthetic because of its dual action of depressing the thalamocortical system and activation of the limbic system.[71] This disruption of the integration of sensory information at the cortical level is responsible for patients reporting an impression of being separated from their surroundings prior to anaesthesia.[72] On the basis of pharmacologic studies, transmission of information through spinal, brain stem and reticular locations should be relatively unaffected.[16] Xylazine is classified as an analgesic as well as a sedative and will produce muscle relaxation. The central nervous system effects of xylazine are thought to be mediated

through activation of central alpha adrenergic receptors.[73]

Xylazine has received almost no attention with the exception of its use in combination with ketamine in one study.[74] Because ketamine is commonly used for restraint of pediatric patients, its effects on BAEP waveforms have been evaluated by several investigators.[39,74-76] The effect of ketamine on BAEP peak latencies is controversial. Two studies have concluded it does not affect BAEP peak latencies.[75,76] Both have design errors, such as failure to control body temperature or limited number of subjects, which could make their conclusions invalid. A more recent study by Church and Gritzke documented prolongation of the waveform latencies which were progressively greater for the latter components.[39] This contradicts the notion that ketamine does not interfere with brain stem function. Impaired synaptic transmission is the proposed mechanism of latency prolongation.[39] Differences in electrode placement, (implanted versus subcutaneous) and species (rat versus cat) make comparison of absolute latency data difficult.[39,75,76]

It is apparent from the contradictory results of these studies that many factors, including anesthetic agents or sedatives, can have effects on electrophysiologic tests. These parameters should be strictly controlled in the experimental setting and comparisons made only between control animals and experimental animals which have been tested using identical experimental protocols. Since many investigators continue to use anesthetic agents to obtain electrophysiologic data, additional studies on the effects of these agents are warranted.

Somatosensory Evoked Potentials

Introduction

Somatosensory evoked potentials (SEP) were first recorded in man by Dawson in 1947.[77] It was not until the advent of the signal averager in the 1950's that this technique found widespread use.[78] Somatosensory evoked potentials have a variety of clinical uses including intra-operative monitoring of orthopedic and neurological procedures, diagnosis of peripheral neuropathies, assessment of spinal cord lesions and evaluation of central nervous system diseases.[79] Since somatosensory pathways extend from the distal aspect of the extremities to the cortex and are readily accessible, they are particularly applicable to neurotoxicological studies. Evoked potentials can be recorded at many levels of the neuraxis.

Waveform Origins

Short latency somatosensory evoked potentials are generated by electrical stimulation of the median or tibial nerves. Median nerve evoked potentials are more easily elicited and recorded than tibial nerve evoked potentials.[20] However, caudal extremities are more vulnerable than upper limbs to distal

neuropathies.[20] Electrical stimulation of the median nerve in the rat creates an action potential that traverses the peripheral nerve, dorsal root ganglia and spinal dorsal column. The dorsal column synapses at the nucleus gracilis and then joins the spinothalamic tract to enter the thalamus. The thalamus then projects radiations to the somatosensory cortex (Figure 3).[80]

Typical recording sites for somatosensory evoked potentials after stimulation of the median nerve include the brachial plexus, cervical spinal cord and contralateral cerebral cortex.[80] The resulting waveforms represent successive anatomic sites through which the sensory impulse passes on its way to the cortex (Figure 3). The peaks are separated from each other because they occur separately in space and time. This allows for localization of deficits to peripheral nerves, sections of the spinal cord, brain stem and cortex. Better deficit localization facilitates thorough characterization of potential neurotoxicants.[12] However, somatosensory evoked potentials have only recently gained acceptance as a method of detecting and characterizing potential neurotoxicants. Technical difficulties in generating the potentials and lack of agreement on the "prototype" SEP waveform have hindered universal acceptance.

Short latency somatosensory potential components are defined as those with latencies less than 25 msec when stimulating at the proximal extremity and recording from the cortex. These are thought to represent far-field dorsal column, medial lemniscus and thalamic activity.[13] Potentials with latencies greater than 25 msec, recorded from the same location, arise from near-field cortical activity.[80] Parameters commonly measured include latency to spinal entry, subcortical activation and brain stem - cortical transit time.[12] The far-field, short latency somatosensory evoked potential recorded from the contralateral cortex of the rat consists of two distinct peaks (Figure 4).[81] The first positive peak (P1) is thought to arise from the brain stem dorsal column nuclei and the medial lemniscus.[82,83] The second negative peak (N1) originates from the thalamus and thalamic radiations.[82] These early components of the SEP produced by stimulation of the median nerve are not affected by level of arousal or depth of anesthesia.[83]

Detection of deficits relies on determining changes in latency and amplitude of the typical peaks. Alterations in latency and amplitude reflect anything from changes in conduction velocity or fiber populations of the peripheral nerve to increased synaptic delay or loss of selected neurons in the spinal cord, brain stem or thalamus.[1] Interpretation of SEP waveforms is more complicated than BAEP waveforms. At least two "typical" configurations have been recorded from normal human subjects.[84] Typical patterns for experimental animals have not been unanimously agreed upon. Technical difficulties encountered in the generation of these potentials include distal extremity temperature and position, stimulus parameters (intensity and rate), stimulator electrode positioning and subject size.[1, 79, 85] Much like the technical problems associated with the BAEP, these must be strictly controlled. Unlike the BAEP, subject size will greatly influence the absolute latency of the SEP waveforms by altering the distance

between the stimulus location (extremity) and recording electrode (scalp). When making comparisons between species or individuals of the same species that differ markedly in size, these discrepancies must be taken into consideration.

NEUROTOXICANTS

ACRYLAMIDE

Introduction

Acrylamide (CH_2=CHCONH$_2$) is a vinyl monomer used for a variety of chemical and industrial purposes. It is an effective flocculent with applications in mining operations, disposal of industrial wastes and purification of water supplies.[86] More recently it has been used as a strengthener during the manufacturing of cardboard and paper products.[87] The neurotoxic properties of acrylamide have been recognized for over 40 years. The monomer of acrylamide is responsible for its neurotoxic effects. Once it has been polymerized the resultant polymer is no longer neurotoxic.[87] Industrial exposure of humans in manufacturing plants has been associated with clinical signs ranging from tingling and numbness of digits, ataxia and weakness of the legs, emotional changes, loss of proprioceptive sensations and hypersomnolence.[86] Early studies on the neurotoxicity of acrylamide documented the first clinical signs as a staggering and uncertain gait, suggesting cerebellar ataxia or sensory deficits.[88] Subsequent studies involving acute high dose exposure demonstrated severe distal degeneration of peripheral nerve fibers which could be associated with the clinically apparent sensorimotor disturbances.[89]

Mechanism of Neurotoxicity

Studies involving long term exposure revealed a distal to proximal dying back axonopathy.[90] Dissimilarities in reports of central nervous system involvement may be related to differences in the dose and time course of acrylamide administration. Experimental procedures in rats have shown high dose exposure over short periods to result in predominately central nervous system signs.[89] Subchronic administration of significantly higher cumulative doses produces essentially peripheral nerve disease and relatively few central nervous system lesions.[89]

Acrylamide appears to have its most profound impact on sensory nerves while motor nerves are affected to a lesser extent.[86,89] Acrylamide neuropathy has been described as a central-peripheral distal axonopathy because the initial degenerative changes affect the distal ends of large diameter axons in both the peripheral and central nervous system. Early studies of Schaumburg et al[91] and Prineas[90] demonstrated that axon degeneration begins with abnormal accumulation of organelles and neurofilaments at the proximal side of distal nodes of Ranvier. Breakdown of the axon and myelin distal to the paranodal swelling ensues.[90,91] Unlike simple Wallerian degeneration, in which the entire distal axon undergoes rapid, simultaneous and irreversible degeneration, acrylamide seems to produce a slowly progressing centripetal degeneration of nerve fibers.[86] This pattern of distal axonopathy occurs in both peripheral

nerves and long central tracts including the spinal dorsal column axons in the medulla and spinocerebellar tracts in the cerebellar vermis.[88]

Proteins and other molecules required for the maintenance and integrity of the axon and nerve ending are synthesized in the cell body. Axonal transport is the mechanism by which these products are delivered to their appropriate destination.[92] Disorders in axonal transport have been implicated in the development of the acrylamide induced peripheral neuropathies.[86,93-97] Studies have demonstrated that alterations in the axonal transport kinetics of various substrates, glycoproteins in particular, are present at the onset of clinical signs but prior to the development of axonal ultrastructural alterations.[86, 93-97] In an effort to elucidate the underlying mechanisms responsible for a variety of "dying back" neuropathies, axonal transport derangements have been the overwhelming focus of current research related to acrylamide neurotoxicity.

Detection of Neurotoxicity

A number of authors have investigated the sensitivity of a battery of functional tests in detecting acrylamide toxicity. Motor dysfunction is easily and consistently detectable at cumulative doses of 100 - 320 mg/kg.[2,6,10,98-100] A recurring theme in many of these studies is that behavioral signs preceded morphologic changes or neuropathologic lesions were considered mild in comparison to observed deficits in the behavioral indices.[2,98,99] This suggests that functional alterations can be detected prior to morphologic changes caused by acrylamide toxicity and emphasizes the need for better characterization of the functional integrity of both the CNS and PNS. Despite this, relatively little work has been dedicated to investigating electrophysiological changes produced by acrylamide in experimentally intoxicated animals. One of the earliest studies of acrylamide neurotoxicity documented electroencephalographic abnormalities in cats.[101] In the late 1970's Goldstein and Lowndes demonstrated deficits in spinal monosynaptic reflexes in the absence of hind limb weakness.[102, 103]

Two studies have assessed the affects of acrylamide intoxication on sensory evoked potentials.[104,105] Schaumburg [105] et al detected delays in the peak latency of the SEP recorded from the gracile tract of the dorsal column system of intoxicated primates prior to any behavioral alterations and coincident with the earliest detectable neuropathological changes in this area.[105] Boyes and Cooper [104] detected delays in SEP waveforms recorded from rats after 16 days of intoxication. Clinical signs were evident at 12 days, however electrophysiologic testing was performed on days 4, 8 and 16.[104] Based on the results of this study no conclusions can be drawn as to which may have occurred first. However, these studies do clearly demonstrate that acrylamide intoxication can produce measurable deficits in CNS function. Boyes and Cooper [104] contend their findings suggest that the extent of CNS damage may be

greater than previously reported.[90,106,107] Areas implicated, which were not previously thought to be involved, included the somatosensory pathway from the dorsal column nuclei to the lateral thalamus and cortical radiations.[104] These findings indicate more extensive electrophysiological testing is needed to define the full extent of CNS damage associated with acrylamide intoxication.

2,5-HEXANEDIONE

Introduction

2,5-Hexanedione (2,5-HD) is the active metabolite of *n*-hexane and methyl *n*-butyl ketone. This compound is thought to be responsible for most, if not all, of the neurological lesions seen as a consequence of repetitive exposure to a variety of hexacarbon solvents.[108] *n*-Hexane is widely used as an industrial solvent in the manufacturing of glues, inks and gasoline. Human exposure has occurred through occupational exposure of workers in manufacturing plants and as a result of intentional inhalation of glue for recreational purposes.[109] Manifestations of hexacarbon neuropathy in humans depends on the severity and mechanism of exposure. Intermittent or low grade industrial exposure is most often characterized by symmetrical sensory dysfunction of the hands and feet which rarely progresses to distal muscle weakness and loss of deep tendon reflexes.[110] Cranial neuropathies and blurred vision have also been reported.[108] In solvent abusers, who tend to have the most intense exposures, the clinical presentation resembles a progressive, symmetrical ascending polyneuropathy accompanied by severe muscle atrophy.[111] The sensory system is much less affected in this type of exposure. Recovery in mild to moderate cases may be prolonged but is usually complete. Advanced cases complicated by muscle atrophy may not resolve fully and residual spasticity may be indicative of corticospinal tract damage.[111]

Mechanisms of Neurotoxicity

The clinical signs of experimental hexacarbon neurotoxicity in a variety of species are similar to those note in human solvent abuse cases. Most models demonstrate greater involvement of motor systems rather than the sensory involvement seen in occupational exposures. Commonly observed effects in experimental models include weakness of the hind limbs with incomplete extension, base wide stance, and drooping of the tail.[111] Neuropathological changes found with hexacarbon intoxication represent the prototypic pattern of distal axonopathies. The characteristic lesion is focal or "giant" axonal swelling in the nerve terminals and axons of peripheral nerves, gracile nucleus, cuneate nucleus, medullary reticular formation, spinal grey matter and neuromuscular junctions.[111] The damage is initially limited to large diameter, long axons in both the peripheral and central nervous system. As the disease progresses,

smaller diameter and shorter axons may be involved.[108] Initially axonal swellings develop on the proximal side of nodes of Ranvier resulting in altered axonal transport in affected regions.[112] Altered axonal transport, inhibition of glycolytic pathways, sterologenesis inhibition and direct interaction with neurofilament proteins have all been postulated as mechanisms of hexacarbon neurotoxicity.[111,113-117]

Detection of Neurotoxicity

Electrophysiologic tests, electromyography (EMG) and nerve conduction velocities, have been used to follow clinical cases in people. Although EMG changes occur early in the disease, nerve conduction velocity decreases are mild and may not document subclinical cases.[111] Several investigators have studied the effects of experimental hexane intoxication on electrophysiologic test parameters in laboratory species. Peripheral nerve conduction velocities and action potential onset and duration are prolonged late in the course of intoxication in most experiments.[109,118,119] This effect is manifested after prolonged exposure in low dose, chronic experiments. The reported onset of detectable alterations varies from five to eighteen weeks after chronic exposure. Results vary depending on the dose and method of exposure. Direct comparisons between experiments are difficult due to the variation in dosing regimens and timing of electrophysiologic testing. Alterations in both the BAEP and SEP of rats intoxicated with hexane have been documented.[118,120,121] Although the routes of exposure (inhalation, subcutaneous injection) have differed, studies have documented prolongation of BAEP waveforms by the third week of exposure. [118,120,121] Somatosensory evoked potentials and brain stem auditory evoked potentials appear to be more sensitive than nerve conduction velocity (NCV) or peripheral nerve action potential (AP) studies early in the course of hexane intoxication.[118,120] However NCV and AP changes persist after cessation of hexane exposure, whereas the BAEP and SEP parameters return to baseline values within the recovery period.[118,120] These alterations in BAEP and SEP document the need to further expand the study of the deleterious effects of hexane to include the central nervous system.

