ABSTRACT

The Auditory Brainstem Response and Autism

Katherine Russell

Director: L. Joseph Achor, M.A., Ph.D.

The goal of this thesis is to explain the use of the auditory brainstem response (ABR) in research on autism and autism spectrum disorders (ASD). Following an explanation of the ABR and its evolution as a diagnostic and research tool in many fields, we concentrate on its role in autism research. ASD is a complex disorder and likewise has a complex etiology including genetic and environmental factors, among which may include brainstem abnormalities. ABR demonstrates these abnormalities; slower conduction and increased latencies have been observed in the ASD population more frequently than in the normal population. These studies have led to a range of theories on the contribution of brainstem and neural development to ASD. Although data has been historically inconsistent at times, patterns have emerged that may prove ABR a useful diagnostic predictor for ASD and reveal differences in subpopulations of patients with ASD, including gender differences.

APPROVED BY DIRECTOR OF HONORS THESIS:

L. Joseph Achor

Dr. L. Joseph Achor, Department of Psychology & Neuroscience

APPROVED BY THE HONORS PROGRAM

Dr. Elizabeth Corey, Director

DATE: _____

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Kate Russell

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CHAPTER ONE

Introduction to the Auditory Brainstem Response

The human auditory system is often underestimated in its complexity. This system allows one to hear the rush of ocean waves against the shore, birds singing softly at sunrise, and the voices of loved ones. Sound waves travel through air to reach the ear canal, which funnels and amplifies waves as they hit the eardrum, causing it to vibrate and initiate a cascade of movement in the tiny ossicle bones. The ossicles' movement against the cochlea ripples fluid within the inner ear, setting into motion thousands of tiny hair cells. Functioning as the sensory receptors of the auditory system, these hair cells are the final conversion point from sound wave to electrical impulse, which will travel through axons throughout a large portion of the brain, beginning in the brainstem.

The electrical activity can be recorded using electrodes attached to the scalp with the use of special computing techniques. The electrical impulses generated in the brainstem and recorded from the scalp in response to auditory stimuli have had many names. Jewett and Williston, founders of the numbering system of the peaks in these recordings, named the impulses "auditory evoked potentials" (Jewett & Williston) in one of the first publications describing the phenomenon in 1971. Achor and Starr (1975) used the term "auditory brainstem response" (ABR) in an early study directed at identifying brain structures involved in producing the electrical impulses (Achor & Starr, 1980). By 1985, an article

published in the Critical Reviews in Biomedical Engineering had begun to solidify information regarding the neural generators of ABR and provided a review of current equipment and analysis techniques used (Boston & Moller, 1985). In 2000, the functional anatomy of the ABR was summarized in detail, though the alternative name “brainstem auditory evoked potential” was used (Biacabe, Chevallier, Avan, & Bonfils, 2001).

Initially, the ABR was used in understanding the neural pathways involved in processing simple auditory stimuli (clicks and tones), but it was later extended to meaningful sounds and speech. The ABR is measured by placing electrodes on the scalp and recording electrical activity produced by the brain when an auditory stimulus such as a tone, click, or speech sound (like /da/) is presented to the subject. Typically, a series of 5-7 peaks occur in the first ten milliseconds following a brief stimulus. The most commonly referenced peaks are labeled I through IV. Peak latency and amplitude are the most commonly used measurements. It was predicted that each peak is elicited by a specific structure along the pathway through which auditory information is processed in the brain. Early investigations into defining the generators of each peak of the ABR used lesion studies on animals such as cats (Achor & Starr, 1980). For example, ABR recordings would be taken in cats with a lesion at the cochlear nuclei and compared to recordings in cats without that lesion. If the lesion altered a specific peak, then it would be assumed that the location of the lesion was responsible for producing that peak.

These early lesion studies showed more complexity than anticipated. Discrete lesions in the auditory pathway often produced changes in several components of the ABR, affecting latency and amplitude differently (Achor & Starr, 1980). A few years after this study was published, it was summarized that each of the different peaks I-IV probably originated from multiple sites. The later peaks, such as IV, were likely to have the most structures within the brainstem contribute (Boston & Moller, 1985). Recent studies affirm this; generators of peak IV likely originate from the superior olivary complex, medial superior olive, and anterior portion of the anteroventral cochlear nucleus, along with some modulation from cells of the medial nucleus of the trapezoid body. Compare this to the single generator of peak I, the spiral ganglion cells of the cochlea (Biacabe et al., 2001). Despite a relatively straightforward pathway into the brain, electrical impulses generated from auditory stimuli face a varied and often-modulated track once inside the brain.

