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COMPUTER MODELLING[1] OF NEURO-OTOXICITY AND AUDITORY PROCESSING DISORDER IN THE RAT AFTER CHRONIC LEAD INTOXICATION

By

SILAS BREWER SMITH

Bachelor of Arts, Communicative Sciences & Disorders, University of Montana, Missoula, MT, 2014
Master of Science, Interdisciplinary Studies, University of Montana, Missoula, MT, 2016

Dissertation

Presented in partial fulfillment of the requirements
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Missoula, MT

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Approved by:

Scott Whittenburg, Dean of The Graduate School
Graduate School

Al Yonovitz, Ph.D., Professor, Committee Chair

School of Speech, Language, Hearing & Occupational Sciences
College of Health Professions & Biomedical Sciences

Nancy Dold, Au.D., Adjunct Assistant Professor, Committee Member
School of Speech, Language, Hearing & Occupational Sciences
College of Health Professions & Biomedical Sciences

Allen Szalda-Petree, Ph.D.,Professor
Department of Psychology

Daniel Denis, Ph.D., Professor
Department of Psychology

Catherine Off, Ph.D., Professor
School of Speech, Language, Hearing & Occupational Sciences
College of Health Professions & Biomedical Sciences

[1]At the present time the world is experiencing an unprecedented pandemic of the corona virus. The disease has been labelled Covid-19. Universities across the United States have locked doors for the remainder of the Spring Semester, 2020. All classes are continuing through distance education. At the University of Montana, there has been a moratorium on beginning any new human or animal research. This study is unique in that it uses a software model (Simulink, Mathworks) in lieu of actual rat subjects.

Declaration

I hereby declare that the work contained in this report has never been submitted for a degree in any other university. To the best of my knowledge, this report contains no material previously published or written by another except where due reference is made within the report itself.

I further declare that the ethical procedures and principles determined by the University of Montana's document on human research and experimentation have been adhered to in the preparation of this report.

Signed Date: April 1, 2020

Acknowledgement

I would first like to thank Al Yonovitz for inspiring me to do research. The mentorship of Dr. Yonovitz has been invaluable. He has demonstrated through countless hours of effort, the selflessness, determination and passion of pursuit that I hope to achieve for myself, and pass on to others in the interest of performing research, and ultimately helping others.

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Silas Smith, Ph.D, Spring Semester, 2020 Speech, Language, Hearing & Occupational Sciences

Computer Modelling of Neuro-Otoxicity and Auditory Processing Disorder in the Rat After Chronic Lead Intoxication

Chairperson: Al Yonovitz, Ph.D.

Committee Member: Allen Szalda-Petree, Ph.D.

Committee Member: Nancy Dold, Au.D.

Committee Member: Daniel Denis, Ph.D.

Committee Member: Catherine Off, Ph.D.

Objective

Past research documents the ototoxicity of inorganic lead (Pb), which in children may include associated auditory processing and learning disabilities. The present investigation aims to explore in computer modelled rats, as a laboratory animal model, the persistent effects of Pb-contaminated drinking water on measures. i.e., wave latency and amplitude, of auditory brainstem function and specifically the response to a known evoked-potential assessment of Backward Masking (BM) as a marker of Auditory Processing Disorder (APD). BM refers to the disruption of an animal's response to a stimulus when succeeded by a later stimulus temporally.

Methods and Study Design

In order to assess the neuro-ototoxic effects and changes in auditory threshold induced from Pb, the early auditory brainstem response (ABR) is typically obtained in anesthetized rats. Unlike the ABR, the middle and late evoked auditory response is modulated by the state of the organism and is not available in anesthetized subjects. It is the middle and late evoked auditory responses that would elucidate a BM effect.

For the software modelling male and female Sprague-Dawley rats will be randomized on the basis of body weight either to 0.0% (Control), 0.2% Pb acetate drinking-water exposures based on findings of an initial Pb dose range-finding trial. All response measures will be modelled with Simulink (MATLAB, Mathworks) and also with a hardware body worn unit. Assessments of ABR and middle-late responses (MID-LATE) will be obtained after 30 days of simulated chronic Pb exposure.

Apparatus

A specially built apparatus has been designed to allow novel methodologies that allow for simultaneous measurement of early, middle and late Evoked Potentials (EP's). The EP's will be measured in active, un-sedated, un-restrained virtual rats. Chronically implanted dural electrodes will be used to obtain data. To accomplish this active measurement, data will be transferred wirelessly from a portable unit to a computer. Specially designed stimuli will yield a clinically applicable method to test for APD's in children. Currently, there are only subjective perceptual tests that are prone to significant error.

Table of Contents **Page**

LIST OF FIGURES

List of Figures

Page

Introduction and Historical Overview of Backward Masking

Auditory Processing Disorders (APDs) affect a diverse range of people. These types of disorders impair auditory function, despite the outer, middle and inner ear maintaining proper function and health (Griffiths, 2002; Howell, Rosen, Hannigan & Rustin, 2000; Musiek & Chemak, 2013). APD is not necessarily related to auditory thresholds. When people with APD have difficulty discriminating sounds in connected speech, it may be due in part, to an effect called Backward Masking (BM) (Marler, Champlin & Gillam, 2002). Masking occurs when one stimulus inhibits another, which can lead to a variety of additional impairments. The neural locus of APDs is not agreed upon, including the specific conditions which cause BM. A better understanding of these processes would lead to a greater ability to provide an intervention and therapy for APD.

The ototoxic effects of Lead (Pb) are well documented (Lurie, Brooks & Gray 2006; Moffitt, Yonovitz & Smolensky, 2018; Zhang, et al., 2019). These effects include auditory processing difficulty and learning disabilities. The current experiment aims to explore in rats, the effects of lead dosage through Pb-contaminated drinking water on the waveform morphology of EEG waves indicative of auditory neurological function. Specifically, the response to a known evoked-potential assessment of Backward Masking (BM) as a marker of Auditory Processing Disorder (APD). BM refers to the disruption of a stimulus when succeeded by a later stimulus temporally.

The central focus of this research is to observe the waveform morphological changes of the auditory evoked potential during a backward masking procedure. It may be possible to objectively measure the electrophysiological Backward Masking (BM) effect in an animal

models using auditory evoked potentials (Lurie et al., 2006). The design of study will allow measurement of the ABR, middle (10-100 msec), and late (100-1000 msec) auditory evoked potentials. This process will allow us to observe the differential electrophysiological responses of evoked potentials during the BM task.

Backward masking refers to the process of raising the sensory threshold for a target stimulus by means of an interfering signal *after* the target stimulus. BM is not unique to the auditory system. Masking effects are similarly exhibited in other perceptual senses as well (Raab, 1963). In simpler and shorter terms, BM is defined by later stimuli affecting earlier stimuli. Masking effects have been documented as early as 1902, when the discovery of the Broca-Sulzer phenomenon established that the effect of length of viewing exposure was related to the apparent luminescence of an object (Raab, 1963). BM has demonstrated high significance for the study of Auditory Processing Disorders or APD's, including but not limited to several learning impairments (Musiek, 2013; Wright et al., 1997). For example, children who stutter have a significantly higher threshold for BM, and the higher masking thresholds correlate with rates of dysfluency (Howell, Rosen & Hannigan, 2000). There is no relation between the impairment of auditory feedback and the structural integrity of the auditory system; therefore, it is believed that the stuttering impairment occurs due to a dysfunction of central auditory processing in the brain (Howell et al, 2000). It has also been shown that children with dyslexia were similarly impaired, and have significantly higher BM thresholds than matched control groups (Rosen & Manganari, 2001).

Auditory Evoked Potentials

Auditory Evoked Potentials (AEP's) are an electrical recording of brain activity. AEP's are recorded from the bioelectric brain potentials. This method records the firing of a population of neurons and then amplified due to the small voltage patterns (neurons firing together to result in a wave). The AEP specifically records the electrical (neuronal) activity in response to a sonic stimulus. These synchronous firing of neurons can be seen along a time/amplitude continuum.

The AEP is comprised of 3 different epochs: the early latency (first 10 msec), middle latency (10-100 msec) and late latency (100-100 msec). The early latency potentials (also known as ABR's) are responses from the auditory nerve and lower-brain structures. The middle latency responses derive from the thalamus and cortex. The late latency responses are cortical - and more susceptible to factors such as consciousness, stimulus type, and recording site/technique.

An example of an early auditory evoked potential – Auditory Brainstem Response (ABR 0-10 msec.) is shown in Figure 1. Each of the peaks in this waveform correspond to specific loci within the brainstem.

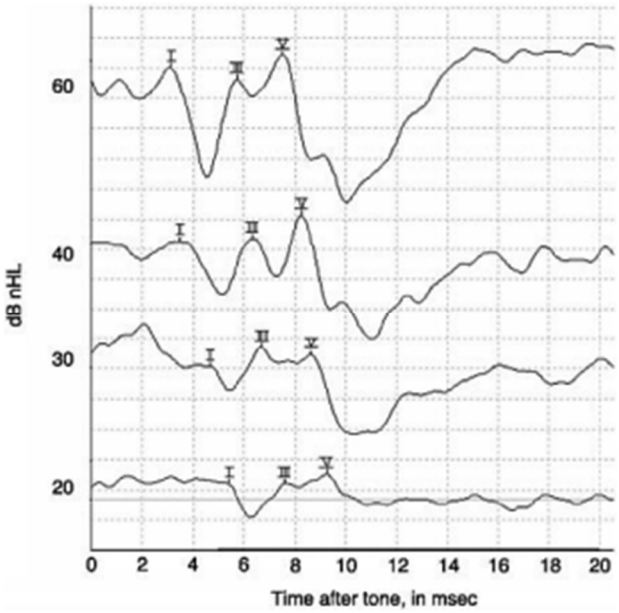


Figure 1 (Above): Early Auditory Brainstem Response (0-10 msec) (Dobie, 2004, p. 97).

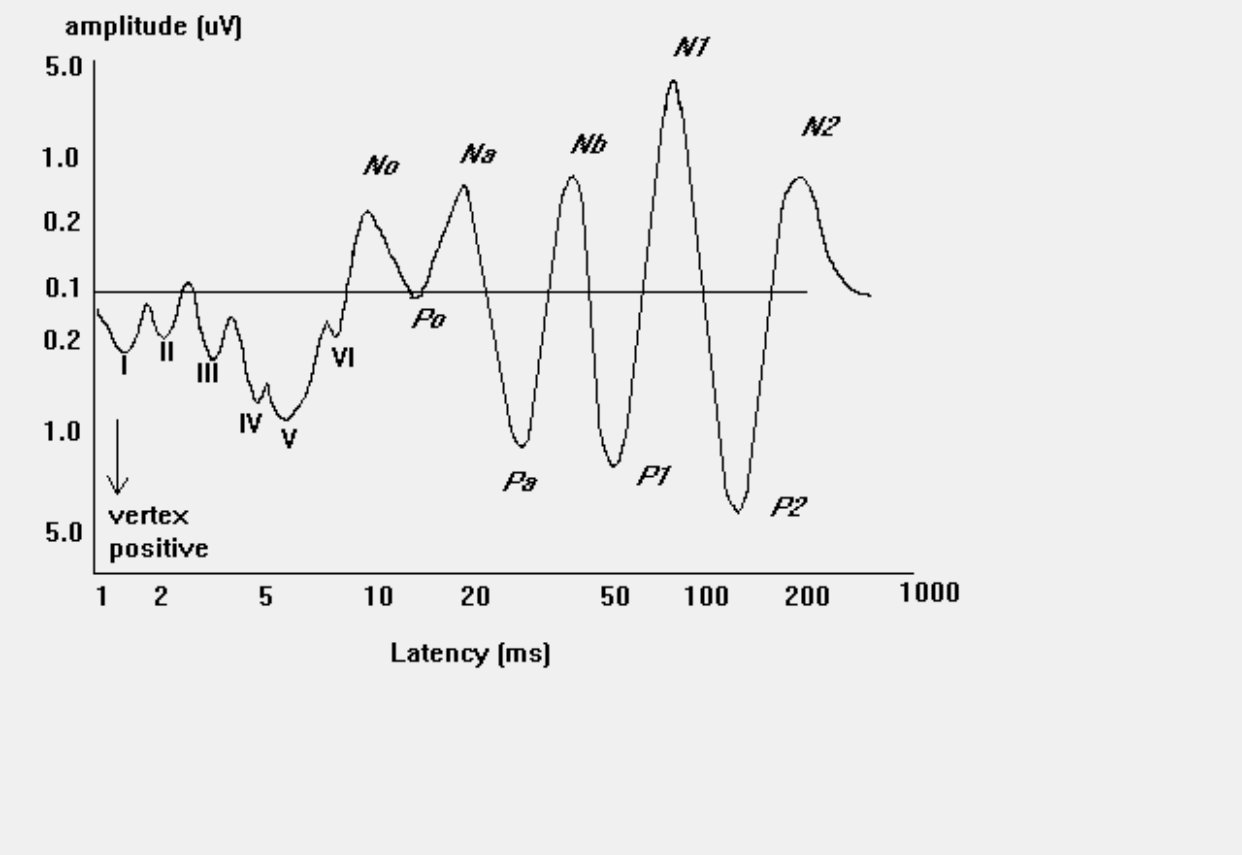


Figure 2 (Above): Early, middle and late auditory evoked potentials, as shown by (Barlow, 1982, p. 124).

It has been hypothesized, and supported by a body of evidence that the BM event disrupts temporal processing at the level of the brainstem (Wrightet al., 1997; Tahaei, Ashayeri, Pourbakht, Kamali & Jahanshahi, 2014). This temporal processing ability may be "mapped" through an Electroencephalographic (EEG) analysis of auditory evoked potentials, measuring brainstem, midbrain and cortical electrophysiological functioning during an auditory task (Natanen, Kujala & Winkler, 2011; Tahaei, Hassan, Akram, Mohammad & Marian, 2014). An example of an early auditory evoked potential – Auditory Brainstem Response (ABR 0-10 msec.) is shown in Figure 1. Each of the peaks in this waveform correspond to specific loci

within the brainstem. An example of an auditory Middle Latency Response (MLR 10-60 msec) is shown in Figure 4. The response origins have been found to be in the midbrain. Figure 5 is an example of early, middle and late auditory evoked potentials.

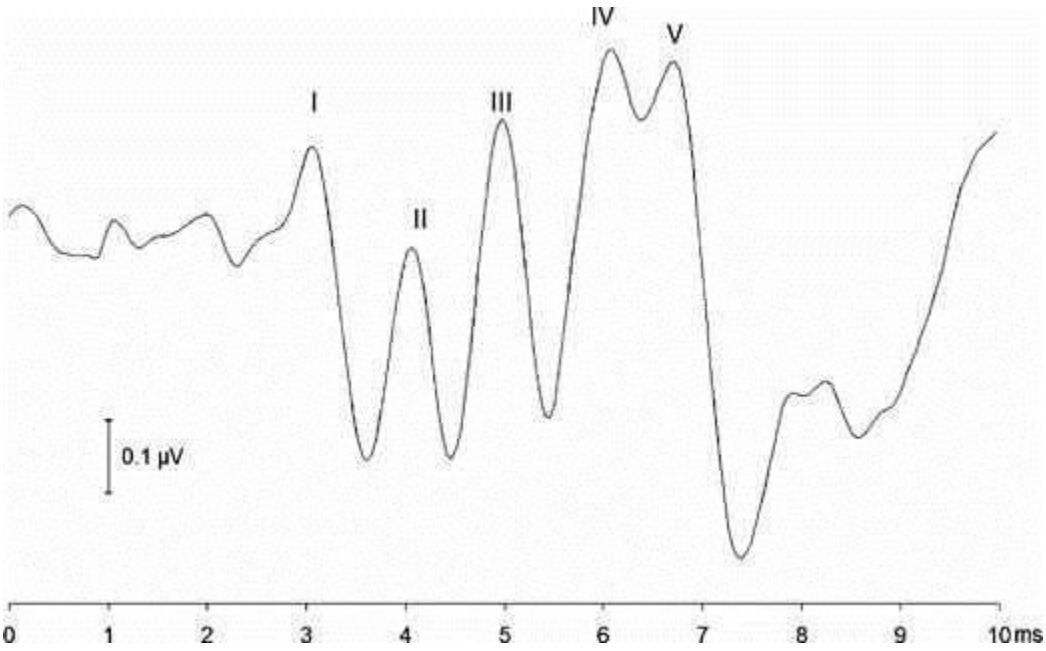
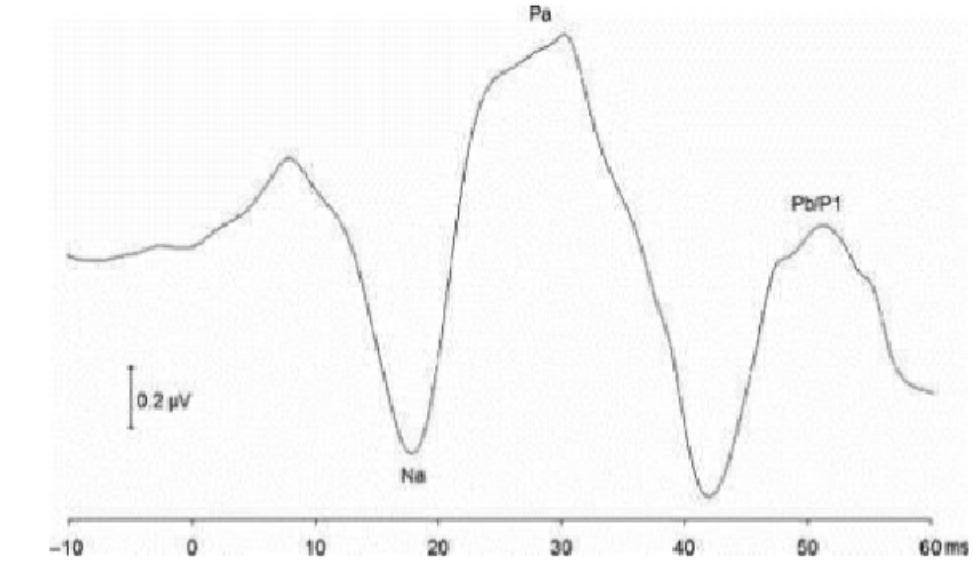


Figure 3 (Above): Early Auditory Evoked Potential (0-10 msec) (Kraus and McGee, 1992).

Figure 4 (Below): Middle Auditory Evoked Potential (Kraus and McGee, 1994)



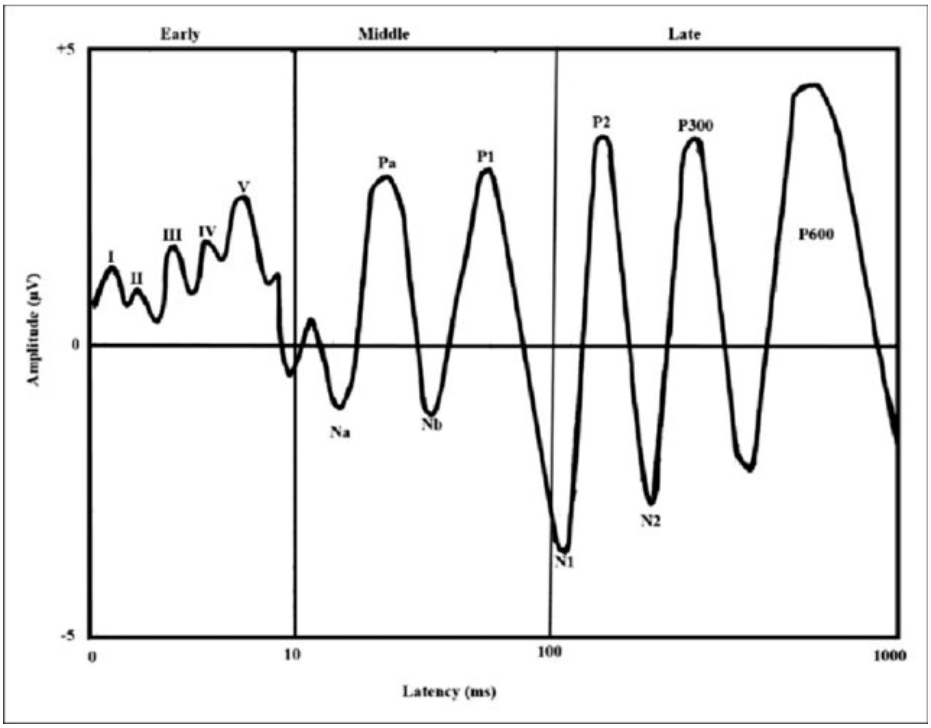


Figure 5 (Above): Early, middle and late auditory evoked potentials, as shown by (Washnik, et al., 2019).