Materials and Methods

Animals

Adult (mean weight at start of project was 337.2 grams) male Long Evans rats were obtained from Charles River Laboratories, Raleigh NC. Rats were acclimated in the Virginia Polytechnic Institute and State University vivarium with a 12 hour light cycle (on from 1800 until 600), free access to feed pellets (Purina 5001 maintenance, Purina Mills Inc., St. Louis, Mo) and fresh water for one week. Rats were then randomly assigned to one of four groups: control (n=15), food restricted (n=15), acrylamide treated (n=15) or 2,5-hexanedione (2,5-HD) treated (n=15). Rats were housed individually in polypropylene cages (48x27x18 cm) for the remainder of the experiment. Light cycle remained the same and the rats were housed in a temperature and humidity controlled room. All rats except the food restricted group were fed free choice powdered rat chow (Purina Mills Inc., St Louis Mo). Powdered rat chow was fed to all rats during the treatment period to allow accurate measurement of the ration provided to food restricted group and maintain the anonymity of the treatment groups. Fresh water via automatic waterers was available to all groups. Food restricted rats were fed powdered rat chow at 50% of the calculated normal intake of 5 grams feed/100 gram body weight/day based on initial weight for the duration of the treatment period (approximately 7 grams per day). The food restricted group was included as a control for the expected weight loss and decreased food intake in rats receiving the neurotoxicants.

Chemicals

Acrylamide (99% pure) was obtained from Sigma Chemical Company, St. Louis, Mo. 2,5-Hexanedione (2,5-HD) (98% pure) was obtained from Aldrich Chemical CO., Milwaukee, WI. Both compounds were dissolved in saline and administered by intraperitoneal injection at a volume of 1 ml/kg. Control and food restricted rats received an equivalent intraperitoneal volume of saline. Acrylamide was dosed at 20 mg/kg/day (total accumulated dose = 420 mg/kg) and 2,5-hexanedione at 350 mg/kg/day (total accumulated dose = 7.35 gm/kg). All groups were injected daily for 21 days.

Electrophysiologic Testing Schedule

Identical procedures were performed on all rats. The principal investigator (TLT) was unaware of the treatment groups during the testing period. Baseline (week 0) values for sensory evoked potentials (SEP), brain stem auditory evoked potentials (BAEP) and functional observational batteries (FOB) were obtained from each rat (n=60). Testing schedule allowed for 15 rats to be tested per day. Each day,

prior to electrophysiologic testing, a separate investigator (L. Backer), evaluated each rat using a previously described functional observational battery (FOB).[98] The functional observational battery is designed to detect changes in locomotion, coordination, muscle tone and general physical characteristics of the rat. These parameters are used to assess functional alterations of the nervous system. Each rat was evaluated on weeks 0, 1, 2 and 3. At the end of the three week treatment period half of each group were killed for histopathologic evaluation. The remaining rats were maintained without treatment for 14 days and were evaluated on weeks 4 and 5. All rats were killed at the termination of the experiment for histopathologic evaluation.

After the FOB evaluation each rat was anesthetized with an intraperitoneal injection of a ketamine and xylazine combination (Vetalar:Rompum; 90mg/10mg/kg).[74] Responses to SEP (two repetitions per trial) and BAEP (two repetitions from each stimulated ear per trial) were recorded. The SEP and BAEP were then repeated such that for each rat on each testing day recordings from two SEP stimulations and four BAEP (2 left side; 2 right side) stimulations were generated. Depth of anesthesia was monitored by assessing responses to pedal (foot pinch) and palpebral reflexes prior to each evoked potential stimulation. Animals were not tested until judged to be at a sufficient plane of anesthesia (absent pedal and palpebral reflexes). A maximum of two additional partial doses of the ketamine : xylazine combination were administered as deemed necessary to achieve the acceptable level of anesthesia prior to testing. Body temperature was maintained between 37°C and 39.5°C by use of gel-filled reheatable thermal pad. Core temperature was continuously monitored by rectal probe inserted 8 cm into the colon.[68] Temperature was recorded at approximately two minute intervals corresponding to the beginning of each evoked potential recording. All electrophysiological data were collected within 25 minutes of induction of anesthesia. The BAEP and SEP were recorded with a Nicolet Pathfinder II. Auditory stimulus was provided by the Nicolet SM 400 Auditory stimulator. Electrical stimulation was provided by the Nicolet SM 100 Stimulus Controller (Nicolet Biomedical Instruments, Madison, WI).

Sensory evoked potentials were recorded following percutaneous stimulation in the area of the median nerve at the left carpus. A 4 mA constant current stimulus of 40 ms duration at a rate of 4.9/second was delivered to the area of the median nerve. Visible twitching of the paw always occurred in response to the stimulus. Recordings were simultaneously obtained from subdermally placed electrodes (platinum needle electrodes, Grass Instruments, Quincy, Mass.) at the brachial plexus area, caudal cervical vertebra and contralateral somatosensory cortex. All active electrodes were referenced to a common electrode inserted at the snout (Figure 5). Filter settings were 5 Hz (low frequency filter) and 3,000 Hz (high frequency filter). Five hundred sweeps of 25 ms duration were averaged for each stimulation. Impedance was monitored prior to each evoked potential recording. All impedance values

were less than 3.6 kilo-ohms. Latency to peak amplitude from stimulus onset was measured for each consistently identifiable waveform generated at the three recording sites (Figure 4).

Brain stem auditory evoked potentials were elicited by monaural click stimuli delivered by way of a deformable foam neonatal ear tip (Nicolet Biomedical Instruments, Madison, WI) placed in each external ear canal. The stimulated ear received rarefaction clicks of 90 dB intensity, 10 ms duration at a rate of 10.9/second. Filter settings were 150 Hz (low frequency filter) and 3,000 Hz (high frequency filter). The nonstimulated ear received 'white noise' at 30 dB intensity. Five hundred repetitions were averaged with a sweep duration of 10 ms for each stimulation. Impedance was monitored and maintained at less than 3.6 kilo-ohms. Each ear was stimulated individually and the auditory responses were recorded from the subdermal at the vertex and referenced to an electrode at the base of the ipsilateral ear. The ground electrode was placed between the scapulae (Figure 6). The latency of each of the four consistently identified waveforms was measured from stimulus onset to peak amplitude (Figure 1).

After electrophysiologic testing on day 21, approximately one half of the rats in each group were allowed to survive and all treatments were stopped. The remaining rats were deeply anesthetized with an intraperitoneal injection of pentobarbital (20mg/100gm body weight) mixed with 500 units of heparin. The rats were then perfused through the ascending aorta with saline for one minute at flow rate of 90 ml/min followed by 4% paraformaldehyde in 0.1 M Na phosphate buffer (12 rats total) or 5% glutaraldehyde in 0.1 M Na phosphate buffer (13 rats total).[3] Paraformaldehyde rats were dissected immediately following perfusion. Samples of the liver, kidney, testicle, small intestine, heart, brain, cervical and lumbosacral spinal cord segments, dorsal root ganglia, distal median nerve, brachial plexus, soleus muscle, sural nerve, tibial nerve and proximal sciatic nerve were obtained and stored in paraformaldehyde for six hours. Samples were then decanted and stored in 70% alcohol prior to embedding in paraffin. Rats perfused with glutaraldehyde were dissected within 24 hours. Specimens were stored in refrigerated glutaraldehyde. Nervous tissue was embedded in Epon epoxy resin. Sections were stained with hematoxylin and eosin and evaluated by light microscopy. The remainder of the rats were allowed to recover for 14 days. They were then killed for histopathologic examination as described.

Only data generated from the electrophysiologic testing aspect of this experiment will be discussed. Functional observational battery data and histopathologic evaluations are not included in this study. They will be assessed by other investigators as adjuncts to the electrophysiologic experiment and will be reported elsewhere.

Statistical Analysis

Somatosensory Evoked Potentials

Due to technical difficulties, adequate data points were not available for all treatment groups to allow for meaningful statistical analysis. Many of the SEP tracings generated during the experiment were not permanently recorded by the computer. This precluded the evaluation and measurement of latencies at a later date. The reason for this failure to record tracings is unknown. The identical procedure was used to store BAEP data successfully. Because of this, the data from this aspect of the experiment were not evaluated beyond reporting the mean and standard deviations of the waveforms recorded from the contralateral cortex (Figure 4). These were the only waveforms that were both consistently identified and preserved.

Brain Stem Auditory Evoked Potentials

Data obtained during the pretreatment week and the three weeks of dosing were statistically analyzed. Data from the recovery period (weeks 4 and 5) were not analyzed. Because of unanticipated deaths in the food restricted (7 rats) and acrylamide (2 rats) groups and planned deaths of half of each group at the end of the three week dosing period, insufficient data were available for meaningful statistical analysis of the results from weeks 4 and 5.

The peak latencies of four consistently identified waveforms were measured (Figure 1). The mean and standard deviation of latencies I through IV and IPL I-IV for each group on a given testing week were calculated. The values obtained from the ipsilateral electrode after stimulations of both the left and right ears, a total of four per rat per testing day, were averaged since the analysis of covariance for ear stimulated was not statistically significant. The interpeak latency I-IV (IPL I-IV or BSTT) was calculated by determining the arithmetic difference of the these two latencies. Data obtained from animals whose body temperature was less than 37.4°C were omitted (43 observations). Temperatures below this normal value have been documented to cause prolongations in BAEP waveform latencies. [62,63]

An incomplete block design was used for comparisons between treatment groups. Experimental design dictated that not all treatment groups were represented each day testing was performed during a given week of treatment. As a result of this design flaw only the 15 rats tested on the same day were used for comparisons of treatment groups (Table 1). Treatment groups not tested on the same day were compared; however, as a result of the study design, the variance was inflated unnecessarily. The increased variance made the comparisons insensitive. Only very large differences between means could have been detected under these circumstances. If results were analyzed without regard to the incomplete

block design, the effect of day of testing could not be separated from the effect of treatment group on the parameters measured. Treatment and day effects were confounded. Because of this insensitivity and the confounding factors, no between group comparisons were reported.

Since each rat was evaluated prior to initiation of therapy and weekly for the duration of the treatment period, each group of rats served as its own control. The general linear models procedure for repeated measures analysis of variance was used to compare the mean peak latency of waveforms I-IV and IPL I-IV for each week of treatment with the corresponding latency from the pretreatment period within each treatment group (ie. week 0 vs week 1, week 0 vs week 2, week 0 vs week 3). The same method was used to detect differences between each successive week (ie. week 1 vs week 2, week 2 vs week 3). Differences were not considered significant unless $p < 0.01$. Time from induction of anesthesia to collection of data, weight of subject, ear stimulated and core body temperature at the time of data collection were considered covariates. The general linear models procedure for analysis of variance was used to determine if the effect of the covariates was statistically significant. Statistical significance was defined as $p < 0.01$.

Results

Somatosensory Evoked Potentials

The most consistently recorded and subsequently retrievable data from the somatosensory evoked potentials were the waveforms recorded from the contralateral somatosensory cortex after median nerve stimulation. Two peaks were consistently identified (Figure 4). By convention the first positive deflection was labeled P1 and the first negative deflection was labeled N1. Readily identifiable waveforms from the brachial plexus and caudal cervical region were not consistently recorded and/or the data was not stored from week to week. Each stimulation trial did not inevitably result in similar traces for these two recording electrodes. This made identification of the prototypic waveform for the brachial plexus and caudal cervical recording positions particularly difficult. This variability in waveform identification, in conjunction with the missing data points prevented meaningful analysis of the data from these recording sites.

Although missing data points also limited the ability to perform meaningful statistical analysis of the waveforms recorded from the contralateral cerebral cortex, mean values for each treatment group for each week were determined (Table 2). The overall mean latency (+/- standard deviation) of P1 from all rats prior to treatment was 14.31 (+/- 1.15) msec. The overall mean latency of N1 from all rats on week 0 was 19.32 (+/- 1.51) msec. This compares with reported results, P1 = 12.3 (+/- 1.5) msec and N1 = 18.8 (+/- 0.7) msec, obtained from rats using identical anesthetic and recording electrode placement protocols.[74] The increased variability noted in this study might reflect irregularities in waveform identification. Attributes of the SEP waveforms were considerably more variable than BAEP waveforms. Comparison of the data from this study with results from studies using different recording protocols is not valid or meaningful since many extrinsic factors can influence the absolute waveform latencies.

The mean latencies of P1 and N1 in the control, food restricted and acrylamide groups were similar for the entire three weeks. The variations from week to week were of the same magnitude as the weekly standard deviations (Table 2). The 2,5-hexanedione group had the largest differences in mean latencies (Table 2). The mean latency of N1 increased from 18.73 (1.57) msec on week 0 to 23.51 (2.14) on week 3. This trend might be indicative of this neurotoxicant's effect on the SEP. No conclusions about the significance of this change can be made given the inability to perform meaningful statistical analysis.

Brain Stem Auditory Evoked Potentials

Control Group

The latencies of the first BAEP waveform and IPL I-IV were not different from baseline at any of the treatment weeks. Latency II was significantly longer than baseline values at weeks 2 ($p = 0.007$) and 3 ($p= 0.0001$) (Table 3). The values from week 2 and 3 were not different from each other ($p = 0.38$) (Table 3). Latency III ($p = 0.002$) and latency IV ($p = 0.006$) were significantly longer than baseline by the third treatment week (Table 3). These results are shown diagrammatically in Figure 7.

Body weight increased steadily from week 0 to week 3 (Figure 8). The mean body weight increased approximately 5% each week for a total mean weight gain of 15.5% of the baseline weight after 3 weeks.

Food Restricted Group

There were no significant differences between any of the latencies measured during the treatment period and the corresponding pretreatment values (Table 4, Figure 9). Rats consistently lost weight during the treatment period. Mean body weight decreased by 22% of baseline value after the three week period. The most rapid weight loss occurred between the second and third weeks when, on the average, rats lost 11% of their week 2 weight. The mean body weight of food restricted rats was 32% less than control rats at the end of three weeks (Figure 8).

Acrylamide Treated Group

The interpeak latency I-IV was not significantly different from the pretreatment value during the treatment weeks 1 through 3. Latency I was significantly shorter than the baseline value on week 1 ($p = 0.001$) then returned to baseline value for weeks 2 and 3 of treatment. Latency III was also significantly shorter than baseline on weeks 1 ($p = 0.001$) and 2 ($p = 0.01$), then returned to baseline value by week 3. Latency II ($p = 0.0001$) and latency IV ($p = 0.01$) were significantly longer on week 3 (Table 5, Figure 10).