It should be noted that the ABR technique itself faces barriers and must be conducted with many factors in mind. Slight differences between recordings, such as changing from a “click” to a “tone” stimulus or recording with slightly different instruments may cause a study to be unreliable. Achieving clear recordings without background noise is another barrier. Biologically, background noise arises from spontaneous electrical activity within the brain and from muscle activity. Nonbiological background noise may be due to electrical interference and/or from stimulus artifact, which is the electromagnetic effect from the

acoustic transducer (Boston & Moller, 1985). In order to correct for unwanted interference, optical or semiconductor isolators may be used.

To reduce background noise and clarify waveforms during analysis, bandpass filtering and averaging techniques are employed. Two types of filtering may be used; low-pass filtering affects peak latency and not waveforms while high-pass filtering affects waveforms. Typically, digital filters are preferred over analog filters because they produce less distortion of waveforms in the ABR. Selective averaging functions much like it sounds—a program attempts to identify and eliminate recordings that deviate far from the median to produce a clearer picture of the waveforms. All of the above techniques may be employed to reduce background noise and allow for easier analysis of the ABR spectrum (Boston & Moller, 1985).

As stated above, the ABR spectrum has been incredibly valuable in the investigation and definition of auditory pathways through the brain. However, information gained by its use has expanded much farther than this. One of the most common clinical applications of the ABR is testing of patients with hearing loss. This recording is particularly useful when studying hearing impairments in infants—patients who cannot describe to physicians what they are able to hear. Numerous studies have been published regarding the benefits of screening infants who are at high risk of hearing impairment (Aiyer & Parikh, 2009) due to factors such as low birth weight, postnatal asphyxia, meningitis, hereditary hearing loss, or others. Certain parameters of the ABR differ in some infants with these high-risks, such as prolonged peak V and inter-peak V-I latency. Because

of the ABR's high usefulness as a diagnostic tool in this situation, it is recommended that all high-risk newborns be tested before leaving the hospital (Galambos & Hecox, 1978).

The ABR's applications are not limited to diagnostics of hearing loss. As will be discussed in Chapter 2, thousands of articles exist that reference the ABR simply in the "methods" section. Since its inception in the early 1970's, the ABR has become useful as an experimental tool in studies investigating everything from celiac disease, an autoimmune disorder of the small intestine, to the effects of specific drugs in the body. Often, ABR data is used to provide information regarding hearing impairment or dysfunction of the brainstem, parts of which are represented by the later peaks, especially IV-V as mentioned above. Variations in waveforms, length of peak latency, or length of inter-peak latency may indicate abnormalities, perhaps due to the presence of tumors, myelination problems, or nerve damage.

This thesis specifically focuses on ABR abnormalities observed in patients diagnosed with autism spectrum disorders (ASD). As we illustrate later, prolonged peak latencies and increases in overall conduction time can provide researchers with valuable information regarding brainstem function. In fact, ABR results have led to the aptly named "brainstem hypothesis," which genetic and anatomical research support through substantiating evidence.

CHAPTER 2

Literature Methods and Review

Due to the extensive use of the auditory brainstem response in both research and diagnosis of auditory impairment and beyond, it was necessary to conduct a thorough review of the literature. The research involved documenting the evolution of the terms used to describe this phenomenon, how ABR was used in pioneering auditory research, and detailing its reference in publications as a method for research indirectly related to the auditory system. This search led to the discovery of over 12,000 articles varied across these specifications.

In order to make the literature search more meaningful, and to help establish a good method of a literature review for the future, most of the research was conducted through an independent study and neuroscience research course. Students recruited for the course searched through articles found based on results from the databases and key phrases found in Table 1 to look for the degree of relevance to ABR. Generally, we found that most early articles, with the exception of some recent reviews, concentrated on describing the ABR and its use. Also, early articles often included lesion studies and measurements from patients with suspected brain tumors and/or psychiatric or neurological disorders. Many studies also presented ABR results from patients with hearing loss, especially in infants. By 1978, researchers were suggesting that all infants with

predisposing risk factors undergo ABR testing to detect any hearing impairment (Galambos & Hecox).