A study of the latencies and amplitudes of the waveforms in the entire evoked potential allow us to determine what structure or structures in the brain are responsible for the BM effect (Paulraj, 2015). The specific loci within the auditory pathway are shown in Figure 6. The repetition rate and the technical issues related simultaneous acquisition of early, middle and late evoked potentials have been solved by Johnson and Yonovitz (2008). The ability to acquire the early, middle, and late components requires a fast sample rate, and a slow repetition rate. While other studies have speculated as to where specifically in the brain where the BM effect occurs, this study will take an expanded approach, allowing in totality, the determination of the neurological pathways affected.

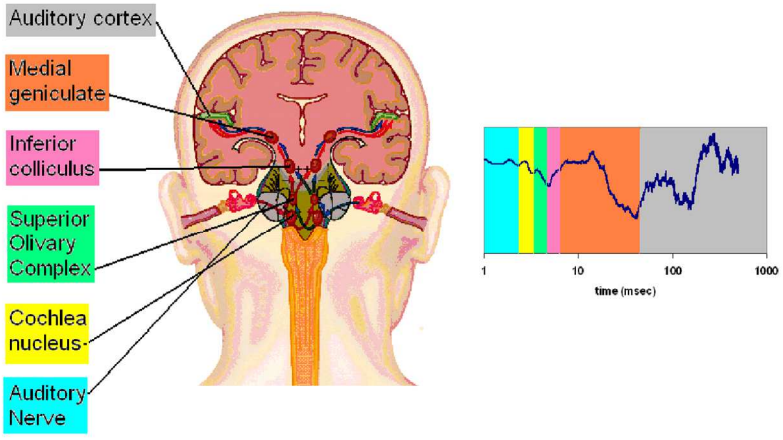


Figure 6: The auditory pathway and the neural generators for the specific waveforms. Adapted from (Kalat, 2007, p. 200)

Auditory Processing Disorder

Sounds are aurally received as more than one individual component or frequency, at least in the majority of real-world situations. The first reporting of human ability to hear multiple

sounds at once was reported in 1843 (Colman, 2008). The first report of a disorder in hearing or perceiving more than one noise at once did not occur until much later. Miller (1947), reported that there are inconsistencies in the ability to respond to multiple frequencies at once. Miller reported three different aspects to the masking effect: relative intensity of both masker and tonal noise, frequency of stimuli, and temporal separation of stimuli. There have been succeeding research endeavors that have cemented these findings as well (Samoilova 1956, Howell et al., 2000). Much of the early research regarding masking and indeed the interference of speech in general, was driven largely by two primary factors: The invention and progression of the telephone, and World War II. These unique circumstances necessitated the understanding and circumvention of the disruption and interference of sound. Miller reported that the greatest interference occurs on a constant, pure-tone signal ranging from 1000-4000 Hz. Miller reviewed a host of different masking noises to reveal which paradigms yielded the most significant masking effects. This report assessed speech noises, pure tones, complex tones and even music, toting that (p. 112) "since much of the popular dance music of the day is (to some people) noisy and annoying, the possibility that it interferes seriously with speech was worth investigating" (Miller, 1947). It was found, however, that music was rather unobtrusive in a masking paradigm unless multiple sources of music were played simultaneously. Miller reported that low frequency masking noises were able to mask the full spectrum of audition, while high-frequency masking noises only interfered with a partial domain of audition. It was stated that this disparity is due to high-frequency noises being weaker in energy, and thus easier to produce, allowing also for easier masking. Miller's early work in auditory masking was an important foundation for further endeavors in the field.

The first acknowledgement of what is now known as Auditory Processing Disorder (APD) was published in 1954, in a book simply titled: *Auditory Disorders in Children*, by Helmer Myklebust. Myklebust made a simple, yet novel discovery: that auditory deficits can, and do occur in individuals who present normal audiograms. In simpler terms, those who can hear *single* tones at normal thresholds sometimes fail to hear *multiple* tones at a normal level. This simple discovery led to a great deal of research, and frustration which both continue to this day. In order to understand the foundations and true consequences of these disorders, a basic understanding of auditory functioning must be known (Ahmmed, 2014 Musiek & Chermak, 2007).

The American Speech-Language and Hearing Association (ASHA) considers in the very broadest sense, that Auditory Processing Disorder (APD) refers to how the central nervous system (CNS) uses auditory information. “To avoid confusing APD with other disorders that can affect a person's ability to attend, understand, and remember, it is important to emphasize that APD is an auditory deficit that is not the result of other higher-order cognitive, language, or related disorder” (Bellis, n.d.).

Although the etiology is mostly unknown, APD affects a numerous and diverse population of people across the world (Musiek, 2013). APD is caused by a disruption in auditory processing that occurs in the auditory pathway post-ceding the cochlear response and is generally believed to occur before semantic processing (Griffiths, 2002). This manifests in an inability to properly perceive auditory input when competing stimuli are present. This disruption generally presents in noisy acoustic environments such as restaurants and classrooms. Although it is hopeful that a true causality to central APD may be defined, it must be admitted that the disorder likely results from a number of different factors and may manifest in a number of different ways. The dis (Ahmmed et al., 2014; Griffiths, 2002; Musiek, 2013).

There is a sufficient body of research to promote and enable the current developments of research into the realm of APD (Billiet & Bellis, 2011; Heine & Slone, 2019). One of the central tenants of this research is the significance of temporal processing and backward masking in such disorders (Musiek & Chermak, 2007). It is clear though, that there is much to be learned, unlearned and reworked in this field; the research and etiologies delving into APD's are far from resolved (Ahmmed et al., 2014).

APD is essentially defined as a hearing disorder caused by some function in the brain rather than the peripheral auditory system. This broad definition encompasses a vast number of factors that can cause, and manifest different outcomes that present as APD (Bamiou, 2001). Approximately 5% of children have some form of APD - many of these cases go untreated (Auditory Processing Center, 2019). APD has been clinically diagnosed for approximately 50 years. There has been more research that attends to the symptomatology, rather than the physiological mechanism of these disorders. APD typically is symptomized by difficulty hearing in environments with competing stimuli, and may coexist with disorders of the peripheral hearing system, but is not caused by such conditions (Musiek, 2013). Many individuals with APD will test normally on a pure-tone audiogram (Ahmmed et al., 2014), this is because there are no competing stimuli. APD's are usually diagnosed when a child is in school (Bamiou, 2001).

Diagnosis of APD is not necessarily a simple, or well-defined process, and sometimes requires a broad battery of tests to identify. Tests to diagnose APD may include behavioral and electrophysiological paradigms (Musiek, 2013). APD has been associated with brain tumors (specifically in the CANS) Premature birth/low birth weight and exposure to some metals, among other things (Bamiou, 2001).

In many cases, APD is mistaken for a hearing loss. The diagnosis of APD is most effective with a team-based approach. Members of a team may include teachers, psychologists, speech-pathologists and audiologists with the help of parents (Bellis, n.d.). The treatment of APD is as complex as the diagnosis. Ultimately, the official diagnosis and treatment of APD is performed by an audiologist. The approach to treating APD is to remediate the effects rather than cure the disorder. However, treatment can be successful to the point where no symptoms of APD are exhibited.

There is a reliable history of subjective evidence that has proven the relevance of BM to APD. The first recorded evidence of auditory BM was reported by Miller (1947). Miller tested auditory thresholds in a forward masking procedure for periodic tone bursts which were preceded with masking stimuli of varying intensity. It was found that when a tone was preceded by the masking signal, the threshold for audibility of the tone was significantly higher (i.e. poorer). The effects of auditory BM were further expanded on by Samoilova (1956), who reported that these masking effects were intensified when the amplitude of the masking signal was raised. Samoilova also reported that masking effects were increased when the duration of the pure-tone stimulus was abbreviated, and when the interval between the stimulus and masking noise was decreased (Raab, 1963). This research marks the first subjective assessments of masking signals. Samoilova determined the relevant parameters of pure-tone stimuli length were 20 to 100 msec, with an Inter-Stimulus Interval (ISI) of length 1-100 msec, masking amplitude of 10-100 dB and masking noise frequency of 650-6000 Hz. The maximum amount of masking amplitude achieved in these experiments was 70 dB at an ISI of 2 msec and pure tone stimulus of 20 msec in length according to subjective assessments.

Although there is no agreement to, and likely no simple or single cause of APD, there continues to be valuable revisions as new research and technological advancements emerge. In the meantime, there are several hypothesized models that aim to encapsulate the effects and etiologies based on known evidence. There are several theories regarding the relationship of temporal resolution difficulties to speech and language impairments in APD (Billiet & Bellis, 2011; Hall et al., 2002; Mcanally & Stein, 1996; Merzenich et al., 1996). The BM related impairments were originally attributed to difficulty in fundamental-frequency discrimination in rapid tasks (Reed, 1989). In a test measuring fundamental frequency discrimination ability in speech versus non-speech acoustic sounds, non-speech phonemes, even with complex frequency shifts were not as affected in a temporal resolution paradigm in children with dyslexia. This evidences that the phenomenon (BM) may be speech-specific (Rosen & Manganari, 2001). These investigators asked the question: "could a non-speech deficit in children with dyslexia be used to predict performance in speech contrasts?" Rosen & Manganari, 2001 also remarked on the difficulty of capturing the predictive power of two stimuli that acoustically are very different. The non-speech broadband masking noise is much different than "real-speech" noises.

Studies have hypothesized that based on previous data, BM disrupts phonological processes, and that these phonological interruptions are the driving factor behind temporal impairment (Heath, Hogben & Clark, 1999). This disruption is presented in disabled readers with a comorbid language disorder. Accordingly, they stated that the temporal processing deficits were not present in disabled readers without a comorbid language delay (oral).

In another widely cited study, Marler et al. (2002) reported a comprehensive review of auditory memory in children with a language impairment by means of a backward masking task. These researchers defined an auditory temporal processing disorder as "an impaired ability to

separate sounds in time." Marler et al. (2002) contended that higher backward masking thresholds are correlated with Language Learning Impairment (LLI), and therefore delayed perceptual learning in children. To prove this, these researchers measured backward masking thresholds in children with LLI. Marler et al. (2002) stated: "The question remains whether the disruption was at a sensory, memory, or cognitive level." Marler et al. (2002) at first replicated the findings of other psychoacoustic assessments of children with LLI. The researchers solidified the model of backward masking related to APD. This research furthered the model by measuring the backward masking effect objectively as well as subjectively through electrophysiological recordings. This research used stimuli consisting of a 10 msec 1 KHz pure-tone signal with a 5 msec rise/fall envelope followed immediately by a 150 msec narrow-band masker (.6 KHz – 1.4 KHz). Stimuli were presented monaurally. These researchers observed that both behaviorally and objectively, there were statistically significant differences between the language-impaired group and the control group. Higher backward masking thresholds were observed in the LLI group, and the Mismatch Negativity (MMN) electrophysiological response was delayed and reduced in amplitude. Marler et al. (2002) asserted that the disruptions in the MMN response was due to a disruption of early auditory memory. They stated that there are two cortical regions associated with the mismatch negativity response: small specialized regions in the auditory cortex that process varying aspects of acoustic stimuli, and independent stimulus processing region of the frontal lobe, which may also play a role in attention-switching processes. The MMN disruption also supports the model of impaired auditory encoding.

Lead Toxicity

Lead was one of the first metals used by humans, and its effects on human health have been documented throughout history, although the totality of deficits are not particularly known.

It is estimated that approximately 450,000 children in the United States under the age of 6 have blood-lead levels that exceed the Center for Disease Control (CDC) definition of low-level lead exposure (5µg/dl) (CDC, 2012, p. 10). Known lead-containing items include: scrap metal, mining byproducts, automobile-batteries, ammunition, pipes, cable covering, construction/building material, solder, radiation shielding, collapsible tubes, fishing weights, ceramic glazes, plastics, paint, airplane fuel and many more. Humans primarily receive lead products through ingestion and inhalation (OSHA, n.d., para. 1). Lead-containing dust is the primary occupational concern, while the Occupational Safety and Health Administration (OSHA) states that the primary source of lead exposure in young children is from deteriorating paint. OSHA also states that children of ages 6 and under are at the highest risk for lead-related deficits, and are subject to effects even at minimal blood-lead levels (OSHA, n.d., para. 6). Due to the increased knowledge of lead poisoning and its consequent disruptions when ingested (or inhaled), the continuum of research has switched focus to the effects of low-level toxicity; mainly to the Central Auditory Nervous System (CANS). A major disruption in the CANS manifests in an inability to process competing auditory stimuli.

Lead exposure in children has been correlated with a number of deficits including: Central and nervous system damage, kidney damage, a cohort of learning/attention disorders, lower intelligence, motor deficits, speech/language disabilities, decreased muscle coordination and muscular growth, bone-growth dysfunction, hearing damage, seizures and death. Lead exposure in adults has been correlated with: Fetal brain damage, fertility dysfunction in both men and women, high blood pressure, digestive dysfunction, nerve dysfunction, memory and attention problems and muscle and joint dysfunction (Sanders et al., 2009).

There are many recent examples of prevalent lead exposure, including the Animas river spill in Colorado, and the contaminated water crisis recently uncovered in Flint, Michigan

(Moffitt, 2018). Much of the population of Flint has been exposed to lead through water pipes; this exposure was correlated to "longest water residence times in pipes", "oldest house age" and "poorest neighborhood housing conditions." 99.1% of the houses in Flint were built before the lead ban in 1978. This exposure to lead has been correlated to numerous social and cognitive deficits in the population of Flint (Kennedy et al., 2016). In August of 2015, the Animas River was compromised by the Gold King mine spill in San Juan Colorado, exposing many people to low concentrations of lead (Rodriguez-Freire et al., 2016). A plug trapping water in a dilapidated mine was destroyed accidentally during construction. This exposed 3 million gallons of heavy metal-contaminated water into a tributary of the Animas River. Although lead has long been known as a neurotoxin, many people today are still exposed to the effects. It has been historically reported also that Ludwig Von Beethoven's deafness (and symptomologies of deafness) were due to an extensive exposure to lead (lead was added to wine) over a period of time (Stevens, Jacobsen & Crofts, 2013). It was reported in the autopsy that the cochlear nerves were reduced in size, measured by high lead levels in the bone tissue.

The degree to which exposure to lead toxicity effects the auditory system is unfortunately as relevant now as it ever has been. Although lead has been removed from many common substances, it is still present in some new and some deteriorating materials, and these lower levels of toxicity coincide with different symptomologies that are sometimes harder to detect. There has been a great deal of evidence demonstrating correlation between blood Pb levels and a variety of impairments in the central and peripheral nervous system. Lead toxicity has been strongly associated with the abnormal Auditory Brainstem Response (ABR) (Gray & Holian, 1999; Haskins, 2008; Lurie, 2006; Moffitt, 1983; Sanders et al., 2009).

APD and Lead Exposure

Specifically related to auditory processing, lead exposure has been correlated to, among others intelligence, ADD/ADHD, dyslexia, sensory-perceptual deficits, and speech/language deficits (Musiek, 2013). These disorders have been evidenced to be partially resulting from APD, defined and tested in part by disruption of normal backward masking levels.

A study in 2006, using an avian species, concluded that backward masking and temporal processing are definitive characteristics of low-level lead toxicity (Lurie et al., 2006). These authors reported that lead exposure has been correlated with lower IQ scores and dyslexia, along with a host of other sensory difficulties and generalized the result from the avian subjects they tested to humans. It was stated that cellular modification in the auditory system leads to dysfunctions in the same regions. This study cited specific deficits in temporal auditory function, not in amount of neurons, but in the weight of the neurofilament protein; proposedly accounting for the lack of temporal processing abilities. These researchers stated that children exposed to lead toxicity present many of the same dysfunctions as those of an APD (without the presence of lead toxicity) (Lurie et al., 2006).

An animal model of lead-toxicity and measurement of temporal processing was developed in mice (Haskins, 2008). In a previously published study (Gray & Holian, 1999), a causal effect between lead exposure and disrupted backward masking thresholds was obtained using a similar animal model with chicks. The effects remained relatively constant across the two animal models. Perception in both lead-affected individuals and children with APD (sometimes being the same) is disrupted in quickly changing sounds rapidly succeeding others. It was suggested that there is a causal link between APD's, backward masking and early lead exposure. Haskins injected a lead-acetate amalgamate into fertilized chicken eggs, and tested an electrophysiological assessment of APD (Haskins, 2008). Four days after the eggs were hatched

auditory evoked potentials were measured. Researchers recorded a significant difference during ABR testing. ABR testing yielded significant differences between lead exposed and non-lead-exposed groups with discrepancies both pre and post-natal. They reported latency differences in waves I, II and III.

Backward Masking and Auditory Processing Disorder

Temporal Processing and Backward Masking

An early study of backward masking, and in fact titled *Backward Masking*, was published by Pickett (1959). This was a seminal study, which touched on paradigms of BM incuding ISI and amplitude of signals. Pickett touched upon Samoilova's earlier work in 1956, reporting that when the ISI decreased to 1 msec, the threshold was lowered (improved) to 60 dB. Pickett reported results distinctly similar to those of Samoilova three years previously, opening the door for a continued study of the backward masking phenomenon. Both of these authors stated that there is a clear, and even a relatively linear correlation between ISI length, target stimulus length, stimulus amplitude, masking noise amplitude and threshold.

There have been many succeeding experiments and studies that have provided a well-established foundation for the backward masking phenomenon (Musiek, 2007). Subjective assessments for the effect have allowed a greater understanding of the role of temporal processing in audition.

Age Reports in Backward Masking

It is well-known that many aspects of audition change with age, this remains true with BM. Several studies have shown that BM performance declines over age, as does audition in general (Gehr & Sommers, 1999; Cobb, Jacobson, Newman, Kretscher & Donnelly, 1993). However, even with normal hearing, aging ears generally show defective BM functions. In 1993,

a study aimed to corroborate the previous evidence for age related increasing BM thresholds. These researchers reported a robust evidencing of digenesis in BM function related to age both in terms of decibel threshold and inter-stimulus interval effects. It was reported that the younger group performed significantly better. Also reported was an interaction between age-related digenesis and ISI) (Cobb et al., 1993).

In 1999, two researchers examined the progressive age-related effects in the backward masking paradigm (Gehr & Sommers, 1999). They reported robust findings of age effects in the data taken. Higher BM thresholds in two groups of individuals were measured in a subjective BM task using a 10 msec sine wave (.5 kHz) and a masking broadband noise (50 msec). There was clear evidence of correlation between age and backward masking thresholds. These researchers found that in the younger group, with an inter-stimulus interval between tone and masking noise in the region of 6-8 msec and beyond, the BM effects were almost nonexistent. In comparison, the older age group exhibited backward masking effects even at the longest measured inter-stimulus interval (20 msec) (Gehr & Sommers, 1999).

Backward masking has been shown to follow auditory development (Hartley, Wright, Hogan & Moore, 2000). Temporal resolution – the relationship between speed of stimuli and accuracy of processing was the focus of this research. The main goal of study to measure the hypothesized improved temporal resolution thresholds in 10 year olds relative to 6 year olds. This plan of study followed the assumption that temporal resolution, and auditory performance in general, are improved in that particular range of development. It is reported that auditory function equivalent to an adult's is not achieved until around age 11 or so on average (Hartley et al., 2000) There was a reported 34 dB threshold advantage attributed to the older group. Age-related improvements were seen in auditory backward masking in 6 to 10 year old children.

(Hartley et al., 2000). This evidence of causality between age and temporal/backward masking thresholds corroborated previous publications that have reported similar data. In this study, a correlation between lower IQ and increased backward masking thresholds was also reported, this aligns with Wright's work 3 years earlier which showed a 45 dB backward masking threshold elevation between older and younger groups. It should be noted though, that it is true that auditory function is improved in the 6-10 year old age range, however, the cause is not known. It may include factors genetic, external, or a combination of the pair; findings in that area must be regarded with some caution.