The mean body weight changed very little over the three week period. Mean pretreatment weight was 330.46 grams while mean weight at the end of the third week was 327.17 grams. This represents a 0.9% decrease from baseline. Mean body weight of the acrylamide treated rats was 14% less than the control rats at the end of three weeks (Figure 8).

2,5-Hexanedione Treated Group

Latencies of all of the waveforms (I-IV and IPL I-IV) were significantly longer than pretreatment levels on weeks 2 and 3. All except latency I were also significantly longer than baseline at week 1 (Table 6). The effect on latency IV was progressive in that each week the latency was significantly longer than in the preceding week. Latencies of II, III and IPL I-IV from week 2 were not significantly different from week 1; however, all latencies showed significant prolongations by week 3 (Table 6). The prolongation of latency I was stable after week 2 (Table 6, Figure 11).

Mean body weight decreased by approximately 5% each week which accounted for the overall decrease of approximately 15% by week 3. The mean body weight of 2,5-HD treated rats was 26% less than control rats at the end of three weeks (Figure 8). This rate and degree of weight loss were comparable to the weight loss in the food restricted group.

Covariates

Covariates of body temperature, stimulated ear, body weight and time from induction of anesthesia to testing procedure were analyzed across treatment groups. Normal body temperature for the rat is in the range of 37.5°C to 38.5°C. The mean body temperatures of each group are shown in Figure 12. Despite exclusion of body temperatures less than 37.4°C, temperature had a significant affect on the IPL I-IV on week 0, latency II on week 2, latency III on weeks 1 and 3, and latency IV on weeks 0 and 3. Time from induction of anesthesia was only significant for latency I on week 2. Body weight did not significantly influence any of the latencies at any testing period.

Discussion

The results of this study demonstrate that brain stem auditory evoked potentials recorded from subcutaneously placed electrodes in anesthetized rats can discern the deleterious effects of acrylamide and 2,5-hexanedione on the central nervous system. No comments can be made about the ability of the somatosensory evoked potential, as recorded in this study, to detect neurotoxic effects of acrylamide and 2,5-hexanedione.

Somatosensory Evoked Potentials

The inability to document SEP changes stems predominately from technical difficulties experienced during the storing of waveform data and is not necessarily a reflection of the inherent ability of this technique to generate data. The trend evident in the 2,5-hexanedione treated group may have been statistically significant if sufficient data points had been available for analysis. However, the increased variability and difficulty in consistently identifying prototypic SEP waveforms may have limited the usefulness of this test. This variation may have resulted from the subtle deviations in electrode placement with each recording session. It would be of interest to compare control data obtained from subcutaneously placed electrodes with results from rats with implanted electrodes. If there was more homology in the implanted group, then perhaps subcutaneous recording of the SEP would not be the appropriate technique for studying potential neurotoxicants.

Brain Stem Auditory Evoked Potentials

Control Group

The statistically significant but numerically small prolongations of latency II at week 2 and latencies II, III and IV by the third week of treatment is disconcerting (Table 3, Figure 7). Identical testing protocols and parameters were used during the entire testing period. Variables which could be incriminated as a cause of these unexpected results include core body temperature, electrode placement, body size and depth and duration of anesthesia.

The effect of temperature variation was minimized both by maintaining core body temperature during the recording sessions and by omitting any data obtained from rats with core temperatures less than 37.4°C. Despite this, analysis of temperature as a covariate revealed a significant affect across all treatment groups on latency II at week 2 and latencies III and IV on week 3. Although temperature changes might account for the observed differences, the changes in body temperature were minimal (Figure 12). No estimation of the extent of the interaction between temperature and latency could be

made from this data. Latency increases of 1.0 msec for wave II, 1.75 msec for wave III and 2.0 msec for wave IV have been reported when the body temperature of a rat is systematically lowered from 37.0°C to 27.0°C.[122] In humans it has been estimated that wave V (analogous to rat BAEP wave IV) increases 0.16 msec for each 1°C drop in body temperature. [123]

If electrode positioning were of such critical importance, a much larger standard deviation within each group and greater variation in baseline values between groups would be expected. Standard deviations for all latencies across all groups and treatment weeks ranged from 0.06 to 0.30 msec, with the majority (49/64) in the range of 0.06 to 0.15 msec (Tables 3-6). Since the magnitude of latency prolongations (0.04 to 0.09 msec) were within the standard deviation of the tests, the importance of these statistically significant differences could be questioned.

Body size should not have had effect on the latencies even though control rats gained 15% of their original body weight by week 3. The only reported effect of size on BAEP is related to gender differences. Both human and rat females have been documented to have shorter BAEP latencies. These gender differences have been attributed to brain size. [16,38,50] The rats used in this experiment were all mature, male adults so changes in brain size should not have contributed to these differences.

The most likely candidate and the variable most difficult to control was depth of anesthesia at time of recording. Depth of anesthesia was subjectively estimated by evaluating toe pinch and palpebral response. These are admittedly insensitive measures. However, in a study designed to monitor the level of anesthesia via electroencephalography concurrently with these subjective parameters the two were found to correlate.[74] The period from 15 to 30 minutes after an injection of ketamine:xylazine (90 mg: 10 mg/kg ip) was characterized by high voltage slow wave activity on the electroencephalogram and absence of pedal and palpebral reflexes. Changes in the EEG pattern after 30 minutes were accompanied by appearance of weak palpebral and pedal reflexes.[74] In this study, all recordings were made while both pedal and palpebral reflexes were absent and within 25 minutes of injection of the ketamine:xylazine anesthetic. Therefore, similar depths of anesthesia were likely.

Prolongations in all latencies have been reported after repeated doses of ketamine. [39] These latency shifts were progressive and had the greatest effects on the later waveforms (IV-VI). In an effort to assess the effects of multiple injections of the ketamine:xylazine anesthetic, control rats and food restricted rats were compared. Food restricted rats were used for comparison because there were no statistically significant differences in any latencies during any week for this group. During testing on weeks 2 and 3, a combined total of 5 rats in the control group and 11 rats in the food restricted group were given an additional injection of the combination anesthetic. If the higher dose of anesthetic or corresponding level of anesthesia were the cause of the observed latency changes in the control group,

then statistically significant changes would have been expected in the food restricted group since nearly twice as many rats were given additional doses of the anesthetic combination, but as noted above, this was not the case. In this group there appears to be no logical explanation for this apparent discrepancy. These differences found in the control group may represent statistically but not biologically significant results.

The differences in latencies II, III and IV in the control group may be attributed to an inflated type I error rate. Type I errors occur when a pair of means is judged significantly different when in reality the means are equal.[124] The proportion of type I errors in a set of significant results is a function of the significance level, the power of the tests and the proportion of cases in which the alternative hypothesis is true. [124] Setting the significance level at $p < 0.01$ rather than 0.05 decreases the likelihood of committing type I errors. In other words, the probability of committing a type I error is reduced from 5% to 1%. The significance level for this experiment was set at $p < 0.01$. The p values for significant results were much less than this value.

The magnitude of difference between latency II in week 3 and week 0 was 0.09 msec (Figure 7). This was the largest of the variations within the control group considered significantly different. The smallest variation considered significantly different was 0.05 msec (latency II week 2 vs latency II week 0). Detection of statistical significant in this small magnitude of difference between means reflects a high degree of sensitivity for this test. This sensitivity is a result of the narrow range of standard deviations between groups and the large number of data points analyzed, two parameters which will increase the power (and therefore sensitivity) of a given test. Tests with high power have a corresponding higher chance of committing a type I error. [124] A given statistical test is more likely to detect small differences in means if there is minimal variation in the set of data points being analyzed.

Increased variability within a set of data points results in a decrease in the ability of a statistical test to detect differences between means. This is exemplified by the fact that latency IV on week 2 (4.20 +/- 0.30 msec) was determined not to be significantly different from latency IV week 0 (4.12 +/- 0.14 msec, $p = 0.02$), while latency IV on week 3 (4.19 +/- 0.16 msec) was considered significantly different from week 0 (4.12 +/- 0.14 msec, $p = 0.006$) (Table 3). The lack of significance between latency IV on week 2 and week 0 can be attributed to the increased variability in the mean latency on week 2 as reflected by a standard deviation which was approximately twice the standard deviation for the latency on weeks 1 and 3. A similar trend occurs in the analysis of latency III (Table 3). The increase in variability may have been a function of the confounding effect of day of testing and treatment. Since all groups were not represented on each testing day the effect of day of testing and treatment group could not be separated.

The final explanation is that significant differences occurred in the control group from week to week. This would lend credence to the assertion that recording BAEP with subcutaneously placed electrodes is not reliable. If this is true, then variations in BAEP parameters, detected in this study and assumed to result from neurotoxicant effects, must be carefully scrutinized. However, the lack of significant differences in the food restricted group suggests that this method is reliable and additional factors must be involved.

Food Restricted Group

The restriction of food intake by approximately 50% of normal resulted in rats with mean body weights 32% less than the control group at the end of week 3 (Figure 8). This compares with a reported 38% difference in mean body weight of rats after four weeks of 50% reduction in food intake as compared to normally fed controls.[11] There were no statistically significant differences in the means of any of the latencies at any treatment week (Table 4, Figure 9). This is in agreement with the results of Albee et al, who found no significant differences in the BAEP latencies when temperature was analyzed as a covariate.[11]

Although dietary restriction in adult rats can have effects on the results of a functional observational battery, there do not appear to be any detectable changes in the electrophysiologic measures of central nervous system function.[10,11] This correlates with the inability to document histopathologic changes in the central nervous system of diet restricted adult rats. [10, 11] The decreased weights of rats in the 2,5-HD (26% less than controls) and acrylamide groups (14% less than controls) indicated that 50% restriction of dietary intake is a reasonable model for the effects of neurotoxicants on body weight.

Acrylamide Group

The transient decreases in the latencies of peaks I and III are difficult to directly attribute to the neurotoxicant (Table 5). The documented demyelination and axonal damage caused by acrylamide intoxication would be expected to result in delays of the latencies corresponding to affected areas. [87] The only mechanism correlated with decreased latencies is hyperthermia.[125] Given the strict control of temperature during the recordings and a trend for body temperature to decrease during the treatment weeks, hyperthermia is an unlikely explanation (Figure 12).

The weekly mean body weight of acrylamide treated rats was intermediate to the control and food restricted groups (Figure 8). Severe weight loss, as occurred in the food restricted group, had no significant effect on BAEP waveform latencies. It is unlikely, given the reduced magnitude of weight

loss in the acrylamide group, that this parameter would account for the observed changes.

The differences in the means of latency III (weeks 1 and 2) and latency I (week 1) from week 0 were 0.05, 0.04 and 0.09 msec respectively (Figure 10). As occurred in the control data, there is an apparent discrepancy in the comparison of means. Latency III from week 3 (3.31 +/- 0.16 msec) was not statistically different from week 0 (3.28 +/- 0.08 msec,p = 0.08) or from week 2 (3.24 +/- 0.09 msec, p = 1.00). However, latency III from week 2 (3.24 +/- 0.09 msec) was considered statistically different from week 0 (3.28 +/- 0.08 msec, p = 0.011). (Table 5) The same assertions used to explain the importance of the statistical significance detected in the control group may be true in this case. The standard deviation of latency III on week 3 is nearly double the standard deviation on weeks 0 and 2 (Table 5).

The larger magnitude differences detected in latency II and latency IV on week 3 (0.15 and 0.12 msec respectively) may represent true effects of acrylamide (Figure 10). If these findings are considered biologically significant, they suggest pathologic changes in the area of the superior olivary complex and region of the lateral lemniscus in the mesencephalon (Figure 2). Since no other investigators have employed the BAEP as a part of a battery of tests to assess the neurotoxicity of acrylamide, direct comparisons can not be made.

The effects of acrylamide intoxication on SEP latencies have been reported. [104, 105] Statistically significant increases in the latency of three of the four measured peaks corresponding to the medial lemniscus, the thalamus and sensory radiations and the cortex were documented in rats receiving a cumulative dose of approximately 600 mg/kg of acrylamide in 16 days.[104] The early and very subtle alterations in the SEP of primates chronically dosed with acrylamide occurred after a cumulative dose of 150 mg/kg in 15 days. SEP changes were progressive and maximal by 50 days with a total accumulated dose of 500 mg/kg.[105] Given these findings, it is reasonable to postulate that the delay in the latencies of II and IV represent the expected effects of acrylamide.

Functional assessments of rats chronically and subchronically dosed with acrylamide generally require 400 to 700 mg/kg accumulated dose before statistically significant differences are detected.[2,4,6,98,100] Rats in this study received a total cumulative dose of 420 mg/kg over the 21 day treatment period. The slight prolongations of latencies of peaks II and IV on week 3 may have become greater or other latencies may have been affected if dosing had been designed to simulate subacute intoxication. Studies designed to compare the effects of subacute and subchronic administration of acrylamide indicate that manifestations of central nervous system involvement are more pronounced than peripheral nervous system signs in experiments where high doses are given over a short time period.[88,90,91]

2,5-Hexanedione

The body weight of 2,5-HD intoxicated rats was markedly less than controls and baseline means. It is unlikely this had any effect on the BAEP latencies given the lack of significant differences in the food restricted group. 2,5-hexanedione (2,5-HD) had a significant and progressive effect on the latencies of waveforms II, III, IV and IPL I-IV. Latency I was significantly prolonged as compared to baseline on weeks 2 and 3, however the magnitude of these changes was much less than for the other latencies (Table 6, Figure 12). It is difficult to determine if the effects of 2,5-HD on cranial nerve VIII are a result of the neurotoxicant or if the design of this study resulted in the detection of statistical significance which is misleading. Statistically significant prolongations of the first component of the BAEP have been reported in three studies of hexacarbon intoxication in rats.[118,120,121]

The major effect of 2,5-HD exposure was on latency IV (Figure 11). The prolongation of latency IV was progressive and significant for each week of exposure. The increase in latencies II, III and IPL I-IV reached a plateau between weeks 1 and 2. These latencies were significantly longer by week 3 (Table 6). The predominance of effect on the later BAEP waveforms likely reflects the neurotoxic effects of 2,5-HD on the auditory ascending tract in the brain stem. This statement is supported by the significant prolongation of interpeak latency I-IV, which is considered to represent conduction function in the brain stem. These results are in general agreement with previous experiments documenting the preferential delay of later BAEP waveforms in rats exposed to hexane by inhalation and to 2,5-HD by injection.[118,120,121]

Direct comparison of numerical values for the individual waveforms among experiments is limited by variations in recording and exposure protocols. However, the magnitude of the effects can be compared. Rebert[118] et al and Hirata[121] et al reported latency differences between treated and control rats in the order of 0.07 to 0.12 msec to be significant. The magnitude of the significant changes in the later waveforms (III and IV) documented by this experiment ranged from 0.17 to 0.52 msec (Figure 11). This may be a reflection of the larger total dose of 2,5-HD received by rats in this experiment 7,350 mg/kg, as compared to 1,275 mg/kg and inhalation of *n*-hexane which requires metabolism to 2,5-HD.[118,121]

Alterations in the BAEP waveforms due to 2,5-HD occurred earlier than the previously reported onset of detectable changes in neurobehavioral parameters. In a previous study conducted using an identical dosing regimen, significant differences in motor integration activity were noted after 14 days of administration.[98] In a separate study, rats administered 720 mg/kg/day of hexanedione, almost twice

the dose used in the current experiment, only showed significant differences in the more complex behaviors only after 4 weeks of treatment.[126] In this study, the latencies of BAEP waveforms II, III, IV and IPL I-IV became significantly prolonged by 7 days of treatment with 350 mg/kg/day. This suggests that the BAEP is more sensitive to the effects of 2,5-HD than are behavioral tests.