More recent articles that reference the ABR do so in the methods section. It is a popular tool when testing whether potential drugs might have side effects, particularly on hearing. In these cases, ABR is recorded in animals that are administered trial drugs to see whether the drug causes any auditory problems. The ABR may also be a central theme of a scientific paper simply because the researchers are attempting to find any abnormalities within some patient or population group compared to controls. Specific abnormalities may then be used to infer cause or etiology of the symptoms described in the patient or population group tested. These latter types of articles are referenced most often in the forthcoming chapters of this thesis, as most research using ABR in patients with autism spectrum disorders also follows this pattern.

One goal of the literature review was to compare between the effectiveness of two scientific databases in finding articles on the ABR: Web of Science and Scopus. Another goal was to create a comprehensive list of all terms or phrases used to describe the ABR in the literature. Table 1 lists all of the phrases we found for ABR, and how many articles could be found using each phrase in each database. The original list included seven additional terms, but they were eliminated because they produced less than five articles when searched in each database. The most commonly used phrases, and thus the phrases that produced the most articles in the database searches, were “auditory brainstem response,” and the similar “auditory brain stem response” (adding only

a space between “brain” and “stem”), and “brainstem auditory response.” Interestingly, each of these phrases were introduced early on in ABR research: the first two by Achor and Starr (1975), and the latter “brainstem auditory response” by Stockard and Rossiter (1977). However, the phrase has changed over time. In this paper, the “ABR” will be used, representing “auditory brainstem response,” as this phrase continues to be in regular use. In fact, almost every article referenced in the following chapters use “ABR.” It may be that other phrases listed in Table 1 were adopted as the ABR grew in popularity as a research “methods” tool in many different scientific fields of study beyond auditory and neuroscience research.

As can be seen clearly in the total article numbers at the bottom of Table 1, Scopus produced far more unique articles (13,790) than Web of Science (2,058) when searching ABR key phrases (note that here “unique” means that those articles were found only in that specific database). Very little overlap was observed as well; only 376 of the 16,222 articles recorded were found in both databases (duplicates). Although the overlap between the two databases is small, one may miss far more articles if one was to search only using Web of Science. Clearly, Scopus should be used over Web of Science when searching the literature for articles related to the ABR.

Revised numbers can be found in Table 2. First, we removed duplicates found between databases (seen in Table 1). However, during the collection of data, we also realized that many duplicates could exist between the key phrases searched. For example, searching both “auditory brainstem potential” and

"auditory brainstem response" might mention the same articles in some cases. In order to correct for this, all articles were separated by phrase and database. All duplicates between key phrases were removed, and we created a comprehensive list of articles for each database. After review, 4,240 duplicate terms were removed, leaving a total of 12,358 articles—still a large number.

The ABR has expanded tremendously over the past few decades, and has been used in many research fields. However, the most pertinent articles to this thesis relate to the use of ABR data in autism spectrum disorder (ASD) research. In Chapter 3, we will discuss what scientists have discovered through the use of the ABR in this field and their conclusions and inferences regarding this research. Later, we will support ABR findings with evidence from other research. Finally, we will conclude with how the ABR should continue to be used in the ASD field.

Table 1.

ABR Key Search Phrases and Article Numbers for Scopus and Web of Science

Term/Phrase	Unique to Scopus	Unique to Web of Science	Found in Both Databases	Total Articles
“acoustic brain stem response”	11	4	3	18
“acoustic brainstem response”	14	2	3	19
“auditory brain stem response”	1052	594	77	1723
“auditory brainstem response”	1830	809	169	2808
“auditory brain stem potential”	46	4	0	50
“auditory brainstem potential”	46	2	0	48
“brain stem auditory evoked potential”	556	167	15	738
“brainstem auditory evoked potential”	1410	146	42	1598
“brain stem auditory evoked response”	194	137	6	337
“brainstem auditory evoked response”	469	85	36	590
“auditory brain stem evoked potential”	553	7	0	560
“auditory brainstem evoked potential”	135	4	0	139
“auditory brain stem evoked response”	84	41	2	127
“auditory brainstem evoked response”	217	30	12	259
“short latency auditory evoked potential”	42	0	0	42
“short latency auditory evoked response”	10	1	1	10
“brainstem auditory response”	49	3	9	61
“brain stem auditory response”	7023	16	0	7039
“brainstem auditory potential”	26	3	1	30
“brain stem auditory potential”	23	3	0	26
Total:	13,790	2,058	376	16,222

Table 2.