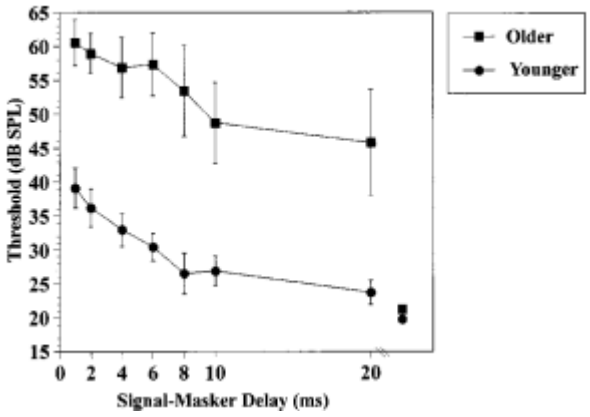


Figure 7 (Above): The BM thresholds for older and younger comparison groups (Gehr & Sommers, 1999, p. 2794)

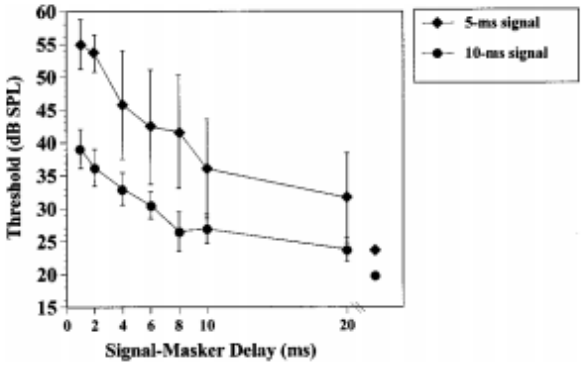


Figure 8 (Above): The comparison of 5msec and 10msec stimuli in the younger group (Gehr & Sommers, 1999, p. 2796).

There have been many historical studies claiming the significance of the improvement of auditory and language process during childhood development, Hartley et al., 2000 showed that backward masking function/processing is similarly developed along these years; pointing to, a significance in the context of audition and auditory processing.

It is clear that the auditory system, and indeed cognition in general undergoes marked improvement before the early teenage years. It has been shown that the auditory system is developed fully by age 11 (on average). Buss, Hall, Grose & Dev (1999) aimed to sequentially test the auditory system in 14 individuals as maturation was reached, and backward masking was the paradigm used to exhibit auditory temporal resolution ability. These researchers measured forward, simultaneous and backward masking. They hypothesized that younger children/individuals show greater variance in threshold detection, and higher thresholds in all 3 masking paradigms.

Two groups were studied, a younger age group of children aged 5 to 11 and an older group of adults aged 23-43. They tested bandwidth masking frequency as a variable with a 10 msec pure-tone stimulus of 1,000 Hz. These studies reported that there was great variance to be

found under the BM paradigm, and relatively less so in the forward and simultaneous masking conditions. A reliable trend was reported in the data, in that masking performance is generally improved in children who develop normally from ages 5 to 11. This was proven true for backward, forward and simultaneous masking conditions. It is worth noting also that the degree to which masking performance was elevated was similar between paradigms (backward, forward and simultaneous).

Buss et al. (1999) claimed that this data provides evidence that the processing deficit is not due to basic auditory system function/processing. The researchers asserted that attention-switching processes are a direct influence on the backward masking response, which indicates a disorder of central processing, and not a deficit in general audition. (Buss et al., 1999).

These studies show a succinct correlation between BM and auditory discrimination ability and development/digenesis. The BM phenomenon is present in all, although individuals with greater auditory confusion (i.e. those older in age) clearly show elevated thresholds for this effect.

Backward Masking, Dysfluency and Dyslexia

Howell et al. (2000) published a study correlating BM performance to the rate of dysfluency in children who stutter. These researchers proposed that the effect was due to a disruption of the auditory feedback loop. There is a marked increase of central auditory processing disorders in people who stutter, however, there is no difference in peripheral hearing evidenced. These researchers assessed professionally diagnosed stutterers as to whether their thresholds for the backward masking effect were different from those without any symptoms of APD. Performance on simultaneous masking assessments was also observed. Researchers used a

subjective measure to report the threshold for effect. These researchers used a 40 dB masking noise with a 300 msec duration. The tone stimulus was a 1,000 Hz sine wave with a duration of 20 msec. Stimulus presentation was monaural. The ISI was 800 msec. These researchers found a distinct difference in the backward masking thresholds between the stuttering groups and the control groups in the backward masking condition. While the simultaneous masking condition remained relatively stagnant across the two groups, the disparity between groups in the backward masking condition was clear. Participants affected by stuttering experienced elevated levels of masking of pure-tone stimuli (that is to say, at higher dB levels) compared to the control group. There is also a much wider degree of variability of masking thresholds in the stuttering group, according to the box plot presented by Howell et al., (2000). There is an outlier belonging to the stuttering group that has a much lower backward masking threshold than either group. This suggests that there are additional unknown factors that are enveloped in the backward masking phenomenon, although it is clear that on average, the masking thresholds are much "worse". It should also be noted that in the simultaneous masking condition, there is apparently a wider degree of variability in the stuttering group as well, although the averages are much more similar to the control group under this condition.

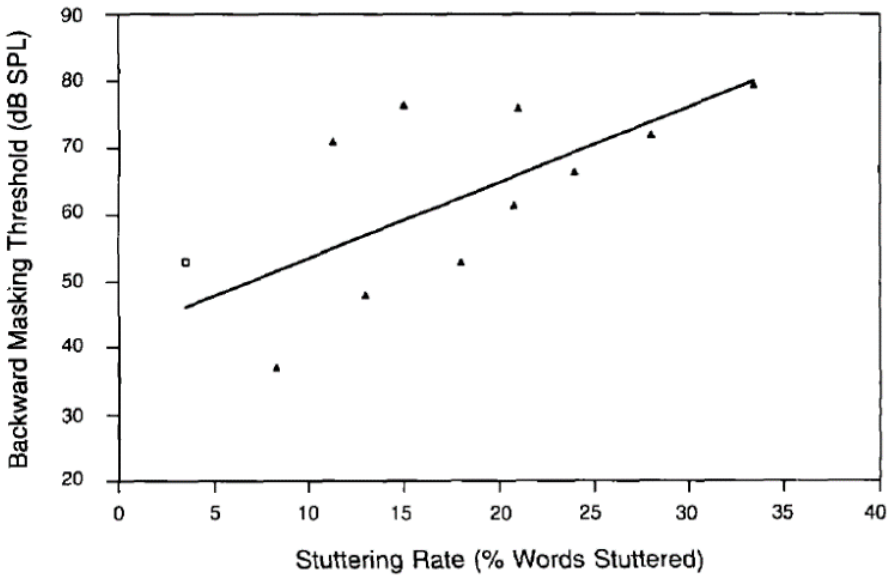


Figure 9 (above): Correlation between stuttering rate and backward masking thresholds (Howell et al., 2000 p. 355)

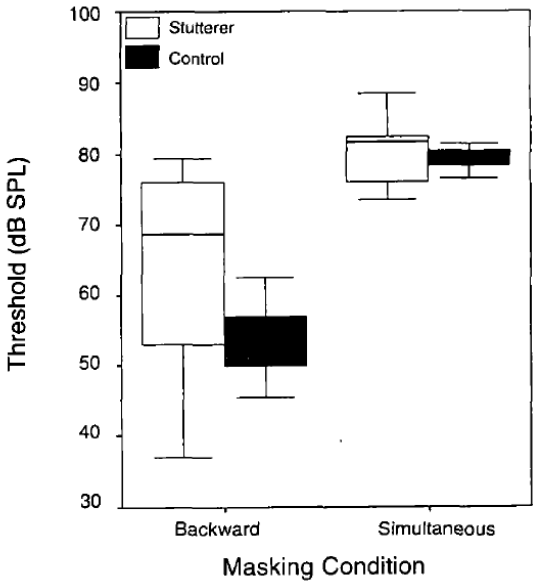


Figure 10: Backward and Simultaneous Masking Threshold Comparison (Howell et al., 2000, p. 355)

The figure above was also presented by Howell in the same study. These researchers evidenced a clear, linear correlation between the stuttering rate of individuals, and poorer backward masking thresholds. This robust evidence has been cited as one of the central supports for the relationship between backward masking and APD.

Backward Masking and Schizophrenia

BM, and forward masking as well have been shown to be correlated to schizophrenia (Kallstrand, Montnemery, Nielzen & Olsson, 2002). One of the common symptoms of Schizophrenia is experiencing auditory hallucinations (among other effects). These researchers reported that schizophrenics performed similar to the control group in a simultaneous masking condition, as do children with language learning impairments. However, in both a forward, and backward masking assessment, sufferers of schizophrenia showed significantly elevated thresholds. Furthermore, those who were more affected by symptoms of schizophrenia, i.e. needing increased residential care, showed increased backward masking. Although the etiology for schizophrenia is truly unknown, backward masking is at the very least correlated to the dysfunction, and may share some significant causal factors (Kallstrand et al., 2002).

Backward Masking and Mental Ability

Researchers reported a correlation between higher mental ability and the P300 wave, specifically in the amplitude and latency of the evoked potential (Beauchamp & Stelmack, 2006). Researchers measured this elusive variable of "higher ability" in terms of discriminatory response time, and specificity/accuracy in a masking task. These researchers also reported discrepancy in the latency of the Mismatch Negativity (MMN) response in a deviant-stimuli task.

It was also stated that the effects are due to an increased ability to access short term working memory that are necessitated by audition, as well as many other activities. They also stated that this resolution/discrimination task is autonomous in nature. Backward masking again was the paradigm investigated for the measurement of auditory resolution. Higher mental ability was deemed attributable to subjects with higher degree of accuracy in responses and faster response time. As other authors have reported, when presented with a short enough ISI, the latencies of the evoked potentials became shorter, rather than longer (Beauchamp & Stelmack, 2006).

These authors concluded that the nature of backward masking to these discriminatory processes is inherent. They noted that backward masking is an effective task to measure response times in a deviant stimuli paradigm. These researchers explored the ISI parameters ranging from 25-150 msec, and white-noise masking stimuli ranging from 800 Hz to 1 kHz. The deviant stimulus was a pure-tone stimulus that varied between 633 Hz, 666 Hz and 700 Hz (Beauchamp & Stelmack, 2006).

Backward Masking in Landau-Kleffner Syndrome

In 1998, a case study was published that explored the temporal processing difficulties that an individual with Landau-Kleffner Syndrome (LKS) exhibited. A specific type of acquired aphasia is manifested in a language disorder accompanied by convulsions. Researchers aimed to identify exactly what sort of lingual/non-lingual deficits occur in this disorder. It was found that William (the afflicted individual) had normal pure-tone audiometric thresholds, and maintained normal middle, outer and inner ear function. However, it was reported that the subject experienced discriminatory deficits when presented with BM condition (Vance & Rosen, 1998).

The most afflicted stimuli were lingual in nature, although some non-lingual stimuli were masked as well, they were not masked to the degree that the lingual stimuli were.

This exposition evidenced yet another language disorder related, or at least correlating to temporal resolution. LKS has been associated with lesions in the temporal lobe, specifically in the auditory cortex. The disorder is also associated with lesions bilaterally in the parietal lobe, superior temporal gyri, and the sylvian fissure. That being said, there is no conclusive definition as to the etiologies of this disorder.

These researchers explored a variety of auditory and language/communicative paradigms. The subject of this case study had apparently normal development until age 3, when his performance dropped dramatically, with unknown cause. William experienced a variety of disabilities - auditory comprehension was affected early on in development, as was speech, although speech abilities were partially intact at times. EEG testing revealed the diagnosis of LKS in this particular individual. Further electrophysiological testing showed nothing significant – MRI evaluations did not detect anything unusual either. William was tested under a common assessment aimed to determine whether individuals process auditory stimuli in a "top-down" or "bottom-up" style learning process. This yields (sometimes) the root functionality of auditory comprehension in an individual (Vance & Rosen, 1998).

Results from the case study are as follows: the individual showed normal auditory function in audiometric pure-tone assessment and auditory brainstem response measurement. William performed also at normal levels for a same/different auditory perception task. In a test involving auditory discrimination and attentional processes – The individual exhibited significant difficulties as compared to normal thresholds for children his age. William also exhibited difficulties on a test (Clinical Evaluation of Language Fundamentals – Revised) involving receptive and expressive

language skills. The subject performed at the skill level equivalent of an 8 year-old when he was 14. William also (at age 14) exhibited difficulties articulating speech, although speech processes were mostly intact.

In the realm of non-linguistic auditory processing tasks, William displayed relatively normal functioning. Auditory gap-detection task results were mixed, the subject displayed a deficit in the right ear, but not the left. In BM tasks, both simultaneous and backward William yielded very poor results. This being one of the most significant findings in the study. In assessments linguistic in nature, William performed very much worse than the control group. In auditory discrimination tasks (both word and non-word) William did not fare well, and performed equally poorly on a lexical decision task (Vance & Rosen, 1998).

APD and the Auditory Evoked Potential – Objective Assessments

It must be noted that the exact neural origins/processes for APD are yet to be discovered. The best current models are based on a conglomerate of research agreed upon by current, devoted minds (Musiek, 2013). One special difficulty in the research of APD is that cortical activity is markedly different in humans and animals. Research in this narrow field must, for the most part, use human subjects. This research is therefore limited in manipulability and nature of variables observed, regardless of whatever relevance they may or may not have. It is in this context, that subjective studies have come to prove especially important in the field of auditory processing research. However, as technological advancements have developed, there have been ventures into electrophysiological markers for auditory processing, primarily through EEG assessments. Musiek (2013) reported on the relevance provided to central auditory processing by certain key features in the auditory brainstem response recorded through EEG testing. According

to a slew of electroencephalographic data, Musiek (2013) stated the specific importance of waves III, IV, and V which mark functioning, or lack thereof, in the brainstem. These malformations in the waveform are likely due to brainstem lesions that affect the central auditory nervous system.

There has been a limited degree of recent developments in ABR measurements, due primarily to the fact that there are more precise methods (e.g. fMRI) that are used to test the most prevalent neural idiosyncrasies. In fact, the main *current* prevalent clinical area where ABR assessments are appropriate is for infant hearing screenings, cochlear implant and hearing aid assessments (when behavioral responses are not usually reliable), as well as the detection of small tumors. However, the few stalwart research ventures in the ABR field have been promising, in that there has been apparent detection of the BM phenomenon in ABR and evoked potential testing (Marler & Champlin, 2005). Auditory brainstem responses in this study were tested concerning children with Language Learning Impairments (LLIs). Marler & Champlin (2005) hypothesized that the greatest morphological significance of the backward masking affect is in the "wave V" of the ABR. These researchers found that the waveform morphology of the two groups were not significantly different when measurements were taken under a no masking condition. However, when tested under a backward masking condition, the LLI group had a reportedly significant delay of the wave V response as hypothesized. Despite being a successful study, there were no formative conclusions drawn to the causality of the backward masking effect, only apparent evidence that the effect can be objectively measured. It must also be noted that the backward masking effect is of extremely small amplitude, and requires a very precise measurement.

De Pascalis & Varriale (2012) reported a study of a late evoked potentials using the mismatched negativity response (MMN), and mental ability in a backward masking paradigm.

They defined this improvement by a measurement of ISI. Those who could hear the "masked" noise with a relatively shorter ISI were posited to have higher mental ability. These researchers proposed that the MMN response to a BM paradigm involves a process they termed "preconscious discrimination". It has been shown that a larger MMN response indicates that sensory discrimination processes are improved (De Pascalis & Varriale, 2012). This response (figure shown below) is related to auditory processing, even to the level of deviating morphologically based on grammatical and semantic changes. These researchers also investigated the effects on the P300 wave. It was hypothesized that the amplitude of the P300 would be greater, and the latency of the mismatched negativity response would be shorter when there is no masking condition present. When there is a masking stimulus present, it is hypothesized that the MMN would have greater latency, and the P300 would have a decreased amplitude; correlating to the intensity of the masking stimulus. The P300, as the name suggests, occurs around 300 msec after onset; it has been highly correlated with consciousness tasks. The P300 is also conveniently the 3rd reliable positive peak in an evoked potential response. This event has been deemed a "task-relevant" response, meaning it manifests as an event-related action potential, as a result of a conscious action. This waveform is usually measured by an oddball task paradigm, where responses to outlier stimuli are focused upon. There is a finite amount of attention-processes available. Attentional processes are strained when there is one more stimulus that needs direct involvement. The p300 has been shown to decrease in amplitude under such conditions (De Pascalis & Varriale, 2012).

Figure 11 (Below): P300 Evoked Potential Response (Rak, R.J., 2012)

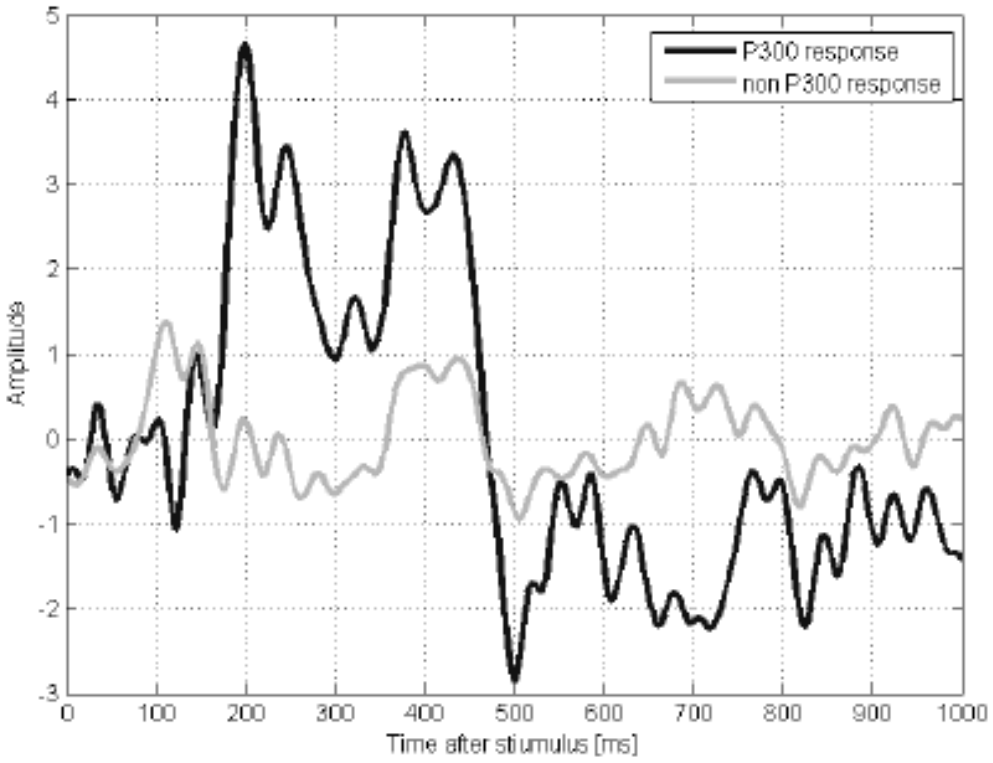
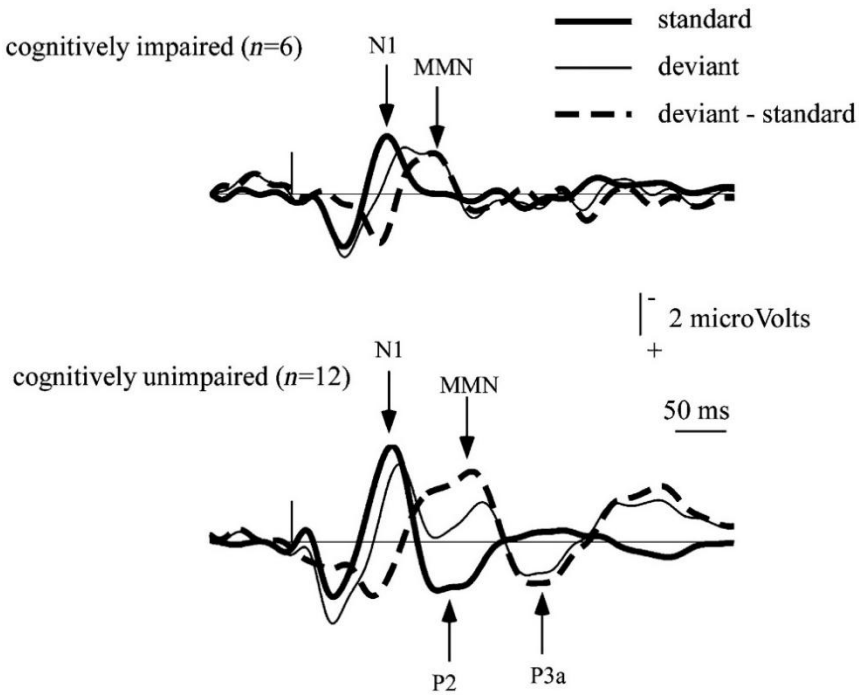


Figure 12 (Below): Comparison of MMN Responses for Impaired and Unimpaired Groups (Natanen et al., 2011, p. 3441).