Summary

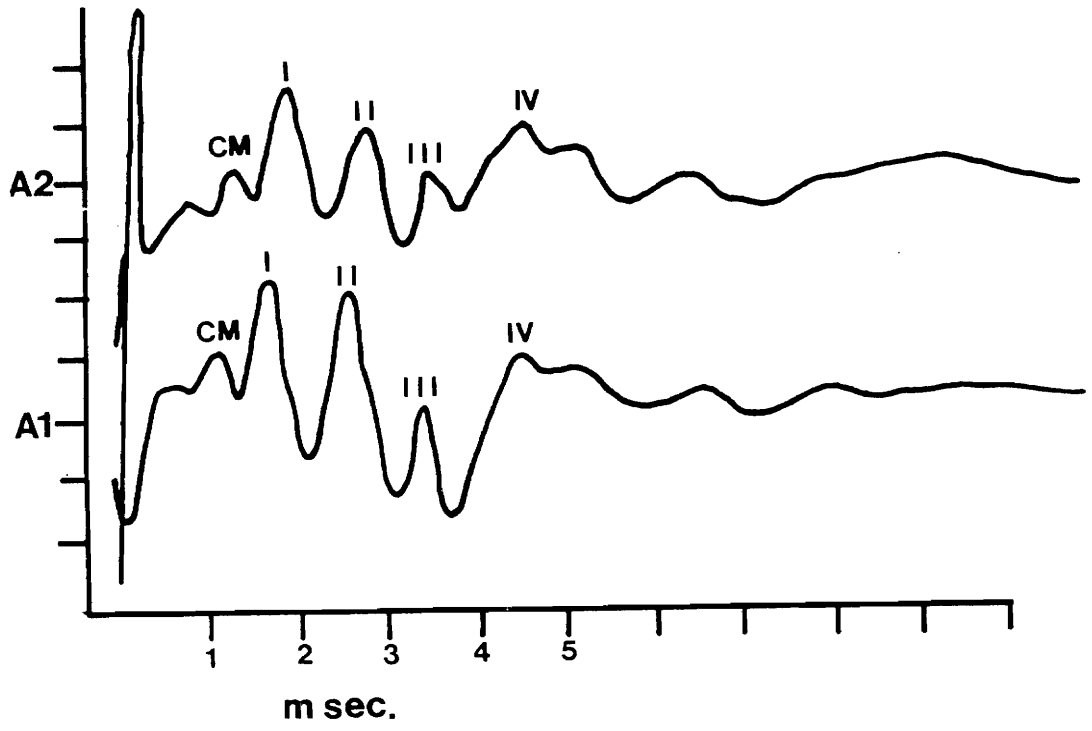
The study of neurotoxicity in laboratory animals is complicated by a multiplicity of factors. The importance of defining and minimizing the confounding factors relates to a need to provide tests which will ultimately prevent human exposure to potentially neurotoxic materials. To achieve this goal, it is imperative that the scientific community develop sensitive and specific screening protocols to test potential neurotoxicants.

This study has demonstrated the feasibility and sensitivity of one type of neurophysiological test used for screening potential neurotoxicants. Recording brain stem auditory evoked potentials from subcutaneously placed electrodes in anesthetized rats successfully demonstrated the deleterious effects of 2,5-hexanedione on the central nervous system earlier than has been documented in the literature using behavioral or histopathologic studies. The effects of acrylamide on the central nervous system were also demonstrated. The acrylamide dosing regime may have limited the extent of damage to the central nervous system which may be responsible for the less dramatic effect on the parameters being studied.

The use of subcutaneously placed electrodes allowed for remarkable repeatability of the BAEP waveform latencies within each treatment group. This is significant since proponents of implanted electrode studies cite increased variability as the major disadvantage of this technique. Unfortunately SEP waveforms recorded in this fashion were not as consistently repeatable or identifiable.

Food restriction was an effective method of mimicking the systemic effects of neurotoxicants. The magnitude of weight loss after three weeks of half maintenance feeding was similar to the weight loss in the intoxicated rats. There were no electrophysiologically detectable deleterious effects of food restriction. The absence of latency alterations in this group suggests that the changes seen in intoxicated rats were not related to decreased food intake or weight loss.

Technical difficulties encountered during this experiment are indicative of the inherent problems associated with employing a single test or type of test to evaluate toxicants which affect a multitude of biological parameters. A variety of biological factors can affect the waveforms produced by an evoked potential response. These include, but are not limited to, the nature and condition of the neural generators, the number of generating elements active, the length and geometry of fiber tracts, the spatial orientation of cell bodies and conductivity of the tissues.[127] In addition, extrinsic factors such as recording and stimulation protocols confound the issue by affecting waveform parameters independent of the biological factors. The relationship of BAEP waveform parameters to some of the extrinsic variables have been documented, however the association between intrinsic biological factors and the BAEP are only barely known.[118] Clarification of these effects is mandatory before meaningful interpretation of changes in the BAEP waveforms induced by toxic agents can be made.



A 1 = Recording from ipsilateral electrode, A 2 = Recording from contralateral electrode. CM = Cochlear Microphonic, I = Latency I,II = Latency II, III = Latency III, IV = Latency IV. Recorded after click stimuli of 90 dB, 10 ms duration, 10.9/sec rate, Filters 150 Hz - 3,000 Hz. 500 repetitions averaged with sweep duration 10 ms, Impedance < 3.6 kilo-ohms

Figure 1 Representative Brain Stem Auditory Evoked Potential Recording from a Normal Rat

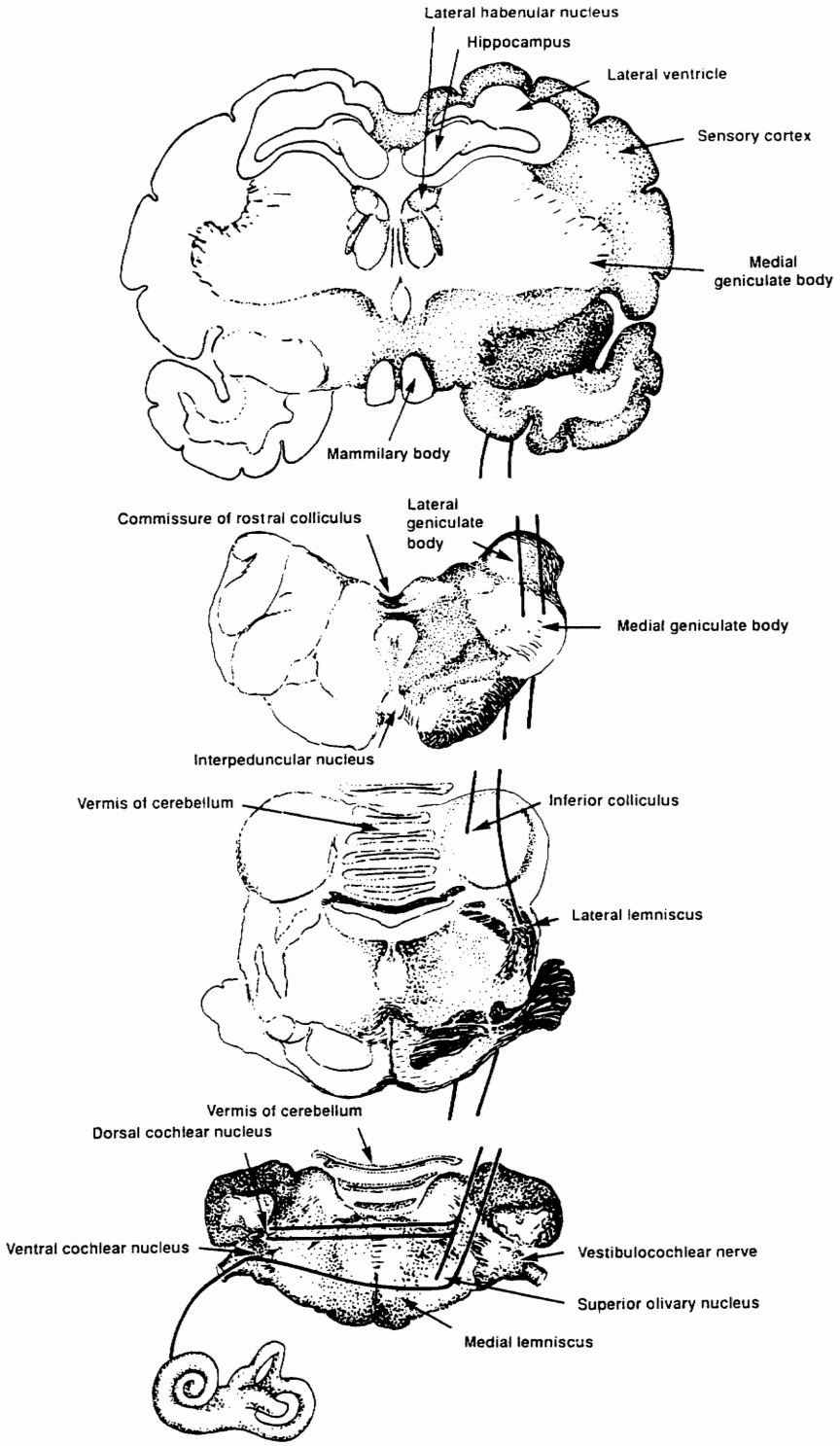


Figure 2 **Schematic Representation of the Brain Stem Auditory Evoked Potential Pathway**

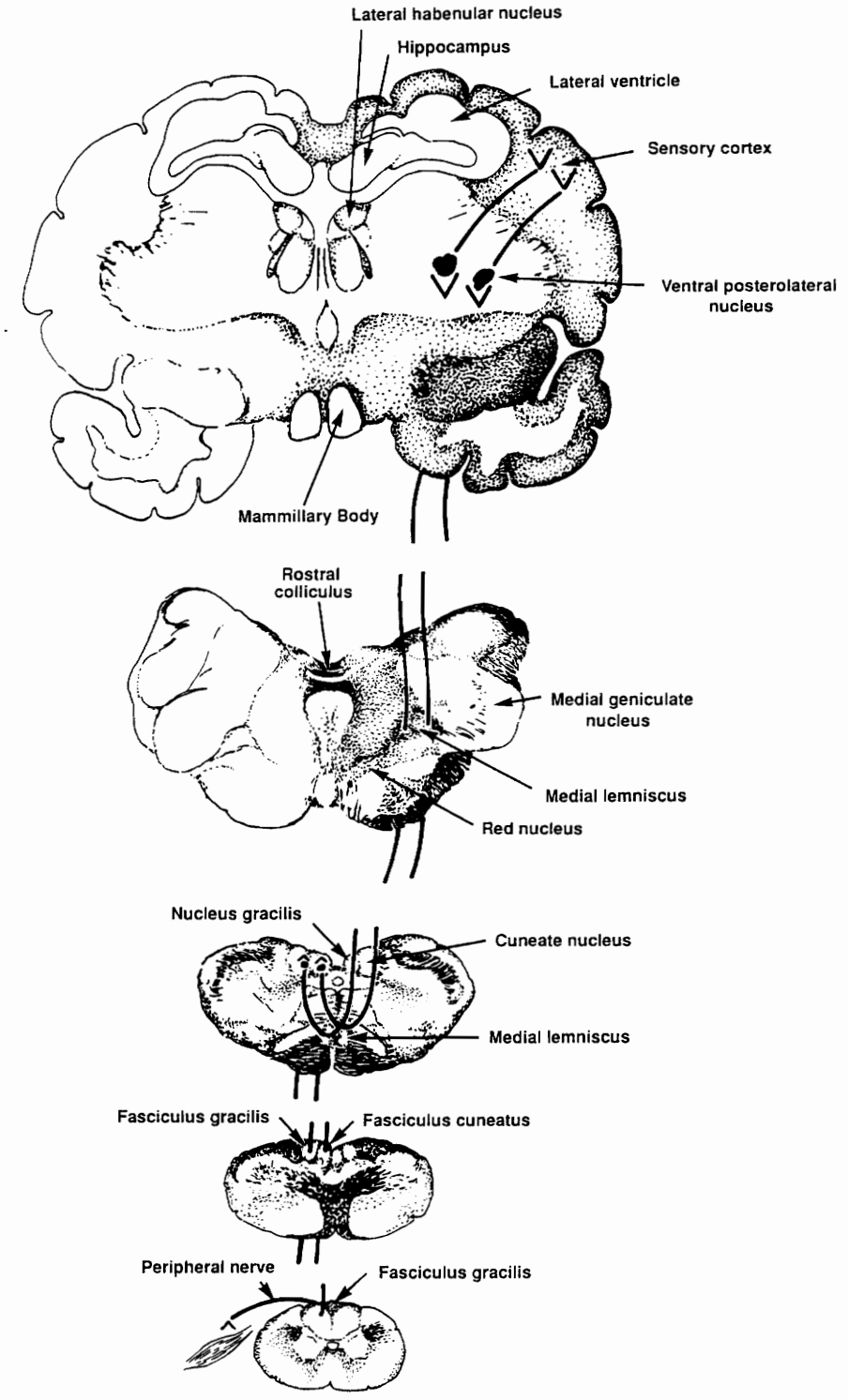
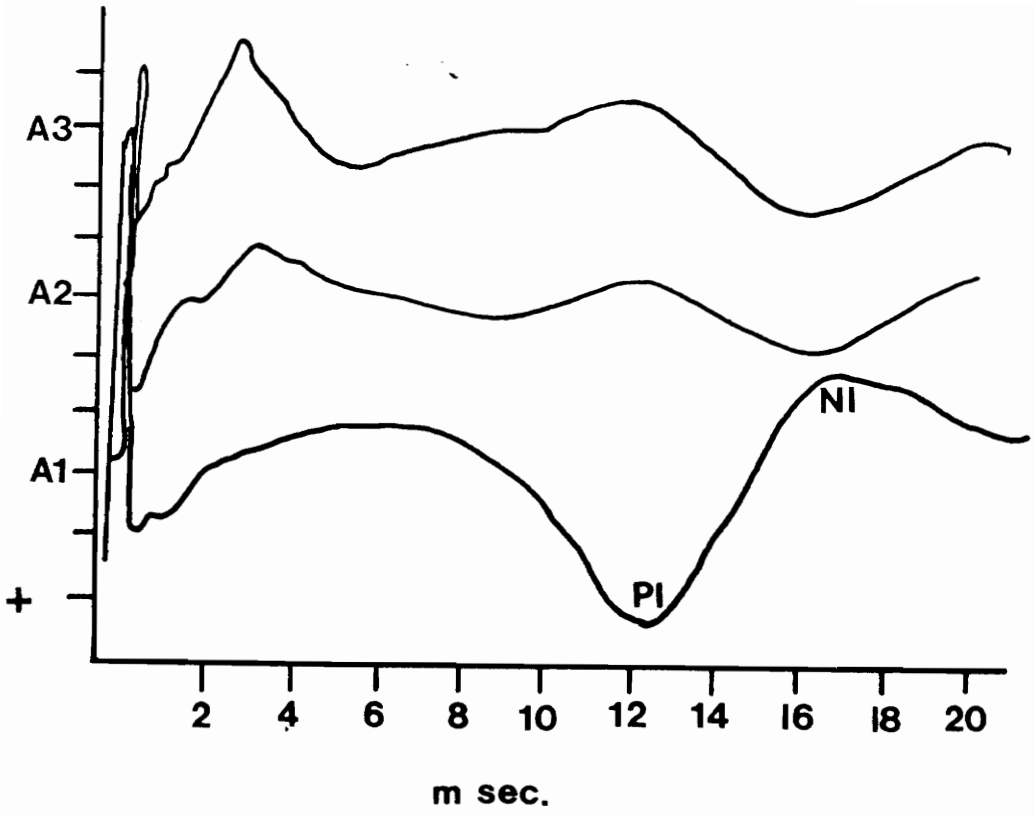
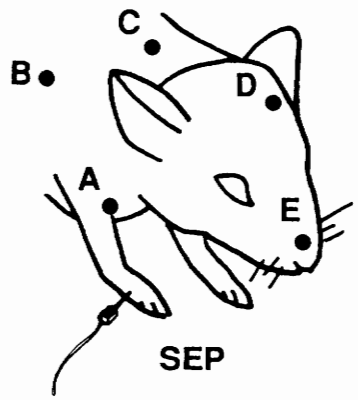