Total ABR Articles in Scopus and Web of Science

Original # of Articles	Duplicates Between Terms	Total Articles w/out Duplicates Between Terms
16,598	4,240	12,358

CHAPTER THREE

ABR Testing in Autism Spectrum Disorders

Pervasive developmental disorder, or PDD, is a term used by the *Diagnostic and Statistical Manual of Mental Disorders – Fourth Edition* (DSM-IV) to encompass autistic disorder, Asperger's disorder, or perhaps pervasive developmental disorder not otherwise specified (PDD-NOS) (Christensen et al., 2016). Due to the variability in the spectrum of autistic disorders, the scientific literature has typically referred to them as "autism spectrum disorders" (ASDs), as has been done here.

ASDs are early-onset developmental disorders that present as difficulties in social communication and interaction, problems with language development and use, and may include repetitive or stereotypy behaviors (Roth, Muchnik, Shabtai, Hildesheimer, and Henkin, 2012). According to a 2012 CDC report, the estimated prevalence of ASD in the United States is at about 1 in 68 children (or 14.6 per 1000 children), with a higher incidence reported in 8-year-old boys (23.6 per 1000) than 8-year-old females (5.3 per 1000) (Christensen et al., 2016).

The auditory brainstem response (ABR) has been used to study ASD for over two decades due to the emerging hypothesis that brainstem dysfunction is involved in ASD development. Language delay and hypersensitivity to sensory stimuli have been consistently observed in patients with ASD, and as a consequence, researchers pursued the idea that impairment of the auditory

system (particularly the brainstem) was involved (Klin, 1993). The ABR was the perfect tool to use in this research, as it does not require active participation from subjects, which would be particularly useful in uncooperative subjects like those often seen with ASD. The ABR is also cheaper and easier to perform than more expensive scans, and it has been used extensively since the 1970's.

Much information can be obtained about the functionality of auditory pathways throughout the brainstem using the ABR. In particular, ASD researchers have focused on wave latencies (typically I-V, although the ABR may record to around VII) and interpeak latencies (IPLs). The waves are numbered in accordance with increasing distance of travel through the auditory system: waves I and II are associated with the auditory nerve, while waves III-V are associated with brainstem structures (Rosenhall, Nordin, Brantberg, and Gillberg, 2003). Wave III is derived from the cochlear nucleus and superior olivary complex of the pons, including the medial olivocochlear system (MOC) (Rosenhall et al., 2003). Wave IV arises from the superior olivary complex and lateral lemniscus area. Wave V comes from activity in ascending axons of the lateral lemniscus (Rosenhall et al., 2003). IPLs reflect time differences between waves of the ABR. The I-III IPL arises from time of conduction through the auditory nerve and synapse into the cochlear nucleus; the III-IV IPL arises from the pathway between the cochlear nucleus and the lateral lemniscus and inferior colliculus, and the III-V IPL arises from activity in the III-IV IPL plus one synaptic delay (Rosenhall et al., 2003).

Many ABR studies were conducted on patients with ASD in the 1980's and 90's, but they often produced inconsistent and sometimes contradictory results. In most studies, the cohort of ASD subjects produced results that were significantly different from age-matched controls, but often the studies were inconsistent across the ASD groups themselves. For example, Wave I latency abnormalities might be observed in some subjects while not in others. It became obvious that something was different in the ASD groups, but ASD groups are intrinsically heterogeneous, and the results were reflecting those differences. At the time, ASD was considered simply "autism" and the disorders we describe as a spectrum now were co-mingled. More clearly defined diagnostic criteria were needed for subjects included in these studies. Other possible flaws included the use of small sample sizes and the lack of information about gender differences, which are present not only in ASD itself, but also in the ABR in general.