The MMN is a late evoked potential that is very reliable in recordings. This is because it does not require the subject to be conscious (De Pascalis & Varriale, 2012). This response is not subject to deviations caused by attention processes, or cortical activity, and can be measured simultaneous to activity of any sort. During experimentation, participants were asked to read a book. The MMN manifests as a negative waveform, and is hypothesized to be the autonomous response to auditory stimuli responsible for temporal resolution. De Pascalis & Varriale, (2012) stated that the mismatched negativity response is reliably larger in individuals with higher auditory discrimination abilities. The BM function is highly correlative to the (MMN), which follows the logic of the theorized autonomous temporal resolution/discrimination hypothesis of the MMN wave. During various backward masking tasks, the MMN has been extinguished entirely under certain paradigms. Data from the De Pascalis and Varriale (2012) study showed significant effects between higher mental ability and shorter length of the mismatched negativity response. Amplitude of MMN response was also shown to be higher in these subjects with

higher mental ability. However, MMN latency decreased when the inter-stimulus interval was decreased, opposing the expectations of the researchers.

It was found that the latency of the MMN was significantly shorter when the ISI was decreased. The authors of this research alluded to the idea that the tones were processed as a "gestalt", a single perceived noise composed of a number of other tones/noises that are compounded (as opposed to a pure tone stimulus). This follows the assumption that the MMN evoked potential is "passive" meaning the processes are initiated subconsciously (De Pascalis & Varriale, 2012).

In another study regarding intelligence and auditory processing speeds, Beauchamp and Stelmack (2006) reported that under a BM condition, the individuals with a "higher mental ability" had better auditory discrimination between the tone and masking noise, as well as having a faster neural response time. These researchers also reported that the higher mental ability group had greater average P300 wave amplitudes, and shorter average latency on the P300 and MMN waveforms. This particular study reported that the intensity of the amplitude, and the length of the ISI were contributing factors to the significance of the differences.

Although not specifically tested under a backward masking paradigm an ABR task evidenced that subjects with Persistent Developmental Stuttering (PDS) have a significantly different evoked potential than subjects with "normal" language functioning (Tahei, Ashayeri, Pourbakht, Kamali & Mohammed, 2014). This current research demonstrates an effect that aligns with the hypothesis stating temporal resolution effects manifest in the central auditory pathway. This same hypothesis, although not stated as definite causality, was these researchers' primary explanation for the differing ABR effects. This study is of importance because the true cause of stuttering is not known, although it is known that the peripheral auditory system is

unaffected (at least due to the stuttering). It is believed that the cause of stuttering is disruption in the auditory feedback loop is due to central auditory processing dysfunction (Howell, 2000). Effects were observed in the latency shift in the onset and offset of the waveform stimuli. Researchers observed markedly significant increased latencies in waves V, A, and O. It was also apparently observed that the V, A waves had a smaller degree of inclination. During data analysis, a strong correlation was drawn between the degree of stuttering present in speech, and the degree of latency in waves A and O. There was an apparent decrease of *synchronicity* as well in the PDS group, where peaks of waves were aligned with less consistency. The study also pointed to the fact the waves specifically related to spectral encoding were unaffected in the stuttering group. This again points to temporal processing as the causal factor for stuttering (Tahei et al., 2014).

One study (Kumar & Singh, 2015) showed that children with APD in a range of ages 8 to 12 have significantly different ABR potentials than those with "normal" auditory processing. The study was extensive, assessing 336 children in total, and performing MANOVA analysis to yield statistical significance of the experiment. The analyses revealed that the latencies of waves V and waves A were delayed. As well, they showed that the slope of waves V and A had a smaller degree of inclination in those affected by APD. These researchers also reported that the first formant was reduced in amplitude when compared to the control group. (Kumar & Singh, 2015). These researchers remarked that in previous studies (including some studies performed by the researchers themselves), the waveforms most significantly affected by APD were waves V, A, C, D, E, F and O. Also reported in other studies was the reduced degree of inclination in the V/A waves.

Banai, Hornickel, Skoe, Nicol, Zecker & Kraus (2009) reported robust evidence that reading skill is correlated with subcortical auditory function by use of ABR. It has long been theorized, and shown through a limited body of research that phonological processing is key to the reading process. (Banai et al., 2009) provided data that defines a correlation between reading and central auditory processing skills. These researchers demonstrated that phonological decoding is apparently directly correlated with the latency of auditory processing morphological waveforms. This is a particularly important study because it develops a real understanding of the relationship between reading and subcortical processes. Banai et al. (2009) correlated scores on reading comprehension tests with subcortical measurements of ABRs to provide statistical evidence that latency delays in peaks V, A, C, D, E, F, and O are apparently correlated to reading ability/function. i.e. those with greater latency delays on the waveforms through electroencephalographic measurement on average, had lower reading ability.

Another nominal study established that there is a correlation between subcortical brainstem functions and performance on auditory processing assessments (Billiet & Bellis, 2011). This study again established a link between phonological processing, reading comprehension and auditory ability. This study showed with specific significance that dyslexia is especially related to APD, and that ABRs may be able to diagnose APD in an objective manner, as opposed to current subjective tasks. This particular study narrowed the focus on dyslexia. This study reported that 30% of children with learning problems related to language (including but not exclusive to auditory processing problems) have significantly different ABR measurements. This study took 32 children with normal hearing sensitivity, and a professional diagnosis of dyslexia ranging in age from 8 to 12 years, and correlated their phonological processing (reading) skills with their evoked brainstem potential recordings. These researchers showed that the ABR

measurements for those diagnosed with dyslexia are significantly different from the ABRs in individuals with "normal" phonological processing. This corroborates the most current research on APD and abnormal brainstem responses. Consistently, it has been found that the brainstem response is at least one of the factors that must be considered when reviewing central auditory processing disorders. This study, again in corroboration with the apparent best, current research, has shown that waves A, C and O are the locus of abnormalities in the brainstem response relating to dyslexia.

In an attempted objective recording measure of the BM task, Van Dijk and Backes, (2002) used functional magnetic resonance imaging (fMRI) to assess the backward masking task in adults with normal hearing. They recorded individuals in both a BM and simultaneous masking condition (as a control). They reported several apparently significant effects in the comparison between masking conditions; those being: greater recorded activity in the cerebellum, left inferior parietal lobe, posterior cingulate cortex and left inferior frontal cortex in the *simultaneous* masking condition than in the backward masking condition. There was reportedly greater activity in the *backward* masking condition in the anterior cingulate cortex and the anterior temporal poles (laterally). These researchers cited this evidence as reason to believe that simultaneous and backward masking respectively activate different neural regions and processes. They went on to state that it is plausible to think that different lingual deficits may be caused by differently affected areas (Van Dijk & Backes, 2002).

Haskins (2008) showed that chicks exposed to lead had auditory brain responses similar to ABRs found in children with language learning impairments. It has been well evidenced that

low levels of exposure to heavy metals like mercury and lead and cause central auditory processing disorders. This particular study is extremely relevant because it shows that the ABRs are very consistent in the resemblance of lead exposed chicks and language impaired humans.

BM has proven to be of high significance in regard to APD. The BM effect has been correlated to language impairment, age-related auditory degeneration, and even in ailments such as schizophrenia. Although it may not be causal to all of these, backward masking is at the very least present in many language disorders, and remains a phenomenon worth investigating. The contributions that may yield from a greater understanding of BM are astounding. The ambiguity in definition and diagnosis of many neurological impairments could potentially be revealed through electrophysiological recordings of the BM procedure. It is blatantly clear that BM processing is an ability that is hindered in individuals with a variety of impairments. It is yet to be discovered though, the exact nature and functioning of BM neurologically.

In a recent study in 2018, ABR's were obtained in rats before, during and after lead exposure (Moffitt, Yonovitz & Smolensky, 2018). These investigators explored the effects of "temporary vs. persistent" exposure. When compared to the control group, a latency and amplitude abnormality was present in several of the peaks of the brainstem evoked potential. It was found that in rats with higher exposure to lead, the deficits spread to the peripheral auditory system (Moffitt et al., 2018). These deficits were somewhat recompensed when ABR's were taken 30 days after exposure to lead was terminated. These authors noted that Pb half-life in blood is approximately 30 days, while Pb half-life in bones can be years or decades, increasing with elevated levels of lead exposure. It was reported that in the first session of ABR's, 45 days after chronic lead exposure, the latencies of waves I, II, and IV were increased. Data also showed that

in the second ABR assessment, post-exposure, these latencies were reduced (Moffitt, Yonovitz & Smolensky, 2018).

Hypothesized Models for Auditory Processing Disorder

There are some recent studies suggesting that there is a comorbidity of auditory processing disorders and impaired phonemic discrimination in the central auditory system (Marler, Champlin & Gillam, 2002). However, the physiological cause or causes of the disruption is not known. The next step in the research of APD is to determine why and how the stimuli "overlap" and interfere in the brain (Wright et al., 1997, Musiek, 2013). Researchers in 2002 reported a nominal study in which the source of language impairment in children was proposed to be a disruption in auditory processing and spectral resolution. These researchers aimed to develop a more precise model to explain the deficits in learning and comprehension when individuals were otherwise unimpaired (Marler et al., 2002). They tested two masking conditions with eight language impaired children, and eight children in the control group with reportedly "normal" language development. There were two especially relevant findings in the data acquired from this research. The first was that perception for the language impaired individuals was disrupted at variable levels in response to different stimulus conditions (Marler et al., 2002). The second important aspect was the reported effect of higher backward masking thresholds in the language impaired group. This study concluded that children who are impaired in their language ability have a varying degree of difficulty discriminating between two sounds within a short timeframe. They also suggested that the specific frequency of a backward masking tonal stimulus and noise affects behavioral results.

In 2001, researchers aimed to determine if BM effects are different under speech and non-speech conditions for auditory stimuli (Rosen & Manganari, 2001). These researchers tested

a group of 8 dyslexic individuals and a control group of similarly aged non-dyslexic individuals. It was determined first that forward masking levels were not significantly different between groups; backward masking thresholds however, were markedly heightened in the dyslexic group. Following this confirmation, they developed a method that tested if these elevated BM thresholds were the cause of dyslexia-related disruption. The authors theorized that if BM is the root of speech misperception, phonemes that contain consonants preceding a vowel will be affected to a higher degree under this condition as opposed to the consonant postceding the vowel. If backward masking was the root of the disruption, morphemes such as "ob" and "od" would not be affected as much as morphemes like "bo" and "ba". However, these researchers found no discrepancies when the change in speech phonemes occurred secondary. Speech recognition should have been adequate on the changed term, because being second in sequence, it would not be subject to backward masking. Although these authors noted that there was initially a better measured ability in the non-dyslexic in terms of better general language ability. Under the backward masking condition, there was no discernable difference. These authors stated that this determines the backward masking task to be irrelevant in this paradigm to basic speech discrimination, but not complex speech noises. It is obvious that sweeping terms are not all that define speech perception, or the backward masking task for that matter.

Researchers in 1999 hypothesized that based on previous data, BM disrupts *phonological* processes, and that these phonological interruptions are the driving factor behind temporal impairment (Heath, Hogben & Clark, 1999). This disruption is presented in disabled readers with a comorbid language disorder. Accordingly, they stated that the temporal processing deficits were *not* present in disabled readers without a comorbid language delay (oral). These researchers aimed to reach a more definite conclusion as to these effects and etiologies.

Data taken from a sample of 7 to 10 year olds found that disabled readers with a co-occurring oral language delay experienced disruption in auditory temporal processing. However, the individuals without oral language delay, even those with reading disorders showed normal temporal processing thresholds (Heath et al., 1999). These authors proceeded to state that there is a plausible correlation between oral/phonological processing and these temporal thresholds. This theory proposed by Heath et al. in 1999 states that the loss of rapid/temporal acuity that is present in auditory processing disorders deters the individuals' phonological awareness.

Naatanen, Kujala & Winkler (2011) reviewed a model of auditory processing regarding "conscious perception". Primary causality of auditory discrimination and processing was deemed to be related to the MMN and N1 evoked potentials in the brain. This study confirmed several others that have evidenced, that central auditory processing is related to certain evoked potentials. The focus of this study in particular was to determine which auditory processes are conscious and which are not.

In a recent attempt to model the auditory pathway through ABR, (Johnson, Nicol, Zecker & Kraus, 2007) described in detail the nature of this paradigm. These researchers linked two theories regarding auditory processing into a single comprehensive model. The two theories included in the research were the "source-filter model of acoustics" and the "cortical sensory processing streams model" (Johnson et al., 2007). The source filter model refers to the constant filtering of speech stimuli in the vocal tract when speech is produced. The cortical sensory processing theory is the more relevant model, at least in regards to the focus of this paper. The sensory processing theory was first shaped in the context of the human visual system. It was proposed (and later evidenced) that there are two separate, but simultaneously functioning pathways that are used to process visual information. These pathways (dorsal and ventral) are

both used to identify objects, but they are focused on different aspects of visual stimuli. Some time later, research was published that evidenced a similarly functioning system in the auditory pathways (Romanski, Tian, Fritz, Mishkin, Goldman-Rakic & Raushecker, 1999). Johnson et al., (2007) went on to define the brainstem response to a complex sound as: "a gauge both of spectrum encoding – which is indicative of the overarching organization scheme of the auditory pathway – and of periodicity encoding". These researchers stated that the brainstem response is replicable and reliable in individuals. Johnson et al. (2007) also reported that the early waves in the auditory pathway (3 msec or less) were especially relevant in diagnosis when presented with an auditory stimulus. These authors went on to say that the process of encoding of frequencies is yielded in the brainstem in an amplitude and latency shift of waveform peaks (Steinschneider, Schroeder, Arezzo & Vaughan, 1993). This study, in addition to observation of early waveforms, observed the "frequency-following response" (FFR) waveform (15-150 msec). It was reported that the FFR is accurate to the point where an EEG taken of the potential following a speech stimulus can be amplified and presented audibly as the same stimulus. The proposed loci of the FFR are the lateral lemniscus and inferior colliculus, although there is still some debate on the matter. The stimulus presented in the experiment was a complex speech sound 40 msec in length. Below is the hypothesized "mapping" of the auditory pathway as proposed by Kraus & Nicol (2005). As evidenced in previous research, specific latency differences are shown between waves and amplitude of waves that constitute the "expected" brainstem response to the speech stimulus.

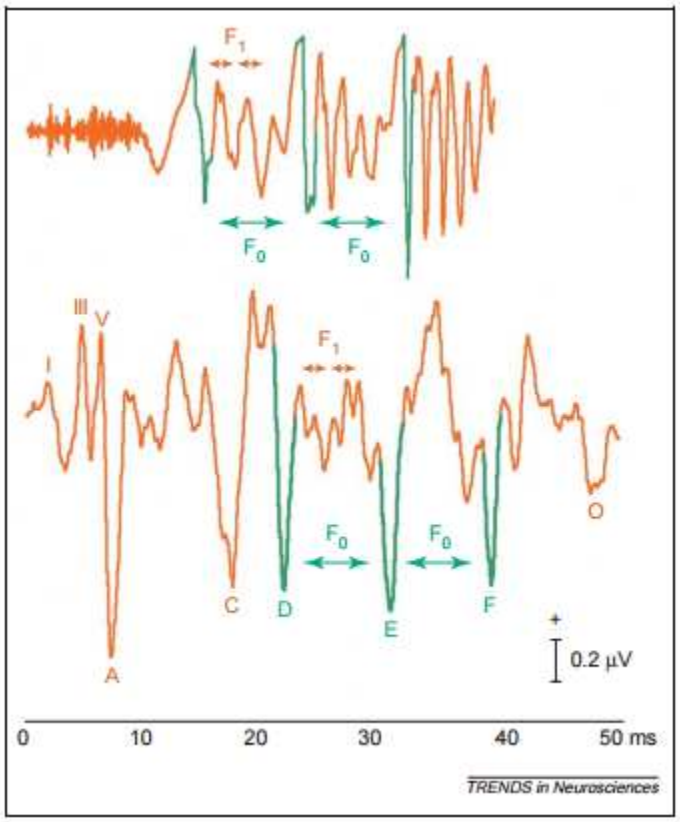


Figure 13 (Above): Early/middle auditory evoked potential with labelling of specific waveform attributes (Kraus & Nicol, 2005, p. 179).

Johnson, Nicol, Zecker & Kraus (2007) completed a study that progressed the knowledge of the relationship between language impairments and the backward masking phenomenon. Researchers reported that children with an assortment of language related discrepancies suffer from a lacking ability in temporal resolution, i.e. sounds/tones in quick succession were perceived with greater difficulty. These researchers proposed that these effects happen in the low-level auditory pathway. A measurement of objective backward masking was tested, and two groups were formed, one of better and one of poorer auditory temporal resolution. The groups were then measured in an objective manner through auditory brainstem evoked potentials. The primary variable in this experiment was the ISI. These researchers stated that this deficit in temporal resolution is not due to a cortical deficit but an "encoding" deficit in

the brainstem related to acoustic cues. The rapidity of the morphology in speech is the key contributor to this auditory confusion. They proposed that if the evoked potential response to a backward masking task would determine if this phenomenon occurs at a subcortical level, or later. These authors purported that between 5, and 10 percent of children with normal peripheral audition are afflicted with some degree of language-related learning disorder. They also reported that these effects are antagonized with increasingly rapid successions of auditory stimuli.

In a comprehensive review published in 2014, the underlying etiologies of central auditory processing disorders were yielded under a factor analysis statistical method. These researchers agreed that there is not a concrete definition available for central auditory processing disorders; they did concede though, that APD is marked primarily by the peripheral auditory system maintaining facility and normal functioning on a pure-tone audiogram (Ahmmed, Ahmmed, Bath, Ferguson, Plack & Moore, 2014). These researchers attempted to complete a totally comprehensive statistical analysis as to the true cause/correlatively to central auditory processing disorders. From these statistical analyses, 3 primary factors were manifested. The first and most prevalent of the 3 these researchers termed “general auditory processing”. The other two, “working memory” and “processing speed.” These three driving forces behind central auditory processing disorders were manifested statistically by means of factor analysis. The “general auditory processing ability” was deemed according to a battery of tests involving backward and simultaneous masking, frequency discrimination and accuracy, and speech processing. “Working memory” was determined by tasks of executive attention, cognitive-related batteries, and listening tests. Processing speed was measured by motor-related input speeds relative to certain tasks. The authors remarked that there is obvious variance in symptoms and abilities, but hoped still to tie together underlying causes and their subsequent effects. They

first aimed to define APD, however, and remarked that generally, the presence of a hearing problem while a normal pure-tone audiogram is maintained marks this particular disorder (Ahmmed et al., 2014).

The terming of such disorders remains somewhat murky however, as many co-occurring disabilities are present in many of these affected individuals. The American Speech and Hearing Association has taken the position that APD is not higher-order in nature i.e. due to cortical malfunctions. However, some more recent research endeavors have explored the idea that a significant effect in evoked potential tests is a result of attentional processing differences, which are inherently "higher-order". Short-term working memory yielded significant correlates as well (Ahmmed et al., 2014). Although there is not consensus, there are a few likely factors that this study aimed to encapsulate.