Figure 3 **Schematic Representation of the Somatosensory Evoked Potential Pathway**



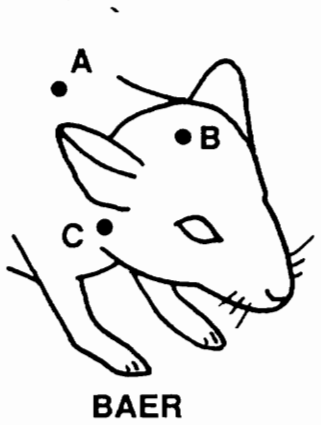
A 1 = Recording from Contralateral cortex electrode, A 2 = Recording from caudal cervical electrode. A 3 = Recording from brachial plexus electrode, P 1 = First vertex positive peak after stimulation. N 1 = First vertex negative peak. Recorded after median nerve stimulation of 4 mA constant current, 40 ms duration, 4.9 / sec rate. Filter settings 5 Hz - 3,000 Hz. 500 sweeps of 25 ms duration were averaged. Impedance < 3.6 kilo-ohms

Figure 4 Representative Somatosensory Evoked Potential Recording from a Normal Rat



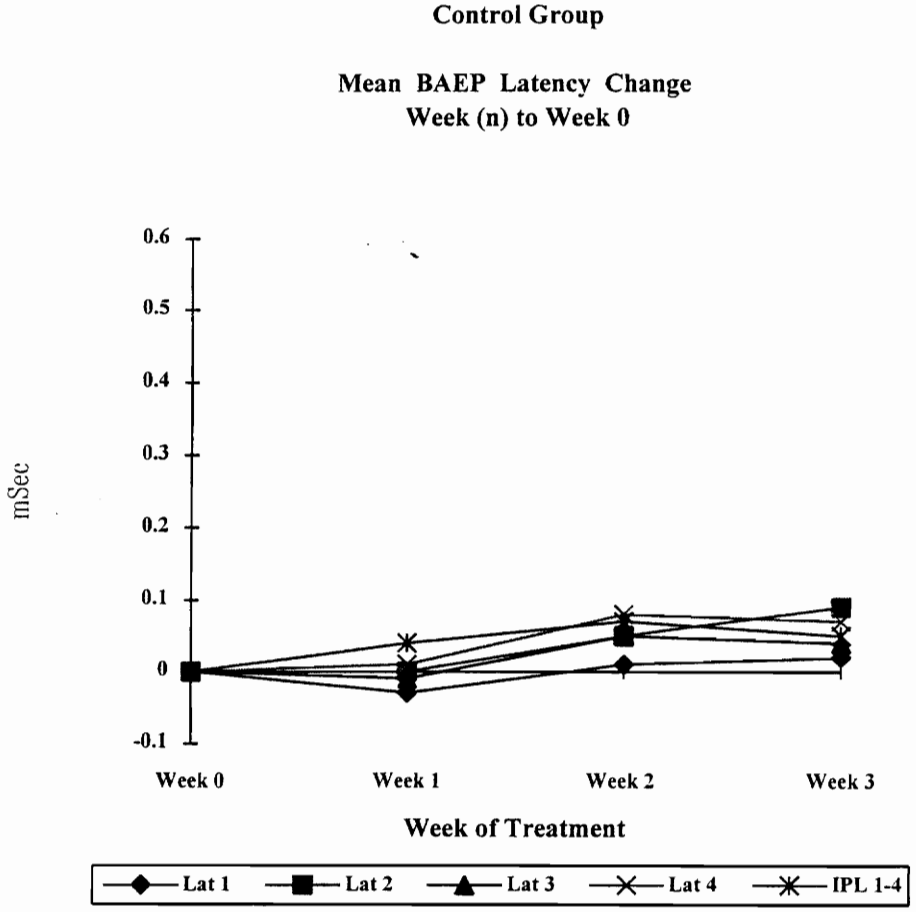
Recording electrode placement for Median nerve stimulation
A = Brachial Plexus, B = Ground Electrode, C = Caudal Cervical Electrode
D = Contralateral Cortex Electrode, E = Reference Electrode

Figure 5 **Somatosensory Evoked Potential Recording Electrode Montage**



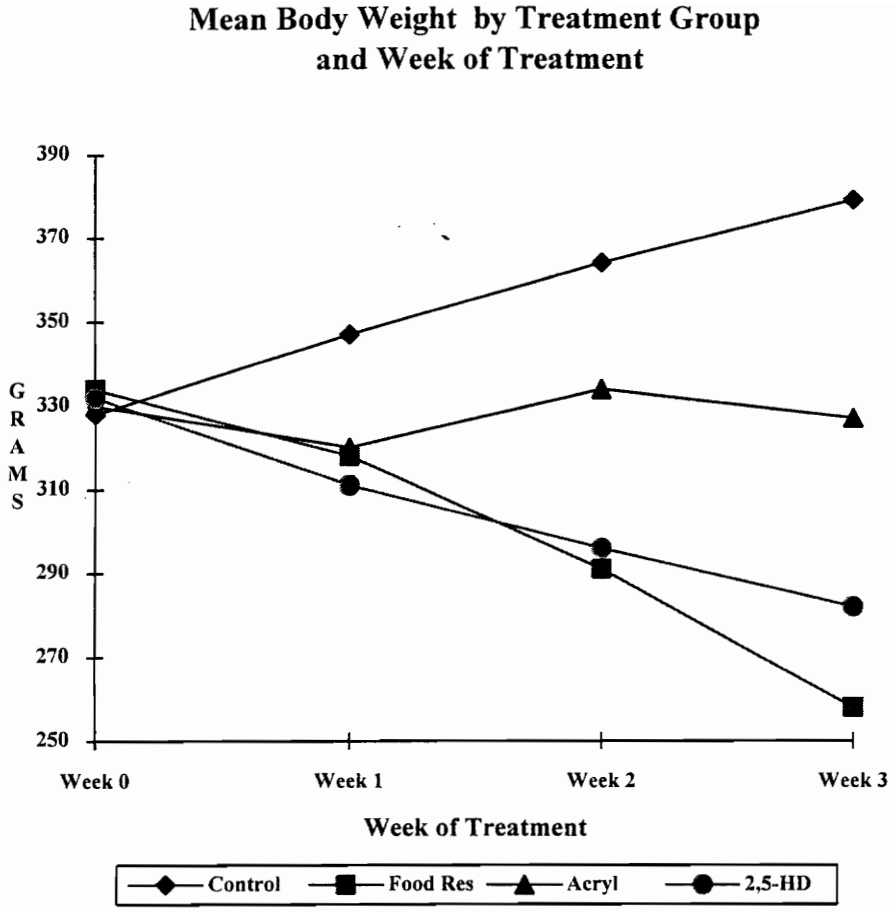
A = Ground electrode between scapulae.
B = Recording electrode at vertex.
C = Reference electrode at base of ipsilateral ear.

Figure 6 **Brain Stem Auditory Evoked Potential Recording Electrode Montage**



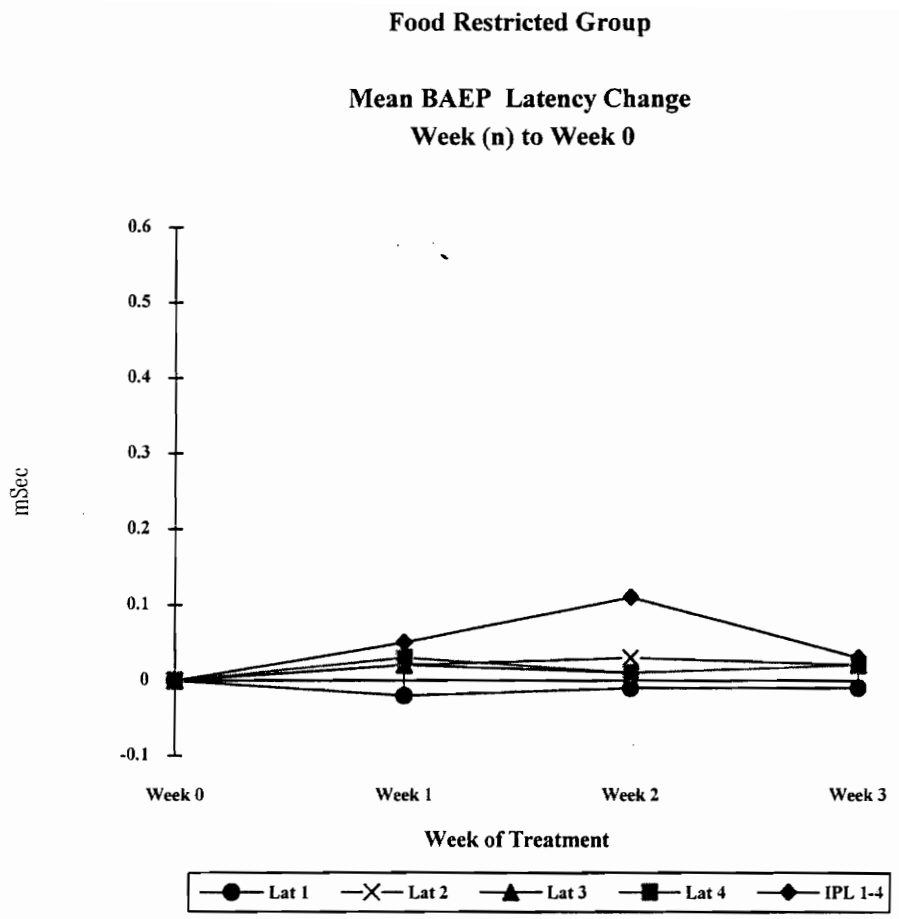
Lat 1 = latency I, Lat 2 = latency II, Lat 3 = latency III,
Lat 4 = latency IV, IPL 1-4 = interpeak latency I - IV.