Increased Conduction Times in ABR

Nonetheless, some general conclusions began to emerge regarding the ABR and ASDs. Although some outlying studies existed, most showed prolonged latencies in one or all ABR waves or IPLs in subjects with ASD versus control subjects. In fact, around 85-90% of all subjects with ASD were showing some type of brainstem abnormality based on this information (Steffenburg, 1991). These prolonged latencies were reflective of increased conductance times throughout the auditory nerve and brainstem. These observations led to the hypothesis that there are myelination problems during development in ASD, which may contribute to structural abnormalities and lead to symptom

development. For example, a 1992 study by McClelland, Eyre, Watson, Calvert, and Sherrard, found increased conduction times through waves I-V in older (14+) ASD subjects, while progressively decreasing conduction times were observed across waves I-V in non-ASD controls. They attributed these findings to an abnormal myelination process, perhaps as a result of perinatal hypoxia (McClelland et al., 1992). They also reasoned that such a myelination defect could explain the late onset of epilepsy in some ASD patients, based on the deficiency of ascending inhibitory input to the cortex (McClelland et al., 1992).

Upon further investigation, the myelination hypothesis turned out to be a clue into the possibility of the role of immune system dysfunction in ASD development. Myelin is a fatty, light-colored substance that gives “white matter” its name and is produced by oligodendrocytes in the central nervous system. Myelin wraps around axons to increase the rate of neural transmission through saltatory conduction. Loss of myelination can be detrimental, as is seen in multiple sclerosis. It is possible that myelination abnormalities contribute to the prolonged conduction times seen in ABR, as noted above. Support for this comes from studies in which antibodies to myelin basic protein (involved in the myelination process) were found to be elevated in the sera of ASD subjects (58%) compared to controls (9%), reflecting the role of the immune system (Singh, Warren, Odell, Warren, and Cole, 1993). Further investigation has revealed other immune function abnormalities, including abnormal T cell activation and/or helper-suppressor lymphocyte ratios (Trottier, Strivastava, and Walker, 1999). Perhaps these results reflect a subset within the ASD population.

The idea that myelination abnormalities may play a role in the development of ASD appears to be supported by some studies involving white matter and general brain volume. A 2001 study conducted by Courchesne et al. investigated cerebral and cerebellar volume differences in ASD versus controls in a range of age groups. Consistent with other studies (Trottier, 1999) (Kallstrand et al., 2010), they found that ASD toddlers (90%) had greater overall brain volume than controls, with 37% qualifying as having macrencephaly. Cerebral and cerebellar white matter was also increased in 2-3 year-olds by 18% and 39%, respectively, while older adolescent ASD subjects did not show such increases (and in some cases, decreases) (Courchesne et al., 2001). Greater cerebral white matter has been documented by Herbert et al. (2003) as well, although the increase noted in the studies (15%) lies between the toddler (18%) and adolescent (not significant) percentages observed by Courchesne et al. (2001). Herbert et al. (2003) proposed that these findings suggest a pattern of overgrowth early on followed by a subsequent downward trajectory of cerebral white matter growth as ASD subjects age. Perhaps this reflects the abnormal myelin maturation described earlier in a subset of ASD patients.

Increased conduction times observed in ABR studies have provided useful insight into the possible contribution of myelination abnormalities in ASD. Much focus has also been given to increased latencies observed in ABR waves and IPLs. In particular, waves III and V, and IPL III-V have shown the most consistent results.

Increased Wave and IPL Latencies in ABR: Wave III

As mentioned above, wave III originates in the cochlear nucleus and superior olivary complex. A recent study by Kallstrand, Olsson, Nehlstedt, Skold, and Nielzen (2010) conducted ABR using "auditory forward masking," which refers to the decrease in ability to detect a stimulus when it is preceded by a masking sound. Based on the idea that ASD subjects often have difficulty perceiving speech in noisy and hyperstimulating environments, Kallstrand et al. (2010) sought to record ABR changes using a reflective technique. The most salient part of this study, however, came from the fact that they compared groups of subjects with Asperger's syndrome, ADHD, schizophrenia, and age-matched controls in an attempt to bring homogeneity to a traditionally heterogenous group of subjects. These somewhat related neurodevelopmental disorder groups were parsed apart, as wave III latencies varied significantly between each (Kastrand et al., 2010), with the Asperger's group showing much shorter latencies. Although the study size was small, this suggests again the idea of sub-populations within the umbrella term "ASD."