Reading abilities were also given importance in this study, and have been a factor in much of APD research current and historic. Again, given the complexity and elusive nature of the reading process, it has been highly disputed if and how auditory processing disorders are related to neurological functioning. The authors of this factor analysis noted that some recent studies have shown no significant relationship between auditory comprehension and reading. It may be that APD occurs alongside many reading disabilities due to some common etiology. At any rate, there can be no definitive conclusions drawn at this point. This study aimed to develop the most appropriate battery of tests that inclusively measure and assess APD (Ahmmed et al., 2014). It was determined that a multimodal approach to this assessment is necessary, despite the admitted murkiness in definition that leads to complications. These authors stated that factor analyses have been performed on auditory processing assessments previously. However, the analyses were done on "out-dated" batteries. These analyses did yield two primary contributing

factors though, “binaural separation/competition” and “composite monaural low-redundancy degradation” (Ahmmed et al., 2014).

Mcanally and Stein (1996) published a paper that posited a controversial opinion – that dyslexia is not a higher-order cortical disorder, but a disorder of early processes occurring in the brainstem. These authors stated two findings that demonstrated this hypothesis. The first being the significance of backward masking functioning and frequency discrimination; The second being the measured evoked potentials. These researchers assessed 23 dyslexic adults, as marked by the difference between nonverbal intelligence and reading ability. These researchers stated that dyslexic individuals exhibit poorer performance relatively when compared with control groups in the presence of rapid auditory tones. It was stated, according to the data taken, that the inter-stimulus interval between the tone and masker did not yield a great effect between the dyslexic group and the control group. Temporal encoding was the factor most discrepant between the control group and the dyslexic group. Temporal encoding refers to the accuracy of the coding of stimuli onset/offset in the brain.

Frequency of stimuli in temporal encoding has been hypothesized to be the result of phase-locked nerve fibers (Mcanally & Stein, 1996). These fibers fire at the same rate as the auditory input for tones 5 kHz and below. It was stated that the dyslexic group under the masking condition had significant difficulties detecting frequency changes in the tonal noise around 1 kHz. This suggests that the impairment resulting in language disorder in dyslexic individuals is a result of a disruption in the temporal encoding of these phase-locked discharges, which in turn effects frequency discrimination.

Mcanally & Stein (1996) found that the greatest masking effects were achieved during a binaural masking condition, where the phase difference of the tone was 180 degrees inter-aurally

presented. This gives support to the idea that phase-effects are related to the disorders presented in dyslexia.

In 2014, an objective assessment of dyslexic individuals was presented in an evoked potential task. Measured was the far field potential: an evoked potential that has been shown to measure directly the firing of the phase-locking neurons. These synapses occur in the brainstem, and it has been shown that lower amplitude in the far field potential correlates with reduced accuracy in phase synchronicity. Evidenced in the data taken in this study, was a significantly lower amplitude for the far-field potential. (Latency of the potential was not significantly affected). The fact that the latencies of the waveform did not differ from the control group lends support that the effect occurs in the brainstem (Ahmmed et al., 2014).

It has long been touted that central auditory processing is not a disorder due to peripheral hearing. Musiek (2013) takes an interesting approach to this model that is not in direct opposition, but claims the process is not quite so simple. Musiek stakes that the greatest masking effect indeed is not due to peripheral dysfunction. Masking effects occur predominantly in a range of 10 msec and below, resolving in the brainstem. However, it has also been shown that BM effects are yielded from the 15-25 msec range as well, and these effects may be related to basilar membrane functioning in the peripheral auditory system. Musiek also cited authors who state that forward masking is more prevalent to peripheral auditory functioning. There is an issue with this model though, in that individuals fitted with a cochlear implant – in other words, those lacking any peripheral processing at all show similar forward masking thresholds.

Marler (2002) considered auditory memory, and specifically its relevance to low-level processing. These researchers specified that the backward masking effects primarily operate on complex (non pure-tone) acoustic stimuli that are nonlinguistic in nature. They provided both

objective and subjective data to defend their case. Marler (2002) tailored two models to encompass auditory processing disorders: The first being a sensory approach, stating that the temporal disruption experienced in APD is due to an incomplete rendering of acoustic waveforms due to some quality of features in the auditory system. The second approach cites low-level auditory memory as the central tenet for temporal disruption. These theories state that the disruption manifests during the encoding/storage of memory processes. This early auditory memory is highly correlated with the MMN response. This potential is not cortical i.e. higher order in constitution.

Marler (2002) stated that an effect in the N1 morphological potential would indicate a sensory disruption. An effect in the MMN would indicate a disruption of low-level auditory memory. After electroencephalographic measurements and data analysis, it was found that the N1 potential was intact, and that the MMN was significantly delayed temporally, and diminished in amplitude. Provided this data, these authors stated that low-level auditory memory pays a key contribution to central language impairments. These researchers described a model based on neural encoding into memory that does not take sensory mechanisms into account. In 2005 Marler continued his studies in the auditory processing field. Marler (2005) made an addendum to his earlier research, reporting that the wave V response is significantly reduced in addition to the MMN response. It was therefore proposed that attentional activity is incorporated in the response. Marler (2005) also remarked that these disruptions appear to be pre-linguistic, meaning they occur before language areas are cortically activated. It is likely then, that the disruption occurs in the brainstem. A misfiring of synapses in certain contexts may produce auditory temporal disruptions.

There has been great a deal of evidence alluding to the idea that auditory stimuli are processed hierarchically. This invokes the idea that primary auditory/language areas have higher neural activation than non-primary areas in a linearly correlated fashion (during a speech/language task). As well, stimuli that are more complex in nature retain greater neural activation (Hall, Johnsrude, Ingrid, Haggard, Palmer, Akeroyd & Summerfield, 2002). In an fMRI paradigm, it was determined that a multi-frequency harmonic tone yields greater neural activation in a few key areas when compared to a pure-tone stimulus. Heschl's gyrus showed higher activation in the right temporal lobe, and the supratemporal plane showed higher activation in both the right and left hemisphere. These researchers cited this evidence, along with previous research to the theory that the auditor cortex is formed hierarchically (Hall et al., 2002).

Escera Leung, and Grimm (2014) purveyed a theory that states that the auditory hierarchy starts as low as the brainstem. They accomplished this using a deviance detection based paradigm. Reported in the data was that the evoked potential related to detection of a deviant/unexpected stimuli was marked by an aberrance in the Mean Latency Response (approximately 10-80 msec after onset) that was distinct from the deviance marked in the MMR response (approx. 100-240 msec after onset), and in the brainstem as well. In other words, the waveform morphology was different for each pathway/response, and the notion that the disruption manifests differently in separate auditory regions aligns with the theory of auditory hierarchy. These researchers also reported similar findings of deviance detection evoked potentials in tested animals.

It must be noted that it takes exact and minute measuring techniques to find significant results in the span of a few microseconds. There have been articles stating there are no

significant effects to be found concerning backward masking and certain language impairments. These studies raise a deal of questions on measurement, reliability and validity.

Training/Attentional Processes relevant to APD

There have been some relevant experiments that have attempted to clinically improve performance on temporal resolution tasks in language impaired children. Some have displayed significant improvement in such endeavors. At the very least, it is worth noting that training may affect the backward masking procedure. It should be noted that although individuals with and without APD exhibit training benefits on backward masking/temporal resolution tasks, those without language impairment show greater potential for improvement.

Merzenich, Jenkins, Johnston, Schreiner, Miller and Tallal (1996) reported that certain cognitive processes, language learning included, can be dramatically improved by means of behavioral training. These improvements were demonstrated subjectively and objectively in an electrophysiological procedure. These researchers evidenced this data to hypothesize that the language disorders related to those temporal deficits are rooted, and manifested from a history/context of poor learning. Temporal/perceptual development may be the causal factor to these language impairments (Merzenich et al., 1996).

In this experiment, researchers attempted to train children with a professionally diagnosed language delay in an attempt to lessen the temporal resolution disruption. They used two different training methods, although both methods were manipulated in an audiovisual realm, presented in the form of a game. The games were reportedly designed to engage the individuals as much as possible, to evidence as much effect as possible on the training variable and with the age range (5-10) and the individuals' unique abilities in mind. The first game the

authors labelled a “perceptual identification task.” This task involved two auditory tonal stimuli played in rapid succession. The second game involved the training of phonetic awareness in the language-impaired individuals. In the first trial of the experiment, training took place over 4 weeks with each individual receiving 19-28 training sessions of length 20 minutes. Five of the seven children tested in the first session exhibited language-learning related benefits, the majority of whom showed increasing benefits as the training continued. Two of the seven children that underwent training even surpassed normal thresholds. Before and after training, the “Tallal Repetition Test” was given, this test being an agreed upon method for assessing temporal processing abilities. The Tallal test showed significant improvement in temporal processing/sequencing abilities. These authors reported that the greatest advantage experienced after training was in the detection of brief stimuli, and under a brief ISI condition. The second test involving phonemic awareness established beneficial results as well. Six of the seven participants performed markedly better after undergoing training. This comprehensive study corroborates previous evidence that temporal processing and some language-related delays/impairments appear to be related.

STATEMENT OF THE PROBLEM

The use of the laboratory rat (*Rattus norvegcus)* has a long history in auditory research especially in auditory and discriminative functions (Henry, 1938; Firstova et al., 2012). Laboratory rats have a lifespan of 2.5 to 3.5 years. This suggests that approximately 11.8 rat days equal one human year. In terms of the middle and inner ear, for the rat, the frequency range is expanded in the higher tones. The rat has been a stable model in many areas of auditory research.

The following questions will be addressed using a computer model of rat hearing. The model is expected to be robust and accurate for issues related to any hearing loss in the model. The issue of aural changes related to lead toxicity and backward masking required assumption as to the neurologic locus of the presumed alterations of the neuro-electric responses.

Question 1: What are the changes in the Auditory Brainstem Response (ABR) that might be expected from a lead toxified group as compared to a control group?

Question 2: What changes in the middle latency responses (mid-late) would be expected for the Backward-Masking functions related to the lead dosage given to animal subjects?

The determination of Question 2 requires the construction of a rat-worn, self-contained evoked potential system. The central focus of this research proposal is to design a system for obtaining evoked potentials in an awake rat. The apparatus will include a miniature BM stimulus-generation system, which allows for acquiring evoked potentials for observing morphological waveform changes of the whole auditory pathway. The electronic apparatus will require very low power surface mount devices. Wireless and micro SD cards will be utilized for acquisition of evoked potential data. Stimulus presentation will occur with the use of a sub-miniature hearing aid speaker inserted in the ear canal of the rat. The design of study will allow observation of the early (0-10 msec), middle (10-100 msec), and late (100-1000 msec) auditory

evoked potentials. This process will allow us to observe the differential electrophysiological responses of evoked potentials during the BM effect.

The principle aims of this study are to assess the modelled neurological auditory pathways including those which sub-serve the full auditory processing functions in the awake animal. These aims include the following:

1) Provide a definition of an advanced electronic device that will acquire early (ABR), and middle, late, and (mid-late) evoked potentials;

2) Obtain auditory evoked potentials from pure tones which include ABR, and mid-late cortical electrical responses after a simulation of rats ingesting lead in the water available to them. The latencies and inter-wave latencies and amplitudes of electrical responses will be utilized to relate the neurological responses to specific loci in the brain;

3) Obtain early, middle and late evoked potentials concurrently using a statistical paradigm that will provide an appropriate comparison for different stimulus conditions; including lead dosage levels.

4) Provide both an auditory assessment of hearing through ABR assessment as well as a backward masking protocol established through middle and late evoked potential assessment.

METHODOLOGY

Animals

The simulated model is based on a single rat of the inbred Sprague-Dawley strain, weighing 200-225 grams. A 12-hour light-dark cycle would be maintained throughout the experimental procedure. The average noise level should be below 51 dB (A) SPL.

Anesthesia

Anesthesia was to be utilized in all surgical procedures for each rat. The anesthetic procedure consisted of an initial intramuscular injection of 50 mg/kg body weight of ketamine hydrochloride followed by a few minutes later by an intraperitoneal injection of 10 mg/kg body weight of xylazine. A rat cadaver fitted with electrode is shown in this figure.

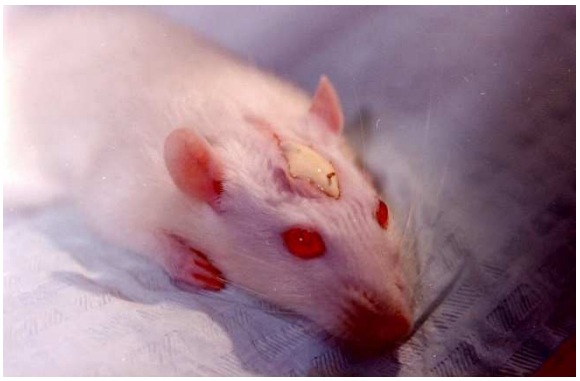


Figure 14. Chronic electrode implanted in rat cadaver.

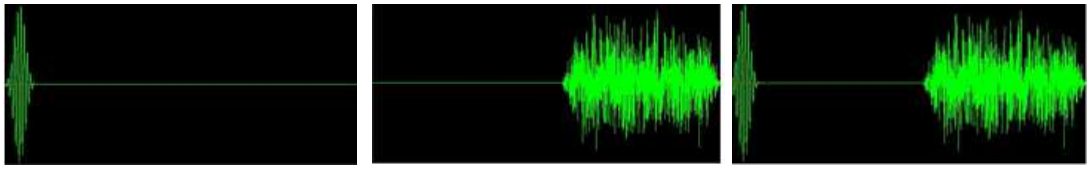
Surgical Procedure

The surgical procedure is based on the studies by Yonovitz (Wassick & Yonovitz, 1985; Fisch and Yonovitz, 1991; Blunston, Yonovitz, Woodahl, and Smolensky, 2015). Surgery consists of the anesthesia procedure described above. Through a small incision, electrodes are implanted. The active electrode are 1 mm to the right of the sagittal suture midway between the

lamda and mid-bregma sutures to a depth where the tip of the electrode just penetrates the dura of the brain. The reference electrode is placed 5 mm anterior to the active electrode and ground electrode is 10 mm posteriorly to the active electrode. The electrode is a 2 mm stainless steel screw with a wire attached and led subcutaneously to an imbedded micro-miniature connector held in place with dental cement.

Backward Masking Stimuli

For the BM determination, an important methodological factor is the target stimulus and noise being temporally accurate for each condition. This study will use randomized stimuli with four different conditions. With a long inter-stimulus interval (20 msec) and high sample rate (25,600 Hz) simultaneous early, middle and late potentials will be obtained. There will be a pure-tone stimulus alone, 1) 4000 Hz, 10 msec with a rise-fall Blackman function), 2) a masking noise alone (white noise, 50 msec, 5 msec rise-fall, 3) the pure-tone followed by the masking noise (50 msec duration) as well as 4) a control condition of baseline evoked potential with no auditory stimulus. This approach, using a randomization will allow any adaptation or habituation to the stimuli to be equally distributed within each condition. Using arithmetic operations on the derived evoked potentials should inform concerning the locus of BM. If the compared waveform morphology for different stimuli are significantly different, a conclusion can be drawn as to which structures are affected during the BM condition.



Stimulus Alone Noise Alone Stimulus and Noise

Figure 15. Stimuli used in this study.

Design and Construction of measuring apparatus

The design definition for the body worn pack by the rat includes the following.

1. The pack must be light and easily attachable with straps to the rat. The pack should stay in place and not be removable by the animal.

2. The electrode array terminates with a small sub-miniature connector. A very small cable from the pack will be attached to the electrode array connector

3. Another cable will connect to a small receiver-speaker such as that used by hearing aid.

Figure 16. The receiver is typically 1.5-2 mm diameter.

4. The controlling microprocessor will be a Raspberry Pi. It uses extremely low-power, a small computer footprint that has wireless capability, micro-SD card, and an I^2C interface that can be connected to a variety of other IC's. A number of A/D converters can be implemented and will achieve the speed necessary to acquire and save auditory evoked potentials. Pre-amplifiers will also be low-power IC's that will provide any operational amplifiers will provide any filtering and signal conditioning.

5. The Raspberry Pi Zero will be interfaced with a miniature MP3 player that will provide the stimuli. It will be used as an attenuator and waveform generator for the presentation of BM stimuli.

6. Data will be transferable via an internet wireless link and/or a micro SD card to a PC.

Hardware implementation

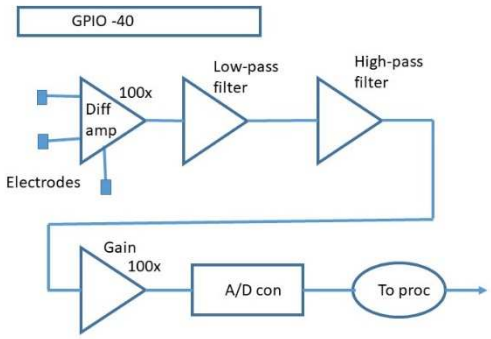


Figure 17. Block diagram of the analog portion of the wearable pack.

Essentially, very low amplitude signals are obtained from the rat and amplified with a differential amplifier (100X). The signals are then filtered with a low and high pass filter to create a bandwidth appropriate for the recorded signal analysis. The signal is then amplified (100X) and this analog signal is converted to digital form that is read by the GPIO-40 connector that is part of the Raspberry Pi Zero.

The filter characteristics are shown in the next two figures. Both the high-pass and lo-pass filters are shown. The filters are equal component Sallen-Key filters with equal component resistors. These resistors are provided digitally under program control.

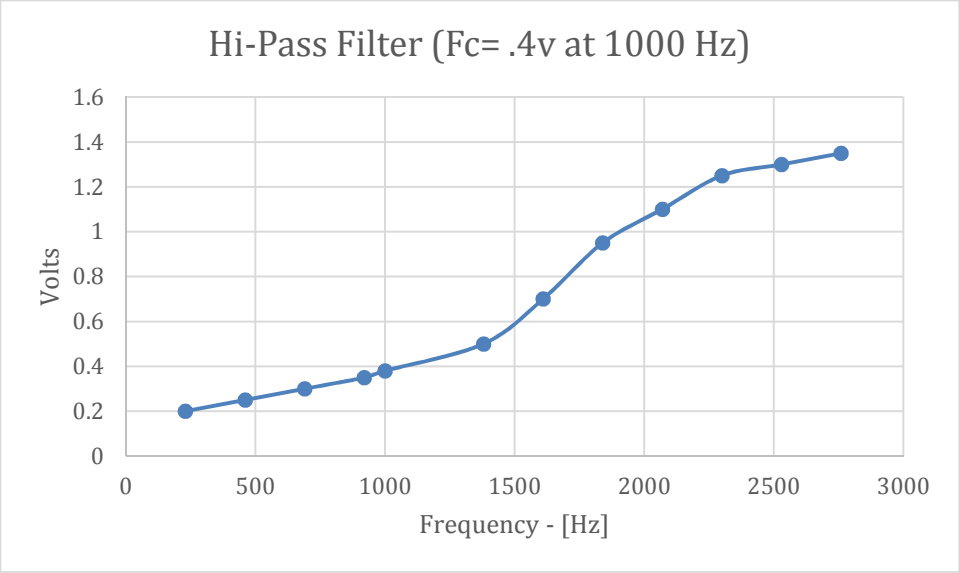


Figure 18. Filter characteristics for the Hi-Pass filter.

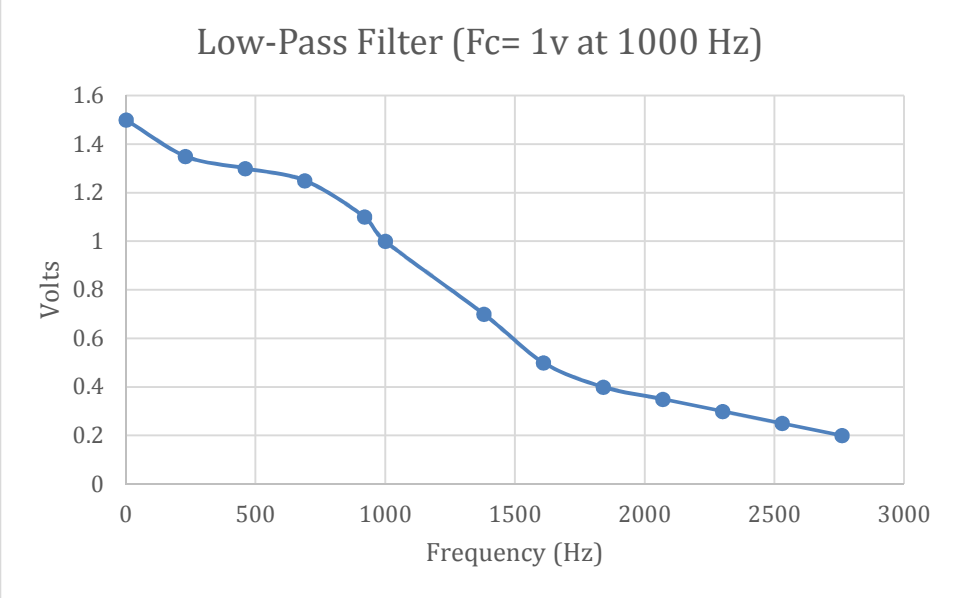


Figure 19. Filter characteristics for the Low-Pass filter.