Figure 7 Change in Mean Latencies by Week: Control Group



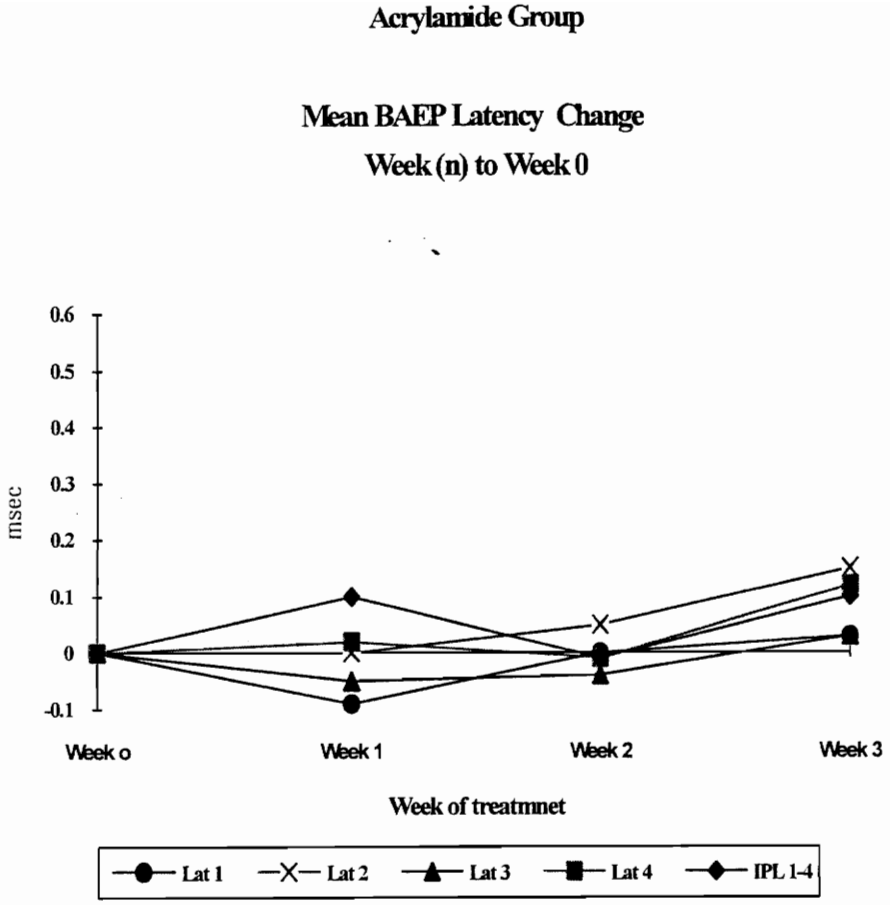
Control = control group, Food Res. = Food restricted group,
Acryl.= Acrylamide group, 2,5-HD = 2,5-Hexanedione group.
Body weight in grams

Figure 8 **Mean Body Weight By Treatment Group**



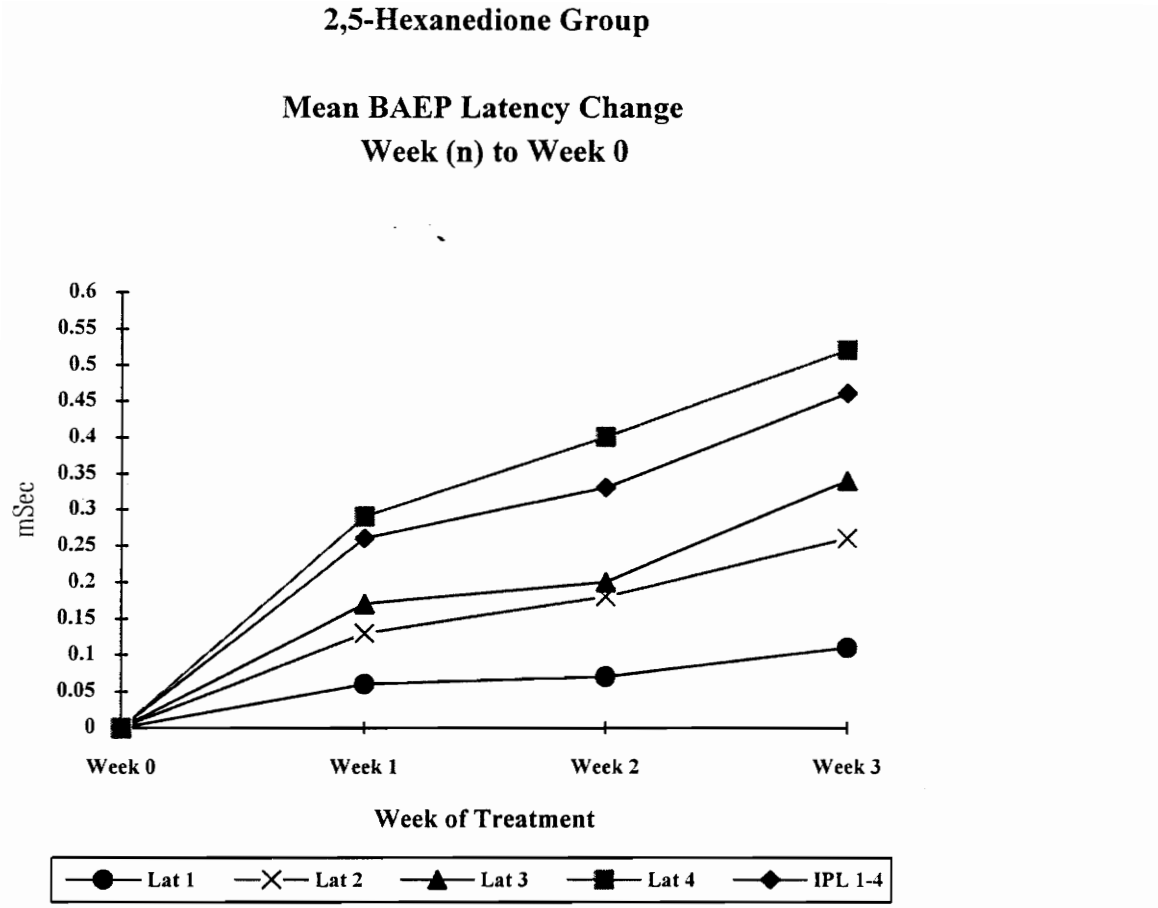
Lat 1 = latency I, Lat 2 = latency II, Lat 3 = latency III,
Lat 4 = latency IV, IPL 1-4 = interpeak latency I - IV.

Figure 9 Change in Mean Latencies by Week: Food Restricted Group



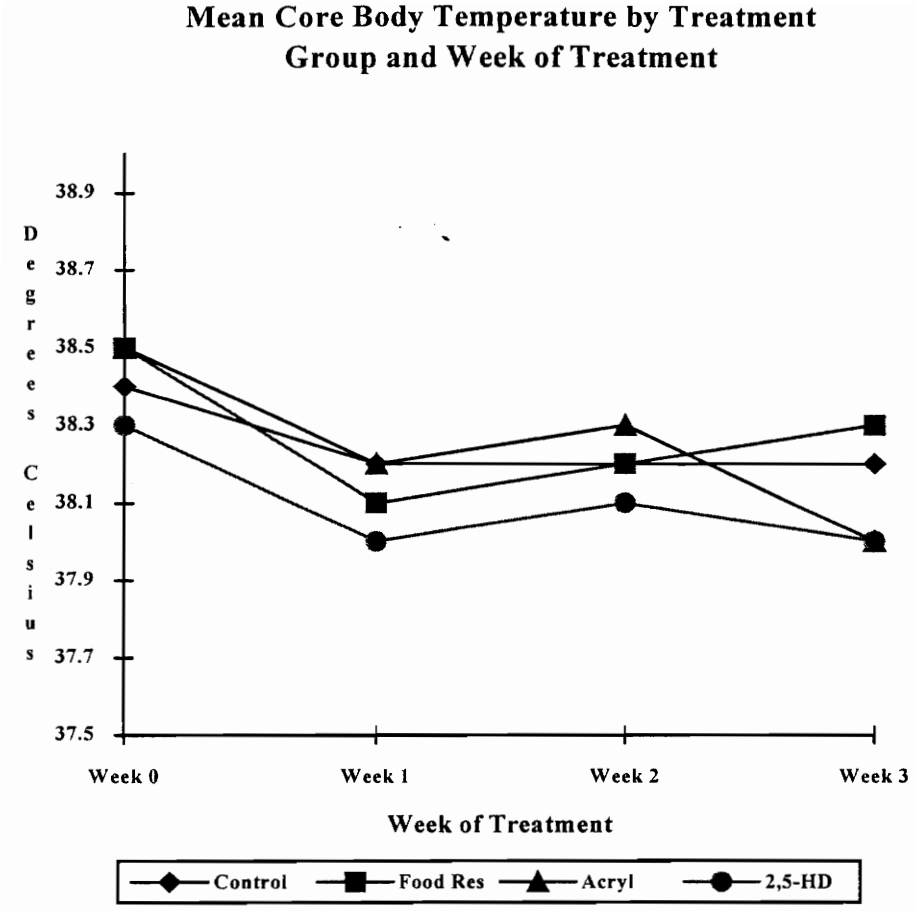
Lat 1 = latency I, Lat 2 = latency II, Lat 3 = latency III,
Lat 4 = latency IV, IPL 1-4 = interpeak latency I - IV.

Figure 10 Change in Mean Latencies by Week: Acrylamide Group



Lat 1 = latency I, Lat 2 = latency II, Lat 3 = latency III,
Lat 4 = latency IV, IPL 1-4 = interpeak latency I - IV.

Figure 11 Change in Mean Latencies by Week: 2,5-Hexanedione Group



Control = control group, Food Res. = Food restricted group,
Acryl.= Acrylamide group, 2,5-HD = 2,5-Hexanedione group.
Rectal temperature in degrees Celsius as measured by rectal probe.

Figure 12 Mean Body Temperature By Treatment Group

Table 1

Statistical Analysis
Incomplete Block Design

Treatment Groups

Block	Control	Food	Acryl	2,5-HD
Red	-	8	7	-
Yellow	7	-	8	-
Blue	-	7	-	8
Green	8	-	-	7

Colors denote groups of animals tested on the same day of each week.
Food = Food Restricted, Acryl = Acrylamide,
2,5-HD = 2,5-Hexanedione

Table 2

Mean +/- Standard Deviation of Somatosensory Evoked Potential Latencies

Control Group

Latency	Week 0	Week 1	Week 2	Week 3
P1	13.38 (0.84) n=26	14.37 (1.34) n=23	14.42 (0.67) n=13	14.78 (1.08) n=16
N1	18.90 (1.30) n=26	18.81 (1.91) n=23	18.99 (0.87) n=13	19.39 (1.2) n=16

Food Restricted Group

Latency	Week 0	Week 1	Week 2	Week 3
P1	14.58 (2.64) n=26	14.92 (1.02) n=19	15.26 (2.3) n=15	15.00 (0.76) n=7
N1	20.04 (1.09) n=26	21.34 (1.09) n=19	21.20 (3.15) n=16	20.41 (1.90) n=8

Acrylamide Group

Latency	Week 0	Week 1	Week 2	Week 3
P1	14.27 (1.15) n=25	14.59 (0.95) n=24	13.54 (0.78) n=17	13.38 (1.86) n=4
N1	19.68 (1.23) n=25	19.02 (1.66) n=18	17.70 (1.19) n=15	18.98 (3.5) n=4

2-5,Hexanedione Group

Latency	Week 0	Week 1	Week 2	Week 3
P1	13.80 (1.15) n=27	16.15 (1.74) n=26	15.75 (1.13) n=27	15.69 (1.22) n=20
N1	18.73 (1.57) n=27	22.85 (1.55) n=25	22.19 (4.5) n=27	23.51 (2.14) n=20

All Latencies in msec +/- ( ) standard deviation and n = number of data points analyzed.
Recorded from contralateral cortex after median nerve stimulation.
P1 = first vertex positive wave.
N1 = first vertex negative wave.

Table 3

Brain Stem Auditory Evoked Potential
Mean +/- Standard Deviation of Waveform Latencies

Control Group

	Week 0	Week 1	Week 2	Week 3
Latency I	1.74 (0.11)	1.71 (0.10)	1.75 (0.10)	1.76 (0.08)
Latency II	2.58 (0.07)	2.58 (0.10)	**2.63 (0.15) [p=0.007] {p=0.007}**	**2.67 (0.11) [p=0.0001]**
Latency III	3.23 (0.08)	3.22 (0.09)	3.28 (0.22)	**3.27 (0.11) [p=0.002]**
Latency IV	4.12 (0.14)	4.13 (0.20)	4.20 (0.30)	**4.19 (0.16) [p=0.006]**
IPL I-IV	2.38 (0.14)	2.42 (0.21)	2.45 (0.32)	2.43 (0.15)

Latencies +/- standard deviation ( ) in msec.
Latencies for week n with p value are significantly different from week 0.
Latencies for week n with p value { } are significantly different from preceding week (n-1).

Table 4

Brain Stem Auditory Evoked PotentiaL
Mean +/- Standard Deviation of the Latencies

Food Restricted Group

	Week 0	Week 1	Week 2	Week 3
Latency I	1.75 (0.14)	1.73 (0.10)	1.74 (0.14)	1.74 (0.12)
Latency II	2.63 (0.09)	2.65 (0.10)	2.66 (0.12)	2.65 (0.15)
Latency III	3.26 (0.06)	3.28 (0.08)	3.27 (0.11)	3.28 (0.13)
Latency IV	4.17 (0.13)	4.20 (0.17)	4.27 (0.21)	4.19 (0.18)
IPL I-IV	2.42 (0.16)	2.47 (0.16)	2.53 (0.20)	2.45 (0.13)

Latencies +/- standard deviation ( ) in msec.
No significant differences were noted between week (n) and week 0 or between week (n) and week (n-1)

Table 5

Brain Stem Auditory Evoked PotentiaL
Mean +/- Standard Deviation of the Latencies

Acrylamide Group

	Week 0	Week 1	Week 2	Week 3
Latency I	1.78 (0.11)	**1.69 (0.13) [0.001] {0.001}**	1.78 (0.10)	1.81 (0.12)
Latency II	2.60 (0.11)	2.60 (0.09)	2.65 (0.12)	**2.75 (0.12) [0.0001] {0.0006}**
Latency III	3.28 (0.08)	**3.23 (0.12) [0.001] {0.001}**	**3.24 (0.09) [0.01]**	3.31 (0.16)
Latency IV	4.18 (0.14)	4.20 (0.22)	4.17 (0.16)	**4.30 (0.21) [0.01] {0.001}**
IPL I-IV	2.40 (0.13)	2.50 (0.30)	2.39 (0.15)	2.50 (0.20)

Latencies +/- standard deviation ( ) in msec.
Latencies for week n with p value are significantly different from week 0.
Latencies for week n with p value { } are significantly different from preceding week (n-1).

Table 6

Brain Stem Auditory Evoked PotentiaL
Mean +/- Standard Deviation of the Latencies

2,5 - Hexanedione Group

	Week 0	Week 1	Week 2	Week 3
Latency I	1.74 (0.10)	1.77 (0.18)	**1.81 (0.12) [p=0.0001]**	**1.85 (0.12) [p=0.0001]**
Latency II	2.55 (0.08)	**2.68 (0.17) [p=0.0001] {p=0.0001}**	**2.73 (0.13) [p=0.0001]**	**2.81 (0.18) [p=0.0001] {p=0.0002}**
Latency III	3.21 (0.08)	**3.38 (0.15) [p=0.0001] {p=0.0001}**	**3.41 (0.11) [p=0.0001)**	**3.55 (0.23) [p=0.0001] {p=0.0001}**
Latency IV	4.09 (0.15)	**4.38 (0.22) [p=0.0001] {p=0.0001}**	**4.49 (0.17) [p=0.0001] {p=0.0001}**	**4.61 (0.27) [p=0.0001] {p=0.0005}**
IPL I-IV	2.35 (0.13)	**2.61 (0.18) [p=0.0001] {p=0.0001}**	**2.68 (0.18) [p=.0001]**	**2.76 (0.24) [p=0.0001] {p=0.004}**

Latencies +/- standard deviation ( ) in msec.
Latencies for week n with p value are significantly different from week 0.
Latencies for week n with p value { } are significantly different from preceding week (n-1).