In addition, Kallstrand et al. (2010) proposes that there are decreases in electrical activity in the superior olivary complex (SOC) based on wave III results. Within the SOC lies the medial olivocochlear (MOC) system, likely the location of filtering ascending auditory input and feedback from higher order auditory nuclei and cortices. This system synapses on and thus modulates the amplification of signals from outer hair cells. Dysfunction of this system might result in lack of control of auditory input, leading to hypersensation. Kallstrand et al. (2010)

suggest that this dysfunction could account for wave III abnormalities and thus symptoms in ASD.

Increased Wave and IPL Latencies in ABR: Wave V

Wave V originates later through the auditory brainstem pathway, in the ascending axons of the lateral lemniscus. As mentioned above, early ABR studies in subjects with ASD often showed inconsistent wave latency results. However, increasingly recent studies have tended to be consistent in showing prolonged wave V latencies. The studies to be mentioned here have relatively large sample sizes (n = 40 to n = 101) and were conducted between 2003 and 2016, after awareness of ASD had grown and after the ASD definition had been updated to reflect clearer diagnoses by clinicians. It should be noted that these studies did not necessarily show prolonged latencies in just wave V, but often included IPL III-V or wave III.

The oldest and largest study conducted by Rosenhall, Nordin, Brantberg, and Gillberg (2003) was a review of data collected on 101 individuals with ASD and age-matched controls over a 12-year period. Over half (58.4%) of the subjects showed ABR abnormalities, with 50.5% showing prolongation of waves I, III, and/or V—the majority of which (37.6%) had pathological lengthening of wave V specifically. Rosenhall et al. (2003) notes that the lengthening of wave V is reflective of brainstem abnormalities, particularly when combined with the evidence of IPL latencies such as III-V. Among the myelination defect hypothesis, they suggest as explanations for the prolonged wave V genetic and anatomical defects, as will be addressed in Chapter 4.

Later studies support Rosenhall et al. (2003)'s findings. Another relatively large (n = 71) experiment conducted by Kwon, Kim, Choe, Ko, and Park (2007) showed significantly prolonged wave V latencies in ASD as compared to age-matched controls, along with IPL III-V and I-V. Roth, Muchnik, Shabtai, Hildesheimer, and Henkin (2012) decided to compare ASD subjects with non-ASD subjects with language delay in hopes of subtracting language delay as a confounding factor in ABR results. Prolonged latencies were observed in both groups, but much more so in waves III and V in the ASD-only patients (62%). 50% of the ASD-only subjects showed significant lengthening of latencies of two or more waves, while only 8% of the language delay-only subjects did. Like other researchers, Roth et al. (2012) suggest abnormal brain growth as a factor in these abnormalities. Interestingly, a smaller study (n = 25) reported gender differences in wave V latencies below 70dB (Dabbous, 2012). These results should be interpreted with caution, however, based on the smaller group size and the fact that significant differences were not observed across all click levels. More research is needed to understand gender differences in ABR responses of subjects with ASD.

The final and most recent study reviewed is unique in that it suggests to the possible use of ABR as an indicator or diagnostic tool for ASD (the neurodevelopmental disorder with historically qualitative rather than quantitative diagnoses). Miron et al. (2015) gathered data from a sample of 70 children later diagnosed with ASD, and observed prolonged latencies in wave V and IPL III-V and I-V. The strongest association was seen with wave V, as it was observed in

70% of the ASD subjects and only in 20% of the age-matched controls. Miron et al. (2015) also calculated how well subjects in the ASD or control groups could be identified based on their wave V latency results. They could be identified—and with 78% positive predictive validity. It should be noted that the ABR tests were performed on infants during the first 3 months of life. These results suggest the potential to use ABR as an estimator for ASD risk. This would be an incredible tool for clinicians and patients, as it would allow intervention therapies to be implemented earlier to help those with ASD. Of course, more large-scale studies are needed to validate these findings and ensure generalizability.

Increased Wave and IPL Latencies in ABR: IPL III-V

A variety of studies describe prolonged IPL III-V in subject groups with ASD. Although IPL I-V is mentioned as well, here we will focus on IPL III-V, as it provides the conduction time from the cochlear nucleus to the lateral lemniscus (or simply the time between waves III and V). Rosehall et al. (2003), Kwon et al. (2007), and Miron et al. (2015) all describe prolonged IPL III-V. A study by Tas et al. (2007) found the same occurrences correlating with older studies such as that by Skoff, Mirsky, and Turner (1980).