A functional schematic diagram is shown in the next figure and contains a full list of the components for the body worn evoked potential unit.

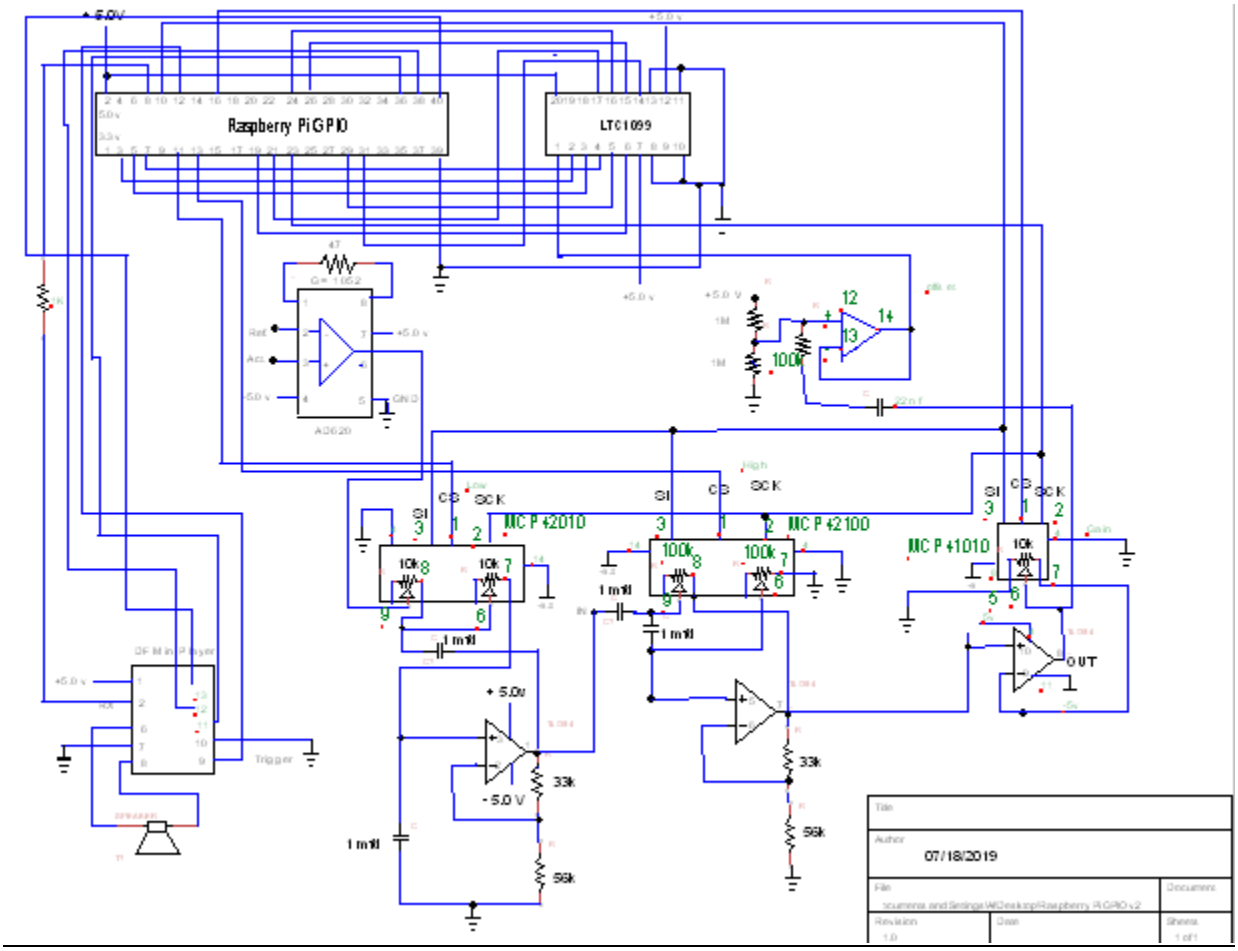


Figure 20. Schematic diagram for the wearable apparatus.

Construction

The construction schematic is shown in the next figure.

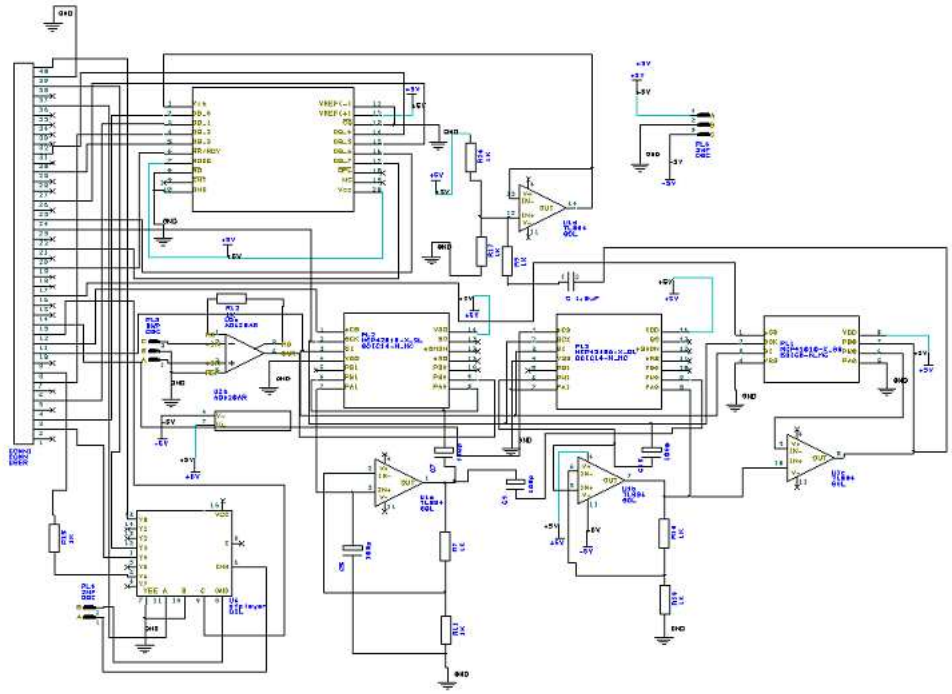


Figure 21. Auto-routing schematic used to produce the printed circuit board.

This schematic forms the basis for an auto-routing algorithm that is used to produce the printed circuit board shown in the next figure (22).

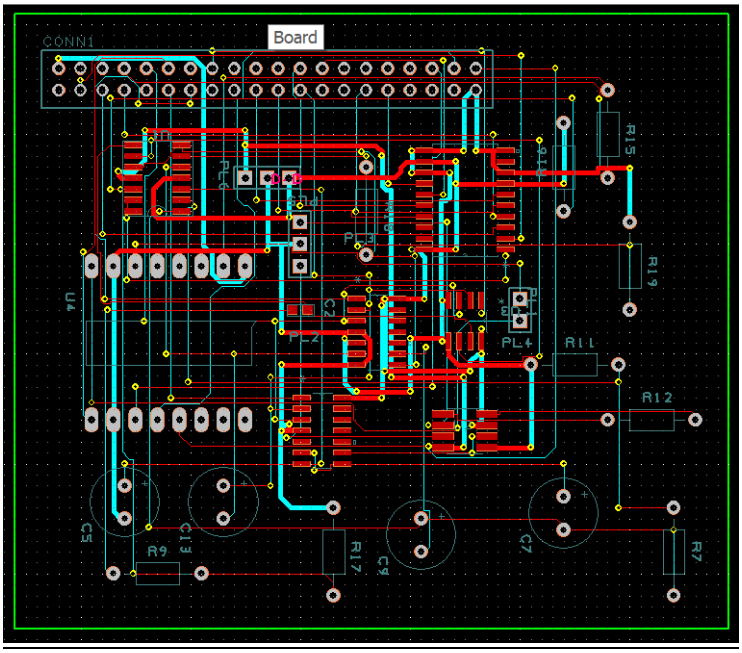


Figure 22. The double sided board used in the design.

The board is a double sided board 3.00 inches by 3.25 inches. Power for the Raspberry Pi Zero and the designed board was provided a 3 package (AAA battery) and four coin cells (CR 2032). The power budget was 450 milliamp hour providing a 2 hour time of operation before the batteries needed recharging. The following figure shows the Raspberry Pi Zero.

Figure 23. The Raspberry Pi Zero.

The final constructed board was carefully soldered by the investigator and is shown in the following two figures.

Figure 24 (above): The completed printed circuit card with mounted components.

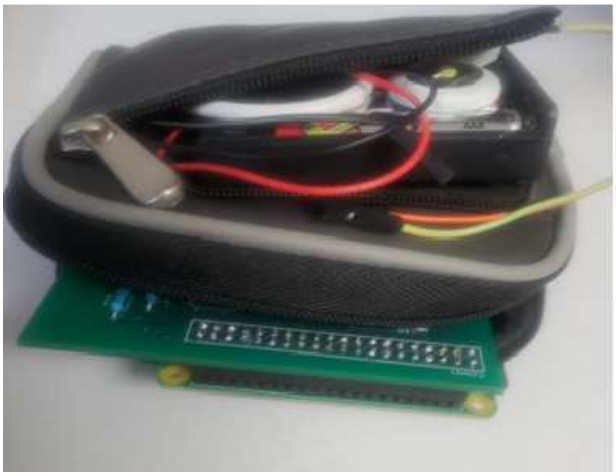


Figure 25: Wearable unit with battery packs.

Software

The software for the Raspberry Pi is Linux. The control program written for the Raspberry Pi was written in “Return to Basic” by Gordon Henderson. This program is shown in the Appendix.

Remote Connection

The Raspberry Pi Zero was connected via the internet remotely to a windows PC laptop using a remote desktop applications for the Raspberry Pi and the PC.

Measurement Procedures

The subtractive paradigm (Smith and Yonovitz, 2017) yields the significance of a tone alone, when compared with the derived response of the “tone plus noise” minus the “noise alone”. When combining evoked potentials in this manner the BM effect is brought forward. The tone plus noise (the masked stimulus) is left when the noise response is subtracted. This graph therefore compares the unmasked “tone alone” (red) to the masked “tone plus noise” (blue).

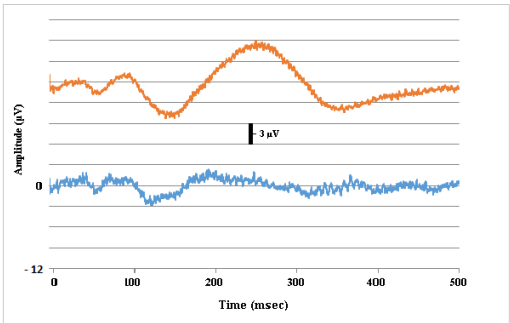


Figure 26: Analysis stragegy for the stimulus conditions.

Evoked potentials will be obtained from a single modelled rat. Each evoked potential was obtained at a silent-gap interval of 20 msec for the target stimulus and noise. The data will yield a BM function.

Analysis Procedures

The analysis procedure used in this experiment is as follows: Modelled stimuli were generated using data for ABR and mid-late evoked potentials taken in previous research from a live rat. This data was fit with a polynomial regression. The coefficients for the polynomial regression were then recalculated to model the effects of lead toxicity on the auditory system. This data generated from real EEG signals in live rats was then input into the novel EEG apparatus to ensure reliability and validity of the device. The output from the EEG device was then observed.

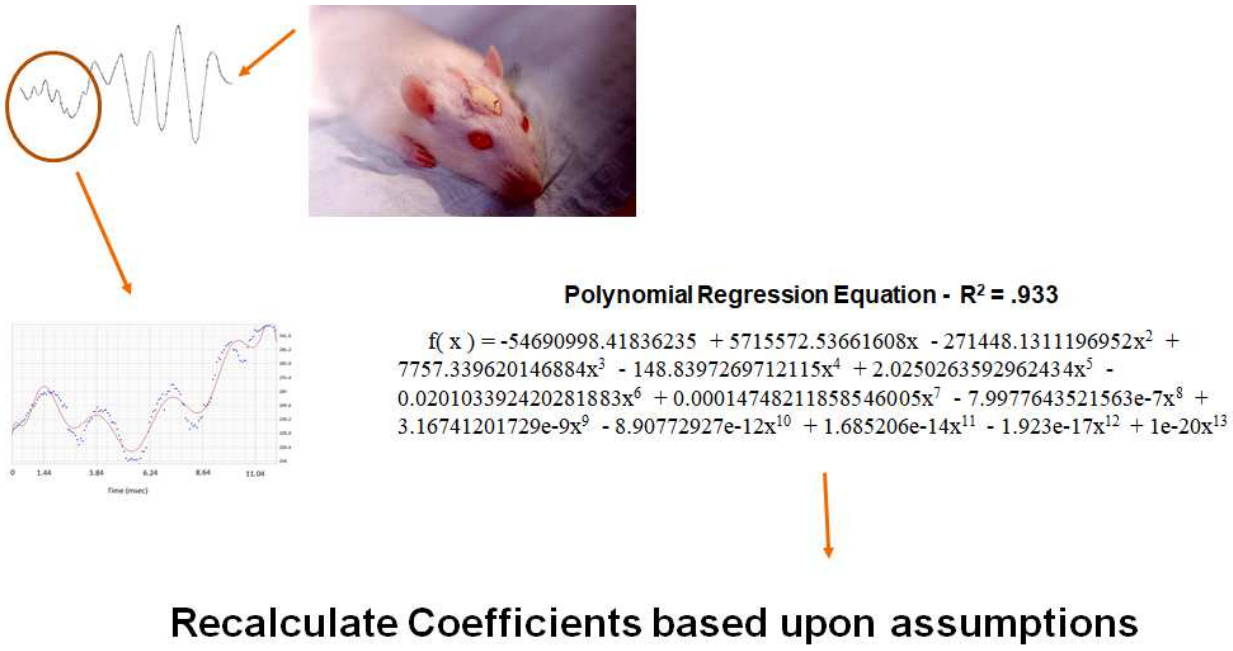


Figure 27: Analysis procedure.

RESULTS

Statistical Treatment of Rat Evoked Potential

In order to establish the function of the rat evoked potential, the EEG was divided into two parts. The first part was the ABR and second was the MID-LATE function. For both the ABR and MID-LATE software emulation, the evoked potential function were obtained, and a hardware evoked potential was derived by signal averaging with the use of the constructed evoked potential unit. The prototype evoked potential and the assumptions that were derived for the lead and BM simulation from Moffitt et al., (2018).

ABR

The ABR (0-10 msec) was digitized and the points are shown in Figure 28 (below). A polynomial regression (Andew Que; http://polynomialregression.drque.net/online.ph) was accomplished for the digitized points.

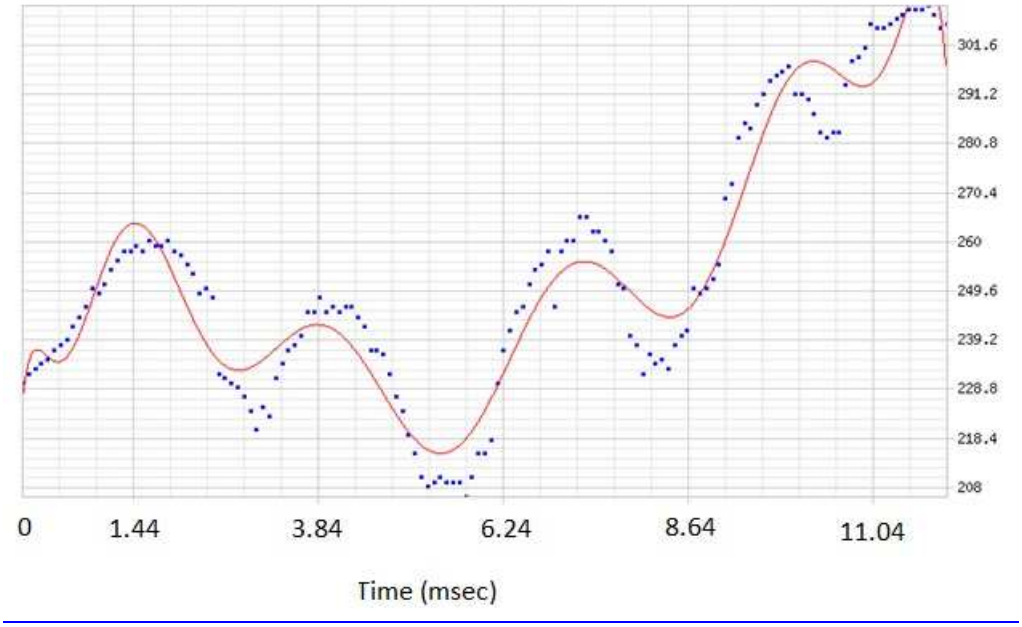


Figure 28 (above): The early ABR with the polynomial fitted line.

The Coefficient of Determination – R^2 was 0.93315759785865. This was a measure of the goodness of fit and indicated that the polynomial fit with 14 coefficients modelled the ABR well.

The polynomial was fit a function with 14 coefficients. The constant and the coefficients were as follows.

Constant	-54690998.41836235035441197685
1)	5715572.536616080131835148 58

2) -271448.13111969521262133969

3) 7757.33962014688372986761

4) -148.83972697121148618587

5) 2.02502635929624333415

6) -0.02010339242028188218

7) 0.00014748211858546006

8) -0.00000079977643521563

9) 0.00000000316741201729

10) -0.00000000000890772927

11) 0.00000000000001685206

12) -0.00000000000000001923

13) 0.00000000000000000001

The regression equation can be expressed as:

$f(x) = -54690998.41836235 + 5715572.53661608x - 271448.1311196952x^2 + 7757.339620146884x^3 - 148.8397269712115x^4 + 2.0250263592962434x^5 - 0.020103392420281883x^6 + 0.00014748211858546005x^7 - 7.9977643521563e\text{-}7x^8 + 3.16741201729e\text{-}9x^9 - 8.90772927e\text{-}12x^{10} + 1.685206e\text{-}14x^{11} - 1.923e\text{-}17x^{12} + 1e\text{-}20x^{13}$

This function was then recalculated with a reduction factor of -10 and -20 dB for the result of the intensity change with the assumption of reduced response to the lead exposure. Figure 29 is shown below.

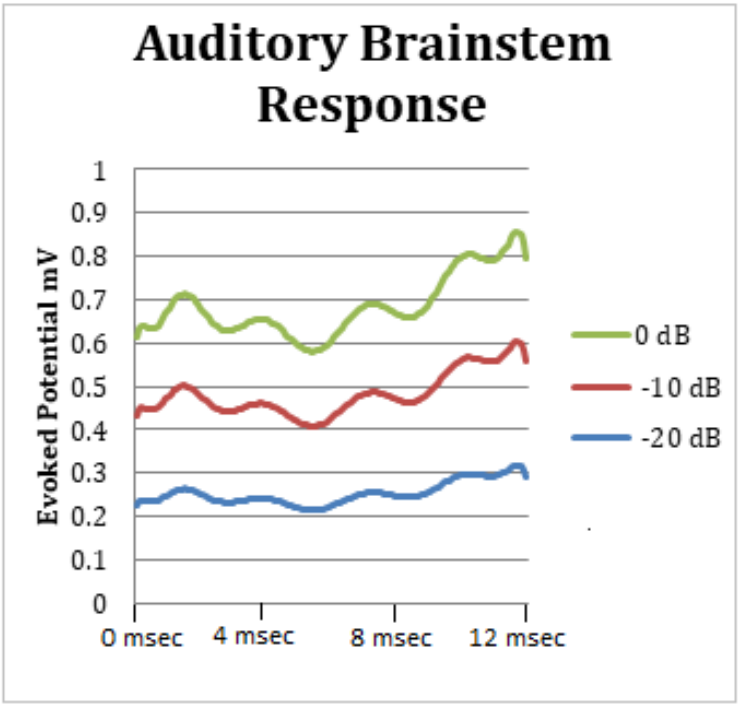


Figure 29: Reduction of ABR response related to simulated lead exposure.