REFERENCES

1. Dyer, R.S., *The use of sensory evoked potentials in toxicology.* Fundam Appl Toxicol, 1985. **5**(1): p. 24-40.

2. Schulze, G.E. and Boysen, B.G., *A neurotoxicity screening battery for use in safety evaluation: effects of acrylamide and 3',3'-iminodipropionitrile.* Fundam Appl Toxicol, 1991. **16**(3): p. 602-15.

3. Spencer, P.S., Bischoff, M.C. and Schaumburg, H.H., *Neuropathological methods for the detection of neurotoxic disease,* in *Experimental and Clinical Neurotoxicology,* P.S. Spencer and H.H. Schaumburg, Editor. 1980, Williams and Wilkins: Baltimore. p. 743-757.

4. Broxup, B., Robinson, K., Losos, G. and Beyrouty, P., *Correlation between behavioral and pathological changes in the evaluation of neurotoxicity.* Toxicol Appl Pharmacol, 1989. **101**(3): p. 510-20.

5. Horobin, R.W., *Why use histochemistry for a better understanding of pharmacology and toxicology?,* in *Histochemical and Immunohistochemical Techniques,* P.H. Bach and J.R. Baker, Editor. 1991, Chapman and Hall: New York. p. 1-9.

6. Pryor, G.T., Uyeno, E.T., Tilson, H.A. and Mitchell, C.L., *Assessment of chemicals using a battery of neurobehavioral tests: a comparative study.* Neurobehav Toxicol Teratol, 1983. **5**(1): p. 91-117.

7. Vorhees, C.V., *Reliability, sensitivity and validity of behavioral indices of neurotoxicity.* Neurotox and Teratol, 1987. **9**: p. 445-464.

8. Mattsson, J.L., Albee, R.R. and Eisenbrandt, D.L., *Neurological approach to neurotoxicological evaluation in laboratory animals.* J Amer Col Toxicol, 1989. **8**(2): p. 271-286.

9. Moser, V.C., *Screening approaches to neurotoxicity: A functional observational battery.* J Amer Col Toxicol, 1989. **8**(1): p. 85-93.

10. Gerber, G.J. and O'Shaughnessy, D., *Comparison of the behavioral effects of neurotoxic and systemically toxic agents: how discriminatory are behavioral tests of neurotoxicity?* Neurobehav Toxicol Teratol, 1986. **8**(6): p. 703-10.

11. Albee, R.R., Mattsson, J.L., Yano, B.L. and Chang, L.W., *Neurobehavioral effects of dietary restriction in rats.* Neurotoxicol Teratol, 1987. **9**(3): p. 203-11.

12. Rebert, C.S., *Multisensory evoked potentials in experimental and applied neurotoxicology.* Nerobehav Toxicol Teratol, 1983. **5**: p. 659-671.

13. Mattsson, J.L., Eisenbrandt, D.L. and Albee, R.R., *Screening for neurotoxicity: Complementarily of functional and morphologic techniques.* Toxicol Pathol, 1990. **18**(1): p. 115-127.

14. Otto, D., Hudnell, K., Boyes, W., Janssen, R. and Dyer, R., *Electrophysiological measures of visual and auditory function as indices of neurotoxicity.* Toxicology, 1988. **49**(2-3): p. 205-18.

15. Dyer, R.S., *The interactions of behavior and neurophysiology,* in *Neurobehavioral Toxicology,* Z. Annau, Editor. 1986, p. 193-213.

16. Shaw, N.A., *The auditory evoked potential in the rat - A review.* Prog Neurobiol, 1988. **31**: p. 19-45.

17. Chiappa, K.H. and Ropper, A.H., *Evoked potentials in clinical medicine.* New Eng J Med, 1982. **306**(19): p. 1140-1150.

18. Sohmer, H. and Feinmesser, M., *Cochlear action potentials recorded from the external ear in man.* Ann. Otol.(St. Louis), 1967. **76**: p. 427-436.

19. Jewett, D.L., Romano, M.N. and Williston, J.S., *Human auditory evoked potentials: Possible brain stem components detected on the scalp.* Science, 1970. **167**: p. 1517-1518.

20. Otto, D.A., *The use of sensory evoked potentials in neurotoxicity testing of workers.* Sem Occ Med, 1986. **1**(3): p. 175-183.

21. Starr, A., *Sensory evoked potentials in clinical disorders of the nervous system.* Ann Rev Neurosci, 1978. **1**: p. 103-127.

22. Tait, C., Miller, J., Cycowicz, Y. and Sohmer, H., *Experimental analyses of the source of ABR wave II.* Arch Otorhinolaryngol, 1987. **244**(1): p. 26-9.

23. Plantz, R.G., Williston, J.S. and Jewett, D.L., *Spatio-temporal distribution of auditory evoked far field potentials in rat and cat.* Brain Res., 1974. **68**: p. 55-71.

24. Jewett, D.L. and Romano, M.N., *Neonatal development of auditory system potentials averaged from the scalp of rat and cat.* Brain Res, 1972. **36**: p. 101-115.

25. Wada, S.I. and Starr, A., *Generation of auditory brain stem responses (ABRs). II. Effects of surgical section of the trapezoid body on the ABR in guinea pigs and cat.* Electroenceph clin Neurophysiol, 1983. **56**: p. 340-351.

26. Funai, H. and Funasaka, S., *Experimental study on the effect of inferior colliculus lesions upon auditory brain stem response.* Audiology, 1983. **22**(1): p. 9-19.

27. Achor, L.J. and Starr, A., *Auditory brain stem responses in the cat. I. Intracranial ad extracranial recordings.* Electroenceph. clin. Neurophysiol., 1980. (48): p. 154-173.

28. Achor, L.J. and Starr, A., *Auditory brain stem responses in the cat. II. Effects of lesions.* Electroenceph. clin. Neurophysiol., 1980. **48**: p. 174-190.

29. Wada, S.I. and Starr, A., *Generation of auditory brain stem responses (ABRs). III. Effects of lesions of the superior olive, lateral lemniscus and inferior colliculus on the ABR in guinea pig.* Electroenceph. clin. Neurophysiol., 1983. **56**: p. 352-366.

30. Mirmiran, M., Brenner, E. and van-Gool, W.A., *Visual and auditory evoked potentials in different areas of rat cerebral cortex.* Neurosci Lett, 1986. **72**(3): p. 272-6.

31. Caird, D., Sontheimer, D. and Klinke, R., *Intra and extracranially recorded auditory evoked potentials in the cat. I. Source location and binaural interaction.* Electroenceph. clin. Neurophysiol., 1985. **61**: p. 50-60.

32. Moller, A.R. and Burgess, J., *Neuronal generators of the brainstem auditory evoked potentials (BAEPs) in the rhesus monkey.* Electroenceph. clin. Neurophysiol., 1986. **65**: p. 361-372.

33. Simpson, G.V. and Knight, R.T., *Multiple brain systems generating the rat auditory evoked potential. II. Dissociation of auditory cortex and non-lemniscal generator systems.* Brain Res, 1992. **602**(2): p. 251-63.

34. Simpson, G.V. and Knight, R.T., *Multiple brain systems generating the rat auditory evoked potential. I. Characterization of the auditory cortex response.* Brain Res, 1992. **602**(2): p. 240-50.

35. Moller, A.R., *On the origin of the compound action potentials (N1, N2) of the cochlea of the rat.* Exp Neurol, 1983. **80**(3): p. 633-44.

36. Bauch, C.D., Rose, D.E. and Harner, S.G., *Brainstem responses to tone pip and click stimuli.* Ear and Hearing, 1980. **1**(4): p. 181-184.

37. Allen, A.R. and Starr, A., *Auditory brain stem potentials in monkey (M. Mulatta) and man.* Electoenceph. clin. Neurophysiol., 1978. **45**: p. 53-63.

38. Church, M.W., Williams, H.L. and Holloway, J.A., *Brain-stem auditory evoked potentials in the rat: effects of gender, stimulus characteristics and ethanol sedation.* Electroencephalogr Clin Neurophysiol, 1984. **59**(4): p. 328-39.

39. Church, M.W. and Gritzke, R., *Effects of ketamine anesthesia on the rat brain-stem auditory evoked potential as a function of dose and stimulus intensity.* Electroencephalogr Clin Neurophysiol, 1987. **67**(6): p. 570-83.

40. Freeman, S., Sohmer, H. and Silver, S., *The effect of stimulus repetition rate on the diagnostic efficacy of the auditory nerve-brain-stem evoked response.* Electroencephalogr Clin Neurophysiol, 1991. **78**(4): p. 284-90.

41. Newton, E.H., Cooper, W., Jr. and Coleman, J.R., *Rate and frequency interactions in the auditory brainstem response of the adult rat.* Hear Res, 1992. **60**(1): p. 73-9.

42. Overbeck, G.W. and Church, M.W., *Effects of tone burst frequency and intensity on the auditory brainstem response (ABR) from albino and pigmented rats.* Hear Res, 1992. **59**(2): p. 129-37.

43. Nuwer, M.R., *Brainstem Auditory Monitoring and Related Techniques,* in *Evoked Potential Monitoring in the Operating Room.* 1986, Raven Press: New York. p. 136-171.

44. Shah, S.N., Bhargava, V.K. and McKean, C.M., *Maturational changes in early auditory evoked potentials and myelination of the inferior colliculus in rats.* Neuroscience, 1978. **3**: p. 561-563.

45. Fabiani, M., Sohmer, H., Tait, C., Gafni, M. and Kinarti, R., *A functional measure of brain activity: brain stem transmission time.* Electroenceph. clin. Neurophysiol., 1979. **47**: p. 483-491.

46. Pratt, J. and Sohmer, H., *Intensity and rate functions of cochlear and brainstem evoked responses to click stimuli in man.* Arch. Otorhinolaryngol., 1976. **6**: p. 85-92.

47. Huang, C.M. and Buchwald, J.S., *Factors that affect the amplitudes and latencies of the vertex short latency acoustic responses in the cat.* Electroenceph. clin. Neurophysiol., 1978. **44**: p. 179-186.

48. Rowe, M.J., *Normal variability of the brain stem auditory evoked response in young and old adult subjects.* Electroenceph. clin. Neurophysiol., 1978. **44**: p. 175-182.

49. Eggermont, J.J. and Don, M., *Analysis of the click-evoked brainstem potentials in humans using high pass noise masking. II Effect of click intensity.* J Acoust Soc Am, 1980. **68**: p. 1671-1675.

50. Stockard, J.E., Stockard, J.J., Westmoreland, B.F. and Corfits, J.L., *Brainstem auditory evoked responses: normal variation as a function of stimulus and subject characteristics.* Arch. Neurol. (Chic.), 1979. **36**: p. 823-831.

51. Coats, A.C., *Human auditory nerve action potentials and brain evoked responses: latency intensity functions in detection of cochlear and retrocochlear abnormality.* Arch. Otolaryng., 1978. **104**: p. 709-717.

52. Picton, T.W., Woods, D.L., Baribeau-Braun, J. and Healey, T.M.G., *Evoked potential audiometry.* J. Otolaryng, 1977. **6**: p. 90-119.

53. Angelo, R. and Moller, A.R., *Response from the inferior colliculus in the rat to tone bursts and amplitude modulated continuous tones.* Audiology, 1990. **29**: p. 336-346.

54. Thornton, A.R.D. and Coleman, M.J., *The adaptation of cochlear and brainstem auditory evoked potentials in humans.* Electroenceph. clin. Neurophysiol., 1975. **39**: p. 399-406.

55. Prijs, V.F., *On peripheral auditory adaptation. II. Comparison of electrically and acoustically evoked action potentials in the guinea pig.* Acustica, 1980. **45**: p. 1-13.

56. Nuwer, M.R., *Basic Electrophysiology: evoked potentials and signal processing,* in *Evoked Potential Monitoring in the Operating Room.* 1986, Raven Press: New York. p. 5-48.

57. Stockard, J.J., Stockard, J.E. and Sharbrough, F.W., *Brainstem auditory evoked potentials in neurology: Methodology, interpretation, clinical application,* in *Electrodiagnosis in Clinical Neurology,* M.J. Aminoff, Editor. 1980, Churchill Livingston: New York. p. 370-413.

58. Shaw, N.A., *The brainstem auditory evoked potential: effects of high frequency filtering.* Electromyogr. clin. Neurophysiol., 1990. **30**: p. 159-163.

59. Janssen, R., Benignus, V.A., Grimes, L.M. and Dyer, R.S., *Unrecognized errors due to analog filtering of the brain-stem auditory evoked response.* Electroencephalogr Clin Neurophysiol, 1986. **65**(3): p. 203-11.

60. Shaw, N.A., *Effects of low pass filtering on the brainstem auditory evoked potential in the rat.* Exp Brain Res, 1987. **65**(3): p. 686-90.

61. Gordon, C.J., Mohler, F.S., Watkinson, W.P. and Rezvani, A.H., *Temperature regulation in laboratory mammals following acute toxic insult.* Toxicology, 1988. **53**: p. 161-178.

62. Williston, J.S. and Jewett, D.L., *The Q10 of auditory brain stem responses in rats under hypothermia.* Audiology, 1982. **21**(6): p. 457-65.

63. Janssen, R., Hetzler, B.E., Creason, J.P. and Dyer, R.S., *Differential impact of hypothermia and pentobarbital on brain-stem auditory evoked responses.* Electroencephalogr Clin Neurophysiol, 1991. **80**(5): p. 412-21.

64. Henry, K.R., *Differential changes of auditory nerve and brain stem short latency evoked potentials in the laboratory mouse.* Electroenceph. clin. Neurophysiol., 1979. **46**: p. 452-459.

65. Stockard, J.J., Sharbrough, F.W. and Tinker, J.A., *Effects of hypothermia on the human brainstem auditory response.* Ann. Neurol., 1978. **3**: p. 368-370.

66. Kaga, K., Takiguchi, T., Myokai, K. and Shiode, A., *Effects of deep hypothermia and circulatory arrest on the auditory brain stem responses.* Arch. Otolaryngol., 1979. **225**: p. 199-205.

67. Samra, S.K. and Lilly, D.J., *Effect of hypothermia on human brain-stem auditory evoked potentials.* Anesthesiology, 1983. **59**: p. A170.