MID-LATE

The Coefficient of Determination – $R^2 = 0.97165196864769$. This was a measure of the goodness of fit and indicated that the polynomial fit with 15 coefficients modeled the MID-LATE as well.

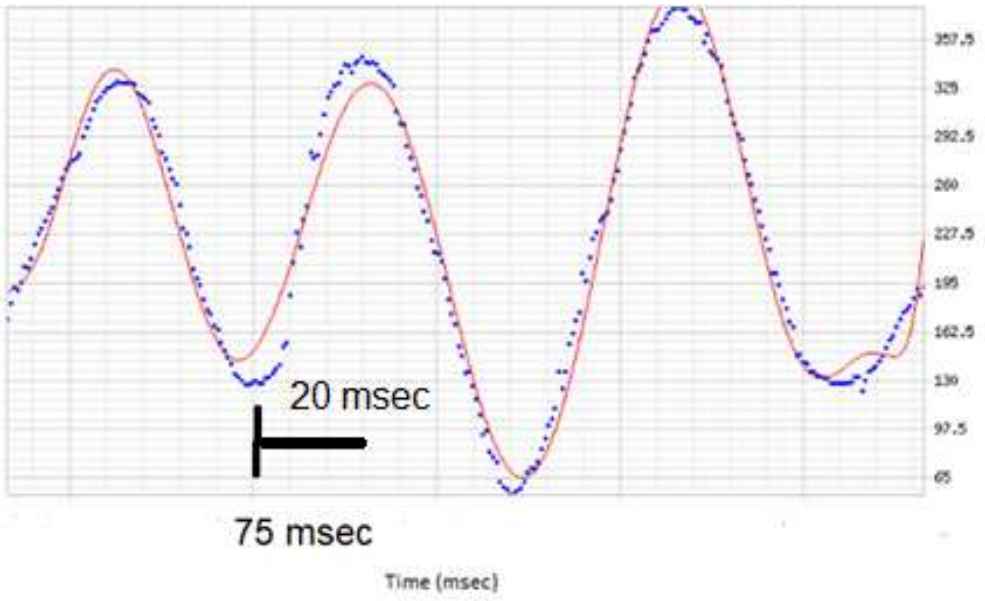


Figure 30; The MID-LATE auditory evoked potential with polynomial fitted line.

The polynomial was fit a function with 15 coefficients. The constant and the coefficients were as follows.

Constant	-41944803599.21700047588622854197
1)	999719873.48563746717548679296
2)	-10779465.18346683729414793803
3)	69407.32467189494893485965
4)	-296.52467448942547207248
5)	0.88258805461130691173
6)	-0.00186758288423461116
7)	0.00000280956679003156

8) -0.00000000294495959501

9) 0.00000000000204847603

8) -0.00000000000000085106

10) 0.0000000000000000016

11) 0.00000000000000000000

12) 0.00000000000000000000

13) 0.00000000000000000000

The regression equation can be expressed as:

f(x) = -41944803599.217 + 999719873.4856374x - 10779465.183466837x2 + 69407.32467189495x3 - 296.5246744894255x4 + 0.882588054611307x5 - 0.001867582884234611x6 + 0.00000280956679003156x7 - 2.94495959501e-9x8 + 2.04847603e-12x9 - 8.5106e-16x10 + 1.6e-19x11

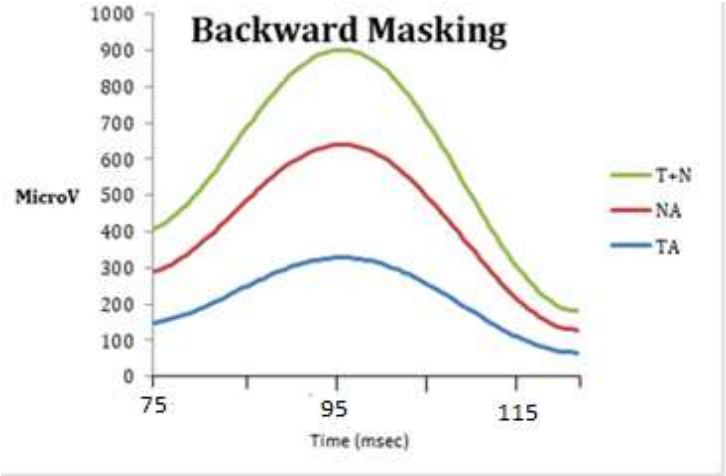


Figure 31 (above): The three random stimuli (Tone plus noise, noise alone, and tone alone), total trials are 400.

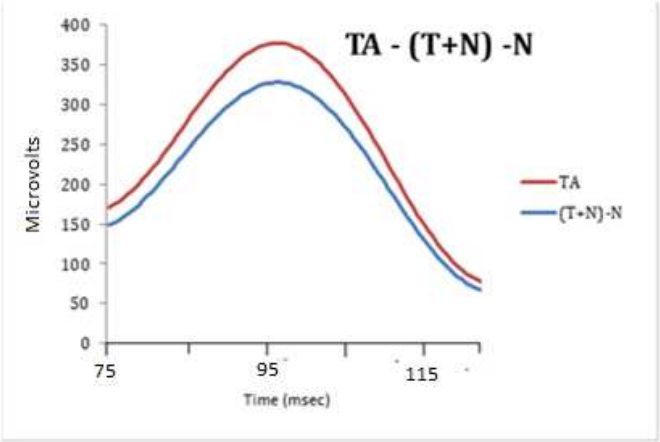


Figure 32: Comparison between Tone Alone (TA) and the Backward Masking Condition (Tone plus noise minus the noise alone).

Discussion and Conclusions

Discussion

It must first be noted that this experiment was not performed on live rats as originally intended. Due to the Coronavirus (COVID-19), animal quarters at the University of Montana were not able to begin any new experiments. Therefore, the present study modelled the effects of lead on the auditory system. The EEG apparatus designed for the study is complete and fully functional. To achieve this model, standardized EEG waveforms were fit with a polynomial regression, and input into the apparatus designed for the experiment, simulating the deleterious effects of lead on the auditory system.

The rat-worn evoked potential system was clearly successful. It was used and implemented by taking the data from the rat model and providing signal averaging and waveform analysis. It is interesting to note that this unit has replaced racks of electronic equipment only in the last 40 years.

Four unique signal types were observed. A 10 msec pure-tone 4,000 Hz wave with a Blackman envelope, a 50 msec white-noise masker, a control condition of silence, and the "masking" condition consisting of the 10 msec pure tone wave followed by a 20 msec silent interval, and the 50 msec white-noise masker. The "masked" condition showed the highest peak amplitude, approximately 900 microvolts.

A reduced detection of the auditory input was modelled using the ABR, simulating a reduced decibel level of perceived noise due to lead exposure.

Two separate time epochs were analyzed, the Auditory Brainstem Response (0-10) msec, and the mid-late evoked potential (approx. 75-115 msec). For the ABR, the reduced detection of the target stimulus due to lead exposure was simulated by -10 dB and -20 dB reductions.

A subtractive paradigm was used to compare the simulated MID-LATE evoked potentials. The response of the tone alone was graphed along with the response to the tone plus noise signal, minus the noise alone signal. This yields the reduced amplitude of the "masked" response to the tone, with the subtraction of the noise alone, the masked tone response is left residual. The BM function is available through this type of analysis.

This approach simulated the effects of lead toxicity in the auditory system, using the outputs from the evoked potential apparatus designed to measure these specific effects. It was shown that both ABR intensity was reduced due to the simulated lead exposure, and that the MID-LATE evoked potentials were reduced amplitude under a backward masking condition.

The current study modelled a reduced amplitude in ABR in response to lead toxicity showing -10 dB, and -20 dB reductions respectively across the waveform. A model of the MID-Late evoked potential also showed a BM effect in the time epoch of 75-115 msec as shown by reduced amplitude in the waveform morphology.

The questions addressed in this study were tested using a model of a live rat. It was shown that a reduction of perception in the auditory pathway is possible to model using real EEG data input through an EEG device. The output from this device is accurate enough to yield the minute electrophysiological changes that are yielded from exposure to lead. It was also shown that a mid-late response to a backward masking task can be modelled using the same paradigm.

Conclusions

There are two central questions addressed in this study. The first: what changes occur in the Auditory Brainstem Response (ABR) in a lead-dosed group as compared to a control group? The second: what changes would occur in the MID-LATE auditory evoked potentials in a lead-dosed group as compared to a control group?

A specifically designed EEG apparatus was designed to test these two questions. To measure middle-late evoked potentials, it is necessary for the animal to be awake. A fully-functional, mobile, backpack-worn device was created to allow measurement of early, middle and late evoked potentials.

Due to the Coronavirus (COVID-19), this experiment was not able to use live rats. Therefore, auditory waveforms reflecting four signal-types under a masking condition were approximated and digitally input through the novel hardware device. A tone alone, noise alone, masking condition and control condition were observed. Simulated ABR outputs showed a model of reduced perception.

This model shows that in the temporal period of 0-10 msec following onset of the auditory stimulus, a lead-effected group will have worse perception as compared to a control group. This is shown in a reduced amplitude of EEG peaks.

The model also showed that in the temporal period 10-1000 msec after onset of auditory stimulus, the MID-LATE latency wave approximately 95 msec after onset exhibited an approximate reduction of 60 microvolts in EEG output during a BM condition. This model locates the disruption of perception in the auditory cortex. In order to observe the response to the masked noise, the response of the "noise alone" was subtracted from the masked "tone plus noise" response. This indicates the masked response to the tone, without the response of the noise on the evoked potential (once subtracted). The tone alone showed a higher amplitude, approximately 75 microvolts at peak, as compared to the masked response of tone plus noise minus the noise alone.

It can be concluded from this study, that a model of lead toxicity in a rodent is possible to achieve with the use of an active EEG device.

Limitations

The main limitation of the current study is clear: the experiment was not able to be carried out on live rats as designed. A study of live rats would possibly have been more robust than using a modelled animal. In this same vein, a better fit of the polynomial regression to generate data would lead to more accurate outputs generated from the EEG device.

Future Directions

In the future, the goal will be to complete this same model of study in a live animal experiment. The apparatus developed for testing this experiment can also be used for many other investigations that require EEG testing in a live rodent, its potential applications are widespread.

The ultimate goal of this research is to understand the mechanism underlying APD. A knowledge of the neural loci of this type of disorder may yield a more effective diagnosis and treatment.

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Silas Smith Vitae

Silas Smith

silas.smith@umontana.edu

PO Box 954, Bonner, MT 59823 (360) 359-2140

Personal Profile

I am an enthusiastic, creative and professional person who can be trusted to complete a task. I work hard, am very determined and able to work unsupervised. I am also able to take initiative and easily adapt to environmental situations. Throughout my career, I have learned the benefit of research and the role of a research posture in approaching clinical work. This has allowed me to develop excellent problem-solving, personal and communication skills, which allows me to relate easily to others. I am reliable, I always strive to keep a positive attitude and am able to learn quickly.

Education

The University of Montana – Missoula, MT
Bachelor of Arts in Communicative Sciences and Disorders, May, 2014
Supervisor: Al Yonovitz, PhD

The University of Montana – Missoula, MT
MS Interdisciplinary Studies -Experimental Psychology and Communicative Sciences & Disorders, May 2016
Supervisor: Al Yonovitz, PhD

The University of Montana – Missoula, MT
Ph.D.
Speech, Language & Hearing Sciences, PhD (May, 2020)
Supervisor: Al Yonovitz, PhD

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Professional Experience

1. Manager at high-end instrument store – Greg Boyd's House of Fine Instruments
2. Taught Acoustics Course at University of Montana
3. Musician

Professional Presentations and Publications

Smith, Silas and Yonovitz, Al. The cortical event related potentials prior to speech: Intra and inter-speaker variation. Presented at the Acoustical Society of America Meeting, 2014.

Quigley, Rita, Joyce, Kara. Smith, Silas, and Yonovitz, Al. The audibility of the cricket, acheta domesticus between 4000-48000 Hz. Presented at the American Entomology Association, 2014.

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Silas Smith Robert Sears and Al Yonovitz. Backward masking determination with simultaneous early, middle and late evoked potentials. Presentation at the Acoustical Society of America meeting, 2016.

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Robert Sears, Silas Smith, Sarah Schied and Al Yonovitz. The ISI critical value in backward masking testing. Presentation at the Acoustical Society of America meeting, 2016.

Robert Sears, Silas Smith and Al Yonovitz. Comparison of Blackman, linear rise-fall, and linear rise-fall chirp signals in backward masking. Presentation at the Acoustical Society of America meeting, 2016.

Allie Cope, Kendra Foster, Silas Smith, Robert Sears and Al Yonovitz. Backward masking of vowel-consonant stimuli. Presentation at the Acoustical Society of America meeting, 2016.

Silas Smith, Robert Sears, Nancy Dold and Al Yonovitz. Backward Masking Determination with Early, Middle and Late Evoked Potentials. Presented at the American Academy of Audiolo gy, 2016.

Robert Sears, Sarah Schied, Silas Smith and Al Yonovitz. The ISI Critical Value in Backward Masking Testing. Presented at the American Academy of Audiology, 2016.

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Silas Smith, Robert Sears, Kendall Alley, Nicole Aline, Taylor Perius, Al Yonovitz. Backward masking determination with simultaneous early, middle and late evoked potentials. Presentation at the American Speech-Language-Hearing Association Convention (2016).

Robert Sears, Silas Smith, Bridget Brannan, Alison Arthun, Al Yonovitz. The clinical determination of the Inter-Stimulus Interval (ISI) Critical Value in Backward Masking Testing. Presentation at the American Speech-Language-Hearing Association Convention (2016).

Robert Sears, Silas Smith, Brittany Galvin and Al Yonovitz. Behavioral and electrophysiological evidence for backward masking. Presented at the Acoustical Society of America Meeting, 2016.

Silas Smith, Robert Sears and Al Yonovitz. Event related speech readiness potentials to spoken words and CV's. Presented at the Acoustical Society of America Meeting, 2016.

Silas Smith and Al Yonovitz. Objective determination of backward masking. Presented at the Acoustical Society of America Meeting, 2017.

Yonovitz, Al, Smith, Silas, and Kincheloe, Harley. The hearing ability of the cricket (*acheta domesticus*) Presented at the American Entomology Society. 2017.

Yonovitz, Al and Smith Silas. Consonant perception and improved S/N ratio using harmonic tracking equalization. Presented at the Audio Engineering Society, 2017.

Smith, Silas and Yonovitz, Al. Backward Masking Determination with Middle and Late-Evoked Potentials. Presented at the American Academy of Audiology, 2018.

Silas Smith and Yonovitz, Al. Event-Related cortical potentials occurring prior to speech initiation. Presented at the Acoustical Society of America Meeting, 2019.

Silas Smith and Yonovitz, Al. Design of a miniature evoked potential testing system for animal assessment of hearing. Presented at the Acoustical Society of America Meeting, 2019.

In Preparation

The cortical event related potentials prior to speech: Intra and inter-speaker variation

The audibility of the cricket, acheta domesticus between 4000-48000 Hz

Real time analysis of electrical brain activity preceding speech

Consonant and distinctive feature perception under backward masking conditions

Backward masking determination with simultaneous early, middle and late evoked potentials

The ISI critical value in backward masking testing

Comparison of Blackman, linear rise-fall, and linear rise-fall chirp signals in backward masking

The clinical determination of the Inter-Stimulus Interval (ISI) Critical Value in Backward Masking Testing

Objective determination of backward masking

Consonant perception and improved S/N ratio using harmonic tracking equalization

Design of a miniature evoked potential testing system for animal assessment of hearing

Work Experience

Greg Boyd's House of Fine Instruments (2010-2020)

Appendix 1

Previous Studies on Backward Masking

Efficacy of three backward masking signals (with R. Sears, A. Yonovitz)

I. Introduction

Individuals that have Auditory Processing Disorders (APD) are shown to have higher levels of auditory Backward Masking (BM) (Hartley & Moore, 2002; McArthur & Hogben, 2001). Therefore, a measure of BM may be diagnostically relevant to those with APD's. This study determined response times of individuals in a BM paradigm with three different BM signal types.

Auditory BM occurs when a target stimulus has reduced auditory perception when paired with a "masking" noise that occurs later temporally (Raab, 1963). This reduction in perception of auditory stimuli manifests in a number of communication disorders (Johnson, et al., 2007; Marler & Champlin, 2005; Marler, et al., 2002). In order to be "masked" a target signal does not need to be completely unheard. Any reduction in target signal caused by a following noise is a result of the masking effect. There may also be forward (target tone after masking noise) and simultaneous (target tone simultaneous with masking noise) masking effects. However, in the auditory system, backward masking has been shown to have the greatest implications (Raab, 1963).

II. Auditory Processing Disorders

APD's involve a disruption in audition in the Central Nervous System (CNS), rather than the peripheral hearing system. It has been proposed that Specific Language Impairment (SLI) has an underlying factor of APD (Johnson, et al., 2007; Marler, & Champlin, 2005). Developmental

disorders and acquired developmental disorders have shown also to be tied with APD (Hampton & Weber-Fox, 2008; Bamiou, et al., 2006; Lew, et al., 2007; Finkelstein, et al., 1998). Therefore, a diagnostic component of BM may prove useful in treatment of many speech and auditory disorders. The diagnosis of APD is critical to many children's well being, and is often overlooked. This is partially due to the fact that they perform within normal limits in a pure-tone audiometry test. In addition to auditory processing, BM has been shown to have an age-related decline in ability to discern temporally close auditory inputs (Buss, et al., 2000). The implications of BM are clear, but remain underrepresented in clinical applications.

It has been shown that EEG measurement of Auditory Brainstem Response (ABR) can effectively measure perception of masked acoustic stimuli. Research has shown that children with SLI have a reduced EEG response that suggests disruption in the auditory pathway (Johnson, et al., 2007; Marler, & Champlin, 2005).

III. Current Study

The present study determined the perception of three different 20 msec signal-types in the presence of a 50 msec Gaussian masking noise. The differential signals were: a linear sweep (chirp) tone (750 Hz-4,000 Hz) with a 5 msec rise and fall, a 1,000 Hz tone with a 5 msec linear rise and fall, and a 1,000 Hz tone with a Blackman function envelope. The response time measured to these tones was determined as an "ease of task" paradigm. This study showed that signal-type has a measurable effect on BM perception. This manifested in changes in response time. The quickest response time was consistently the 1,000 Hz tone with a trapezoidal envelope.

The method of measurement was a simple button-press when the participant heard the target tone (in presence of the masking noise). The threshold of audibility of the target tone was

determined by a 5 dB decrease of tone, until the target was not heard, followed by a 2 dB up/down adjustment. Stimuli were presented monaurally.

Variables examined were signal type and intensity, Inter-Stimulus Interval (ISI) length, perception of target tone (yes or no), response time, and the standard deviation of up/down reversals in order to measure the threshold.

It was shown that signal type had a significant effect on thresholds of audibility in a BM paradigm, although statistical power of the study was low. Response times were consistently faster when presented with the 1,000 Hz tone fitted with a linear rise/fall envelope. It was also shown that signal type affected response time, and an interaction between signal type, response time, and ISI was observed.

This study showed that different signal types variably affect BM perception and response time.

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Backward Masking with Simultaneous Early, Middle and Late Evoked Potentials

I. Introduction

Auditory Processing Disorders (APDs) affect hearing ability, despite intact outer, middle, and inner ear functions (Howell et al., 2000). The most common dysfunction presented in those with APD is a difficulty discriminating auditory inputs that are presented in quick succession. This effect is due in part to a phenomenon known as Backward Masking (BM) (Musiek, 2007). BM in the auditory sense occurs when two or more acoustic stimuli are presented, and the later stimulus obscures the detection of an earlier signal (Raab, 1963). The neural locus of this effect is not known; however, a determination of this effect may lead to improved diagnosis and intervention for many APDs.