68. Gordon, C.J., Fogelson, L. and Highfill, J.W., *Hypothermia and hypometabolism: sensitive indices of whole-body toxicity following exposure to metallic salts in the mouse.* J. Toxicol. Environ. Health, 1990. **29**: p. 185-200.

69. Chen, T.J. and Chen, S.S., *Brain stem auditory evoked potentials in different strains of rodents.* Acta. Physiol. Scand., 1990. **138**: p. 529-538.

70. Welch, T.M., Church, M.W. and Shucard, D.W., *A method for chronically recording brain stem and cortical auditory evoked potentials from unanesthetized mice.* Electroenceph. clin. Neurophysiol., 1985. **56**: p. 352-366.

71. Booth, N.H., *Intravenous and other parenteral anesthetics,* in *Veterinary Pharmacology and Therapeutics,* N.H. Booth and L.E. McDonald, Editor. 1982, The Iowa State University Press: Ames. p. 241-249.

72. Marshall, B.E. and Wollman, H., *General anesthetics,* in *The Pharmacological Basis of Therapeutics,* A.G. Gilman, L.S. Goodman, and A. Gilman, Editor. 1980, MacMillan: New York. p. 267-299.

73. Booth, N.H., *Nonnarcotic Analgesics,* in *Veterinary Pharmacology and Therapeutics,* N.A. Booth and L.E. McDonald, Editor. 1982, The Iowa State University Press: Ames. p. 297-320.

74. Goss-Sampson, M.A. and Kriss, A., *Effects of pentobarbital and ketamine-xylazine anaesthesia on somatosensory, brainstem auditory and peripheral sensory-motor responses in the rat.* Lab Anim, 1991. **25**(4): p. 360-6.

75. Bobbin, R.P., J.G., M. and Lemoine, R.L., *Effects of pentobarbital and ketamine on brain stem auditory potentials.* Arch. Otolaryngol., 1979. **105**: p. 467-470.

76. Cohen, M.S. and Britt, R.H., *Effects of sodium pentobarbital, ketamine, halothane and chloralose on brainstem auditory evoked responses.* Anesthesia and Analgesia, 1982. **61**: p. 338-343.

77. Dawson, G.D., *Investigations in a patient subject to myoclonic seizures after sensory stimulation.* J Neurol Neurosurg and Psych, 1947. **10**: p. 141-162.

78. Eisen, A., *The somatosensory evoked potential.* Can J Neurol Sci, 1982. **9**(2): p. 65-77.

79. Nuwer, M.R., *Other somatosensory techniques,* in *Evoked Potential Monitoring in the Operating Room.* 1986, Raven Press: New York. p. 102-135.

80. Mattsson, J.L. and Albee, R.R., *Sensory evoked potentials in neurotoxicology.* Neurotox and Teratol, 1988. **10**: p. 435-443.

81. Abdulla, F.A., Aneja, I.S. and Bhargava, K.P., *Effect of morphine on far-field somatosensory evoked potentials in the rat.* Neuropharmacology, 1989. **28**(1): p. 69-73.

82. Wiederholt, W.C. and Iragui-Madoz, V.J., *Far field somatosensory potentials in the rat.* Electroenceph clin Neurophysiol, 1977. **42**: p. 456-465.

83. Sen, C.N. and Moller, A.R., *Comparison of somatosensory evoked potentials recorded from the scalp and dorsal column nuclei to upper and lower limb stimulation in the rat.* Electroencephalogr Clin Neurophysiol, 1991. **80**(5): p. 378-83.

84. Giblin, D.R., *Scalp-recorded somatosensory evoked potentials.,* in *Electrodiagnosis in Clinical Neurology,* M.J. Aminoff, Editor. 1980, Churchill Livingston: New York. p. 414-450.

85. Sohmer, H., Gold, S., Cahani, M. and Attias, J., *Effects of hypothermia on auditory brain stem and somatosensory evoked responses. A model of synaptic and axonal lesion.* Electroenceph clin Neurophysiol, 1989. **74**: p. 50-57.

86. Tilson, H.A., *The neurotoxicity of acrylamide: an overview.* Neurobehav Toxicol Teratol, 1981. **3**(4): p. 445-61.

87. Le Quesne, P., *Acrylamide,* in *Experimental and Clinical Neurotoxicology*. p. 309-325.

88. O'Shaughnessy, D.J. and Losos, G.J., *Comparison of central and peripheral nervous system lesions caused by high-dose short-term and low-dose subchronic acrylamide treatment in rats.* Toxicol Pathol, 1986. **14**(4): p. 389-94.

89. Fullerton, P. and Barnes, J., *Peripheral neuropathy in rats produced by acrylamide.* Brit. J. Ind. Med., 1966. **23**: p. 210-221.

90. Prineas, J., *The pathogenesis of dying back polyneuropathies. II. An ultrastructural study of experimental acrylamide intoxication in the cat.* J. Neuropathol. Exp. Neurol., 1969. **28**: p. 598-621.

91. Schaumburg, H.H., Wisniewski, H.M. and Spencer, P.S., *Ultrastructural studies of the dying back process. 1. Peripheral nerve terminal and axon degeneration in systemic acrylamide intoxication.* J. Neuropathol. Exp. Neurol., 1974. **33**: p. 260-284.

92. Harry, G.J., Goodrum, J.F., Bouldin, T.W., Toews, A.D. and Morell, P., *Acrylamide induced increases in deposition of axonally transported glycoproteins in rat sciatic nerve.* J. Neurochem., 1989. **52**: p. 1240-1247.

93. Gold, B.G., Griffin, J.W. and Price, D.L., *Slow axonal transport in acrylamide neuropathy: different abnormalities produced by single-dose and continuous administration.* J Neurosci, 1985. **5**(7): p. 1755-68.

94. Bisby, M.A. and Redshaw, J.D., *Acrylamide neuropathy: changes in the composition of proteins of fast axonal transport resemble those observed in regenerating axons.* J Neurochem, 1987. **48**(3): p. 924-8.

95. Sickles, D.W., *Toxic neurofilamentous axonopathies and fast anterograde axonal transport. III. Recovery from single injections and multiple dosing effects of acrylamide and 2,5-hexanedione.* Toxicol Appl Pharmacol, 1991. **108**(3): p. 390-6.

96. Harry, G.J., Morell, P. and Bouldin, T.W., *Acrylamide exposure preferentially impairs axonal transport of glycoproteins in myelinated axons.* J Neurosci Res, 1992. **31**(3): p. 554-60.

97. Harry, G.J., *Acrylamide-induced alterations in axonal transport. Biochemical and autoradiographic studies.* Mol Neurobiol, 1992. **6**(2-3): p. 203-16.

98. Shell, L., Rozum, M., Jortner, B.S. and Ehrich, M., *Neurotoxicity of acrylamide and 2,5-hexanedione in rats evaluated using a functional observational battery and pathological examination.* Neurotoxicol Teratol, 1992. **14**(4): p. 273-83.

99. Sterman, A.B. and Sheppard, R.C., *A correlative neurobehavioral-morphological model of acrylamide neuropathy.* Neurobehav Toxicol Teratol, 1983. **5**(1): p. 151-9.

100. Tilson, H.A. and Cabe, P.A., *Acrylamide neurotoxicity in rats: A correlated neurobehavioral and pathological study.* Neurotox, 1979. **1**('): p. 89-104.

101. Kupperman, A.S., *Effects of acrylamide on the central nervous system of the cat.* J. Pharmacol. exp. The., 1958. **123**: p. 180-192.

102. Goldstein, B.D. and Lowndes, H.E., *Spinal cord defect in the peripheral neuropathy resulting from acrylamide.* Neurotoxicol., 1979. **1**: p. 75-87.

103. Goldstein, B.D. and Lowndes, H.E., *Primary afferent terminal defect in cats with acrylamide neuropathy.* Soc. Neurosci. Abstr., 1979. **5**: p. 723.

104. Boyes, W.K. and Cooper, G.P., *Acrylamide neurotoxicity: effects on far-field somatosensory evoked potentials in rats.* Neurobehav Toxicol Teratol, 1981. **3**(4): p. 487-90.

105. Schaumburg, H.H., Spencer, P.S. and Arezzo, J.C., *Studies of the primate somatosensory system in experimental acrylamide intoxication.* Neurotoxicology, 1985. **6**(4): p. 3-6.

106. Ghetti, B., Wisniewski, H.M., Cook, R.D. and Schaumburg, H.H., *Changes in the CNS after acute and chronic acrylamide intoxication.* Am. J. Pathol., 1973. **70**: p. 78A.

107. Tandrup, T. and Braendgaard, H., *The number and mean volume of neurons in the cerebral cortex of rats intoxicated with acrylamide.* Neuropathol Appl Neurobiol, 1992. **18**(3): p. 250-8.

108. Spencer, P.S., Couri, D. and Schaumburg, H.H., *n-Hexane and Methyl n-Butyl Ketone,* in *Experimental and Clinical Neurotoxicology,* P.S. Spencer and H.H. Schaumburg, Editor. 1980, Williams and Wilkins: Baltimore. p. 456-475.

109. Anderson, R.J. and Dunham, C.B., *Electrophysiologic deficits in peripheral nerve as a discriminator of early hexacarbon neurotoxicity.* J Toxicol Environ Health, 1984. **13**(4-6): p. 835-43.

110. Kravasage, W.J., O'Donoghue, J.L., Di Vincenzo, G.D. and Terhaar, C.J., *The relative neurotoxicity of methyl-n-butyl ketone, n-butyl ketone, n-hexane and their metabolites.* Toxicol. Appl. Pharmacol., 1980. **52**: p. 433-441.

111. O'Donoghue, J.L., *Alkanes, Alcohols, Ketones and Ethylene Oxide,* in *Neurotoxicity of Industrial and Commercial Chemicals,* J.L. O'Donoghue, Editor. 1985, CRC Press: Cleveland, Ohio. p. 62-89.

112. Griffin, J.W., Price, D.L. and Spencer, P.S., *Fast axonal transport through giant axonal swelling in hexacarbon neuropathy.* J. Neuropathol. Exp, Neurol., 1977. **36**: p. 603.

113. Carden, M.J., Lee, V.M. and Schlaepfer, W.W., *2,5-Hexanedione neuropathy is associated with the covalent crosslinking of neurofilament proteins.* Neurochem Pathol, 1986. **5**(1): p. 25-35.

114. Anthony, D.C., Giangaspero, F. and Graham, D.G., *The spatio-temporal pattern of the axonopathy associated with the neurotoxicity of 3,4-dimethyl-2,5-hexanedione in the rat.* J Neuropathol Exp Neurol, 1983. **42**(5): p. 548-60.

115. Graham, D.G., Anthony, D.C., Szakal-Quin, G., Gottfried, M.R. and Boekelheide, K., *Covalent crosslinking of neurofilaments in the pathogenesis of n-hexane neuropathy.* Neurotoxicology, 1985. **6**(4): p. 55-63.

116. Griffin, J.W., Anthony, D.C., Fahnestock, K.E., Hoffman, P.N. and Graham, D.G., *3,4-Dimethyl-2,5-hexanedione impairs the axonal transport of neurofilament proteins.* J Neurosci, 1984. **4**(6): p. 1516-26.

117. Sahenk, Z. and Mendell, J.R., *Acrylamide and 2,5-hexanedione neuropathies: abnormal bidirectional transport rate in distal axons.* Brain Res, 1981. **219**(2): p. 397-405.

118. Rebert, C.S., Houghton, R.A., Howd, R.A. and Pryor, G.T., *Effects of hexane on the brainstem auditory response and caudal nerve action potential.* Neurobehav. Toxicol. Teratol., 1982. **4**: p. 79-85.

119. Mitolo-Chieppa, D. and Carratu, M.R., *Electrophysiological investigation of 2,5-hexanedione neurotoxicity in rats.* Toxicol Appl Pharmacol, 1986. **84**(2): p. 250-4.

120. Rebert, C.S. and Sorenson, S.S., *Concentration-related effects of hexane on evoked responses from brain and peripheral nerve of the rat.* Neurobehav Toxicol Teratol, 1983. **5**(1): p. 69-76.

121. Hirata, M., *Reduced conduction function in central nervous system by 2,5-hexanedione.* Neurotoxicol Teratol, 1990. **12**(6): p. 623-6.

122. Hirose, G., Chujo, T., Kataoka, S., Kawada, J. and Yoshioka, A., *Acute effects of anticonvulsants on brain-stem auditory evoked potentials in rats.* Electroencephalogr Clin Neurophysiol, 1990. **75**(6): p. 543-7.

123. Marshall, N.K. and Donchin, E., *Circadian variation in the latency of brainstem responses and its relation to body temperature.* Science, 1981. **212**: p. 356-358.

124. Oakes, M., *Statistical Inference.* 1990, Chestnut Hill, MA: Epidemiology Resources Inc. 3-14.

125. Gold, S., Cahani, M., Sohmer, H., Horowitz, M. and Shahar, A., *Effects of body temperature elevation on auditory nerve-brain-stem evoked responses and EEGs in rats.* Electroencephalogr Clin Neurophysiol, 1985. **60**(2): p. 146-53.

126. Sterman, A.B. and Sheppard, R.C., *A neurobehavioral model of 2,5-hexanedione-induced neuropathy.* Neurobehav Toxicol Teratol, 1982. **4**(5): p. 567-72.

127. Huang, C., *A comparative study of the brain stem auditory response in mammals.* Brain Res., 1980. **184**: p. 215-219.

VITA

Todd Leah Towell was born to Jim and Gretchen Howerton on December 1, 1958 in Norfolk, Virginia. Todd graduated from Goucher College with a BA in biology in 1980. Todd received her DVM from the Virginia-Maryland Regional College of Veterinary Medicine in 1990. As a senior veterinary student Todd was inducted into the National Honor Society of Veterinary Medicine, Phi Zeta.

Todd completed a Small Animal Rotating Internship at North Carolina State University College of Veterinary Medicine in 1991. She was voted Most Outstanding Intern 1990-1991. Todd started her combined Master's program and Residency in small animal medicine in the Departments of Veterinary Medical Sciences and Small Animal Clinical Sciences at the Virginia Maryland Regional College of Veterinary Medicine at Virginia Polytechnic Institute and State University under the directions of Drs. Michael Leib and Linda Shell in 1991. Todd received the Pauline Willson-Gunn scholarship in 1993 and 1994. Todd and her long time love and companion, Tim Towell, were married during the second year of her residency.