Electrophysiological responses have been measured in response to masking paradigms (Musiek, 2013). Most electrophysiological measures have been used in a forward-masking paradigm. These electroencephalogical (EEG) measures are determined through recording of electrical signals in the brain, which are then amplified, and filtered to yield a result. Deviance in the latency and amplitude of measured EEG waveforms in response to BM paradigms may indicate the locus of disruption in the auditory pathway. This is due to a standardized waveform morphology in the EEG response to an auditory stimulus.

The present study measured EEG responses in a BM paradigm. The data revealed that an observable change in EEG waveform was present 90-250 msec after the onset of the auditory stimulus. These results indicate that the disruption in the auditory pathway occurs in the midbrain to the auditory cortex.

II. Backward Masking

BM refers to the disrupted perception of a target stimulus when one or more stimuli closely follow temporally. Masking is a phenomenon that occurs in every perceptual sense, and has been observed at least as early as 1902 in a visual paradigm (Raab, 1963).

Some studies claim that BM occludes temporal processing in the brain stem (Wright, 1997; Tahaei, 2014).

Miller (1947) described a disruption in perception of multiple sounds at once, this was identified as “masking”. This masking effect was reported to be manipulated by amplitude of masker relative to target stimulus, frequency(ies) of target and masker, and Inter-Stimulus Interval (ISI) between target tone and masker. It was noted that masking noises at lower frequencies, shorter ISIs, and louder amplitude had larger effects.

It has been shown that BM effects deteriorate with age (Gehr & Sommers, 1999). This has been determined both with decibel threshold and ISI. Accuracy of temporal processing has also been shown to follow the pattern of development in the auditory system (Hartley, Wright, Hogan & Moore, 2000) - as audition improves on a normal development curve, so does temporal processing ability.

III. AEPs

The disruption in temporal processing may be understood through EEG measurements. Through an analysis of Auditory Evoked Potentials (AEPs), the specific site of disruption may be identified. Several waveform “peaks” in EEG response to auditory stimuli are indicative of where in the brain the stimuli are being processed. Musiek (2013) reported several key features in the auditory brainstem response measured through EEG that are indicative of accurate auditory processing. The waves of primary importance were stated as waves III, IV and V which

indicate proper functioning in the brainstem when the amplitudes and latencies are comparable to the standardized EEG waveforms.

Another study determined that in wave V of the EE was disrupted in children with Language Learning Impairments (LLIs) (Marler & Champlin, 2005). This study observed that the control group and LLI groups EEGs were not significantly different under a no masking condition. However, when exposed to a BM task, the LLI group was significantly impaired.

Another waveform significant to temporal processing ability is the Mis-Matched Negativity (MMN) response (De Pascalis & Variale, 2012). In this study mental ability was linked to BM response in terms of ISI times. The MMN response was diminished in amplitude with observants who had lower auditory discrimination ability, and that the MMN response was longer in these participants as well.

IV. CAPDs

Central Auditory Processing Disorders (CAPDs) have been identified at least since 1954. This identification was a hearing disorder that occurs despite a normal pure-tone audiogram (Myklebust, 1954). This means that individuals who are able to hear single noises normally, sometimes fail to hear multiple tones.

CAPDs are generally defined as a disruption in audition occurring after the cochlear response. It is also generally agreed upon that CAPD is not a disorder of higher level cognitive functioning such as attention (Griffiths, 2002). There is sufficient research to show that BM and temporal processing are intertwined with APD.

V. Current Study

The current study observed the masking effects of 4 different signal types: a target noise (pure-tone or pulse), followed by a 10msec ISI, and then a 50 msec masking noise. This condition was compared to the target signal alone, the masking noise alone, and a control condition of silence.

This study revealed that in these masking conditions, an effect was seen in the 90-250 msec range of the waveform morphology. This indicates that the disruption occurred in the midbrain to the auditory cortex.

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Appendix 2: Software

```
110 REM PROGRAM TO TAKE EP'S
120 REM define variables
130 pinMode (19, pinOutput)
140 pinMode (3, pinInput)
150 pinMode (5, pinInput)
160 pinMode (7, pinInput)
170 pinMode (24, pinInput)
180 pinMode (26, pinInput)
190 pinMode (29, pinInput)
200 pinMode (31, pinInput)
210 pinMode (10, pinOutput)
220 pinMode (23, pinOutput)
230 pinMode (21, pinInput)
240 pinMode (13, pinOutput)
250 pinMode (16, pinOutput)
260 pinMode (12, pinOutput)
270 pinMode (11, pinOutput)
280 delob = 5000
300 pi1 = sOpen ("/dev/ttyS0", 9600)
310 REM initialize dfplyer
320 REM Gain SI=10 - CS=16 - SCK=23
330 REM hi SI=10 - CS=13 SCK=23
340 REM lo SI=10  - CS=11 - SCK=23
350 REM control pin dfplayer
360 digitalWrite (12, 1)
362 PRINT "Subject Code (6 Char): ";
364 INPUT code$
366 PRINT "Variable fixed stimulus (v or stim val: ";
368 INPUT stim$
370 PRINT "Enter number of trials: ";
380 INPUT n
390 PRINT "Enter - Lat=1- Mid=2 - Ear=3: ";
400 INPUT eptype
410 PRINT "Enter Gain (1-254): ";
420 INPUT gain
430 PRINT "Enter Volume (0-30): ";
440 INPUT vol
490 REM reset df player
500 sPut (pi1, 126)
510 sPut (pi1, 255)
520 sPut (pi1, 6)
530 sPut (pi1, 12)
540 sPut (pi1, 0)
550 sPut (pi1, 0)
560 sPut (pi1, 239)
570 WAIT (.1)
580 WAIT (5)
590 REM Norw for df player
600 sPut (pi1, 126)
610 sPut (pi1, 255)
620 sPut (pi1, 6)
630 sPut (pi1, 11)
```

```
 640 sPut (pi1, 0)
 650 sPut (pi1, 0)
 660 sPut (pi1, 0)
 670 sPut (pi1, 239)
 680 WAIT (.1)
 690 REM Sp for df player
 700 sPut (pi1, 126)
 710 sPut (pi1, 255)
 720 sPut (pi1, 6)
 730 sPut (pi1, 9)
 740 sPut (pi1, 0)
 750 sPut (pi1, 0)
 760 sPut (pi1, 2)
 770 sPut (pi1, 239)
 780 WAIT (.1)
 790 REM volume df player
 800 sPut (pi1, 126)
 810 sPut (pi1, 255)
 820 sPut (pi1, 6)
 830 sPut (pi1, 6)
 840 sPut (pi1, 0)
 850 sPut (pi1, 0)
 860 sPut (pi1, vol)
 870 sPut (pi1, 239)
 880 WAIT (.1)
 890 REM digitalWrite (12, 0)
 900 REM WAIT (.1)
 910 REM digitalWrite (12, 1)
 920 REM Gain Control Pins
 930 digitalWrite (16, 1)
 940 digitalWrite (23, 0)
 950 digitalWrite (10, 0)
 960 REM hi control pins
 970 digitalWrite (13, 1)
 980 digitalWrite (23, 0)
 990 digitalWrite (10, 0)
1000 REM lo contol pins
1010 digitalWrite (11, 1)
1020 digitalWrite (23, 0)
1030 digitalWrite (10, 0)
1040 REM A/D pins
1050 digitalWrite (19, 0)
1060 DIM rawdat(255)
1070 DIM epavg(255)
1080 DIM epsum(255)
1085 DIM rand(1000)
1090 DIM A(15)
1100 DIM pexp(16)
1102 DIM sum1(256)
1104 DIM sum2(256)
1106 DIM sum3(256)
1108 DIM sum4(256)
1110 hnum1 = 0
1112 hnum2 = 0
```

```
1114 hnum3 = 0
1116 hnum4 = 0
1120 REM clear variables
1130 FOR j = 0 TO 255 CYCLE
1140   rawdat(j) = 0
1150   epavg(j) = 0
1160   epsum(j) = 0
1170 REPEAT
1180 FOR j = 0 TO 15 CYCLE
1190   pexp(j) = 0
1200   A(j) = 0
1202 REPEAT
1204 zzz$ = "RANDORD.TXT"
1206 myfile0 = openIn (zzz$)
1208 FOR j = 1 TO 1000 CYCLE
1210   input# myfile0, dat1
1220   rand(j) = dat1
1222 REPEAT
1224 close (myfile0)
1228 hgr
1230 RAD
1240 origin (128, 0)
1250 colour = Black
1260 FOR j = 0 TO 255 CYCLE
1270   FOR k = 0 TO 255 CYCLE
1280     plot (k, j)
1290   REPEAT
1300 REPEAT
1310 IF eptype = 1 THEN hi = 255
1320 IF eptype = 2 THEN hi = 128
1330 IF eptype = 3 THEN hi = 3
1340 REM IF eptype = 1 THEN lo = 143
1350 IF eptype = 1 THEN lo = 254
1360 IF eptype = 2 THEN lo = 245
1370 IF eptype = 3 THEN lo = 255
1380 IF eptype = 1 THEN delep = 4000
1390 IF eptype = 2 THEN delep = 1300
1400 IF eptype = 3 THEN delep = 1
1410 REM gain spi
1420 GOSUB 2850
1430 pexp(3) = 1
1440 pexp(7) = 1
1450 digitalWrite (16, 0)
1460 FOR j = 0 TO 15 CYCLE
1470   digitalWrite (10, pexp(j))
1480   WAIT (.01)
1490   digitalWrite (23, 1)
1500   WAIT (.01)
1510   digitalWrite (23, 0)
1520   WAIT (.01)
1530 REPEAT
1540 digitalWrite (16, 1)
1550 REM hi spi
1560 gain = hi
```

```
 1570 GOSUB 2850
 1580 pexp(3) = 1
 1590 pexp(6) = 1
 1600 pexp(7) = 1
 1610 digitalWrite (13, 0)
 1620 FOR j = 0 TO 15 CYCLE
 1630   WAIT (.01)
 1640   digitalWrite (23, 1)
 1650   WAIT (.01)
 1660   digitalWrite (23, 0)
 1670   WAIT (.01)
 1680 REPEAT
 1690 digitalWrite (13, 1)
 1700 REM lo spi
 1710 gain = lo
 1720 GOSUB 2850
 1730 pexp(3) = 1
 1740 pexp(4) = 1
 1750 pexp(7) = 1
 1760 digitalWrite (11, 0)
 1770 FOR j = 0 TO 15 CYCLE
 1780   digitalWrite (10, pexp(j))
 1790   WAIT (.01)
 1800   digitalWrite (23, 1)
 1810   gain = lo
 1820   WAIT (.01)
 1830   digitalWrite (23, 0)
 1840   WAIT (.01)
 1850 REPEAT
 1860 digitalWrite (11, 1)
 1870 colour = Black
 1880 FOR j = 1 TO 254 CYCLE
 1890   FOR k = 1 TO 254 CYCLE
 1900     plot (k, j)
 1910   REPEAT
 1920 REPEAT
 1930 update
 1940 FOR g = 0 TO 255 CYCLE
 1950   FOR del50 = 1 TO delob CYCLE
 1960   REPEAT
 1970   digitalWrite (19, 1)
 1980   digitalWrite (19, 0)
 1990   a0 = digitalRead (3)
 2000   a1 = digitalRead (5)
 2010   a2 = digitalRead (7)
 2020   a3 = digitalRead (29)
 2030   a4 = digitalRead (31)
 2040   a5 = digitalRead (26)
 2050   a6 = digitalRead (24)
 2060   a7 = digitalRead (21)
 2070   rawdat(g) = (a0 * 1) + (a1 * 2) + (a2 * 4) + (a3 * 8) + (a4 * 16)
+ (a5 * 32) + (a6 * 64) + (a7 * 128)
 2080 REPEAT
 2090 colour = White
```

```
 2100 rect (0, 0, 256, 256, FALSE)
 2110 k = 0
 2120 FOR j = 0 TO 255 CYCLE
 2130   plot (k, rawdat(j))
 2140   k = k + 1
 2150 REPEAT
 2160 update
 2170 WHILE INKEY = -1 CYCLE
 2180   GOTO 1870
 2190 REPEAT
 2200 FOR h = 1 TO n CYCLE
 2202   IF stim$ <> "v" THEN rand(h) = VAL (stim$)
 2210   hvTab (30, 20)
 2220   PRINT "           ";  ;
 2222   IF rand(h) = 1 THEN lable$ = "TA"
 2223   IF rand(h) = 2 THEN lable$ = "NA"
 2224   IF rand(h) = 3 THEN lable$ = "TN"
 2225   IF rand(h) = 4 THEN lable$ = "SI"
 2230   PRINT h;  "    ";  lable$;
 2240   colour = Black
 2250   FOR j = 1 TO 254 CYCLE
 2260     FOR k = 1 TO 254 CYCLE
 2270       plot (k, j)
 2280     REPEAT
 2290   REPEAT
 2300   REM play bak df player
 2310   sPut (pi1, 126)
 2320   sPut (pi1, 255)
 2330   sPut (pi1, 6)
 2340   sPut (pi1, 15)
 2350   sPut (pi1, 0)
 2360   sPut (pi1, 1)
 2370   sPut (pi1, rand(h))
 2380   sPut (pi1, 239)
 2390   WAIT (.1)
 2400   FOR g = 0 TO 255 CYCLE
 2410     FOR del50 = 1 TO delep CYCLE
 2420     REPEAT
 2430     digitalWrite (19, 1)
 2440     digitalWrite (19, 0)
 2450     a0 = digitalRead (3)
 2460     a1 = digitalRead (5)
 2470     a2 = digitalRead (7)
 2480     a3 = digitalRead (29)
 2490     a4 = digitalRead (31)
 2500     a5 = digitalRead (26)
 2510     a6 = digitalRead (24)
 2520     a7 = digitalRead (21)
 2530     rawdat(g) = (a0 * 1) + (a1 * 2) + (a2 * 4) + (a3 * 8) + (a4 *
16) + (a5 * 32) + (a6 * 64) + (a7 * 128)
 2540   REPEAT
 2550   colour = White
 2560   rect (0, 0, 256, 256, FALSE)
 2562   IF rand(h) = 1 THEN GOTO 4000
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```
 2564   IF rand(h) = 2 THEN GOTO 4500
 2566   IF rand(h) = 3 THEN GOTO 5000
 2568   IF rand(h) = 4 THEN GOTO 5500
 2600   FOR j = 0 TO 255 CYCLE
 2610     epavg(j) = INT (epsum(j) / hnum)
 2620     k = k + 1
 2630   REPEAT
 2650   k = 0
 2660   FOR j = 0 TO 255 CYCLE
 2670     plot (k, epavg(j))
 2680     k = k + 1
 2690     update
 2700   REPEAT
 2710   WAIT (2)
 2730   colour = Black
 2740   FOR j = 1 TO 254 CYCLE
 2750     FOR k = 1 TO 254 CYCLE
 2760       plot (k, j)
 2770     REPEAT
 2780   REPEAT
 2790   update
 2800 REPEAT
 2802 PRINT "         Save (y or n): ";
 2804 INPUT sav$
 2805 IF sav$ = "n" THEN GOTO 6240
 2807 IF sav$ = "y" THEN GOTO 6000
 2819 END
 2850 REM Decimal to Binary
 2860 fdiv = gain / 128
 2870 IF fdiv >= 1 THEN A(0) = 1
 2880 sdiv = (gain - (A(0) * 128)) / 64
 2890 IF sdiv >= 1 THEN A(1) = 1
 2900 tdiv = (gain - ((A(0) * 128) + (A(1) * 64))) / 32
 2910 IF tdiv >= 1 THEN A(2) = 1
 2920 fourthdiv = (gain - ((A(0) * 128) + (A(1) * 64) + (A(2) * 32))) / 16
 2930 IF fourthdiv >= 1 THEN A(3) = 1
 2940 fifthdiv = (gain - ((A(0) * 128) + (A(1) * 64) + (A(2) * 32) + (A(3)
* 16))) / 8
 2950 IF fifthdiv >= 1 THEN A(4) = 1
 2960 sixthdiv = (gain - ((A(0) * 128) + (A(1) * 64) + (A(2) * 32) + (A(3)
* 16) + (A(4) * 8))) / 4
 2970 IF sixthdiv >= 1 THEN A(5) = 1
 2980 seventhdiv = (gain - ((A(0) * 128) + (A(1) * 64) + (A(2) * 32) +
(A(3) * 16) + (A(4) * 8) + (A(5) * 4))) / 2
 2990 IF seventhdiv >= 1 THEN A(6) = 1
 3000 eigthdiv = (gain - ((A(0) * 128) + (A(1) * 64) + (A(2) * 32) + (A(3)
* 16) + (A(4) * 8) + (A(5) * 4) + (A(6) * 2))) / 1
 3010 IF eigthdiv >= 1 THEN A(7) = 1
 3020 pexp(8) = A(0)
 3030 pexp(9) = A(1)
 3040 pexp(10) = A(2)
 3050 pexp(13) = A(5)
 3060 pexp(11) = A(3)
 3070 pexp(12) = A(4)
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```
 3080 pexp(13) = A(5)
 3090 pexp(14) = A(6)
 3100 pexp(15) = A(7)
 3110 PRINT pexp(8);  pexp(9);  pexp(10);  pexp(11);  pexp(12);  pexp(13);
pexp(14);  pexp(15)
 3120 RETURN
 3130 END
 4000 REM Sum1
 4002 hnum1 = hnum1 + 1
 4004 hnum = hnum1
 4010 FOR g = 0 TO 255 CYCLE
 4020   sum1(g) = sum1(g) + rawdat(g)
 4030 REPEAT
 4040 FOR g = 0 TO 255 CYCLE
 4050   epsum(g) = sum1(g)
 4052 REPEAT
 4054 colour = Yellow
 4060 GOTO 2600
 4500 REM Sum2
 4502 hnum2 = hnum2 + 1
 4504 hnum = hnum2
 4510 FOR g = 0 TO 255 CYCLE
 4520   sum2(g) = sum2(g) + rawdat(g)
 4530 REPEAT
 4540 FOR g = 0 TO 255 CYCLE
 4550   epsum(g) = sum2(g)
 4552 REPEAT
 4554 colour = Red
 4560 GOTO 2600
 5000 REM Sum3
 5002 hnum3 = hnum3 + 1
 5004 hnum = hnum3
 5010 FOR g = 0 TO 255 CYCLE
 5020   sum3(g) = sum3(g) + rawdat(g)
 5030 REPEAT
 5040 FOR g = 0 TO 255 CYCLE
 5050   epsum(g) = sum3(g)
 5052 REPEAT
 5054 colour = Blue
 5060 GOTO 2600
 5500 REM Sum4
 5502 hnum4 = hnum4 + 1
 5504 hnum = hnum4
 5510 FOR g = 0 TO 255 CYCLE
 5520   sum4(g) = sum4(g) + rawdat(g)
 5530 REPEAT
 5540 FOR g = 0 TO 255 CYCLE
 5550   epsum(g) = sum4(g)
 5552 REPEAT
 5554 colour = Green
 5560 GOTO 2600
 6000 REM 6000 Save
 6010 code1$ = code$ + "1.txt"
 6020 code2$ = code$ + "2.txt"
```

```
6030 code3$ = code$ + "3.txt"
6040 code4$ = code$ + "4.txt"
6050 myfile1 = openUp (code1$)
6055 FOR g = 0 TO 255 CYCLE
6060   print# myfile1, sum1(g)
6070 REPEAT
6080 close (myfile1)
6090 myfile1 = openUp (code2$)
6100 FOR g = 0 TO 255 CYCLE
6110   print# myfile1, sum2(g)
6120 REPEAT
6130 close (myfile1)
6140 myfile1 = openUp (code3$)
6150 FOR g = 0 TO 255 CYCLE
6160   print# myfile1, sum3(g)
6170 REPEAT
6180 close (myfile1)
6190 myfile1 = openUp (code4$)
6200 FOR g = 0 TO 255 CYCLE
6210   print# myfile1, sum4(g)
6220 REPEAT
6230 close (myfile1)
6240 END
```