The prolongation of IPL III-V is significant as support for the hypothesis that brainstem dysfunction is involved in ASD development. Waves I and II tell us about what is happening in the auditory nerve, shortly after sound has hit our eardrum, vibrated our middle-ear ossicles, and frequencies have been sorted by the cochlea and transduced by our hair cells. Damage or dysfunction of the auditory nerve would likely affect hearing or the vestibular system. Although

some research shows a slightly higher incidence of hearing impairment in patients with ASD than in the normal population (Rosenhall, Nordin, Sandstrom, Ahlsen, and Gillberg, 1999), results have been inconsistent and there is currently no consensus that an increased prevalence exists here (Beers, McBoyle, Kakande, Dar Santos, and Kozak, 2014). Additionally, most ABR studies have shown latency abnormalities in later waves than I and II.

Waves III-V involve higher order functioning, where auditory information is now being processed and filtered by the brainstem so it can be further processed by cortical structures. Dysfunctions here might affect how we respond in a noisy environment or whether we are filtering out the correct sensory stimuli and focusing on what we need to. Higher-order cortical input might not be as effective if the brainstem structures feeding it information is dysfunctional. Even in the presence of normal sensory receptor and auditory nerve function, these more central auditory structures can have problems (Beers et al., 2014). The problems just described fit well with the symptoms of ASD: difficulty hearing or focusing on the intended stimuli in a noisy environment, experiencing hyperacusis, in which certain frequencies and volumes of sounds are unbearable, or simply hyper-responsiveness to auditory stimuli, where the sensory input is overwhelming (Beers et al., 2014).

Abnormalities in ABR recordings of IPL III-V (and really, waves III and V as well) support dysfunction in these central auditory brainstem structures. Data provided by ABR gives us a structure to focus on in the pursuit of understanding the causes of ASD.

ABR Studies Have Led to Hypotheses of ASD Etiology

Since brainstem dysfunction was suspected of underlying the cause of ASD, the ABR has been a critical research tool for understanding the neurodevelopmental disorder. Increased conduction times through the auditory processing pathway led to the idea of myelination abnormalities, which may be caused by genetic predispositions. Anatomical changes such as increased cerebral white matter, and inappropriate timing of under and overgrowth of brain size have been observed as a result. Prolonged latencies in wave III have led to investigation of the medial olivocochlear system in the superior olivary complex, whose dysfunction may play a role in the hypersensitivity to sensory stimuli seen in patients with ASD. Prolonged latencies in wave V produced by ascending auditory afferent input via the lateral lemniscus further pushes researchers to study brain growth abnormalities. Abnormal wave V latencies may be specifically unique to ASD subjects and perhaps could function as an indicator of ASD risk in infants.

ASD is a complex neurodevelopmental disorder, so the brainstem dysfunctions discovered by ABR only provide some insight into its causes. Genetic, neurochemical, immune, and other factors contribute to our understanding of ASD and our interpretation of ABR results. The broader etiology of ASD and its relation to the information ABR data has provided us with will be discussed next.

CHAPTER FOUR

Support of the Brainstem Hypothesis in ASD

The ABR has been a very useful tool in the search for causes and contributing factors to ASD and its symptoms. Prolonged wave latencies and IPLs have led us to the "brainstem hypothesis," in which dysfunction of the brainstem is either the source of or a main contributor to ASD symptoms. Bauman and Kemper (2005) agree, stating, "...there is no region but the brainstem for which so many lines of evidence indicate a role in autism." ABR has brought us here; now what are the "many lines of evidence" corroborating ABR's story?

RSA and The Polyvagal Theory

The vagal (or vagus) nerve, known for its diverse autonomic functions, provides afferent and efferent connections to nuclei in the medulla of the brainstem. A particular output, the "myelinated vagus", provides input to the heart's sinoatrial node to control heart rate. This input has earned another name based on this control over the heart—the "vagal brake." The more vagal input to the heart, the more heart rate will be suppressed. High heart rate is generally associated with higher arousal and anxious psychological states, while lower heart rate, such as that induced by the vagal brake, allows for low arousal states. This is the basis for understanding the polyvagal theory as proposed by Stephen Porges (2007).

The polyvagal theory proposes that humans have three levels of neural control over heart rate that increase in phylogenetic age (the first stage evolved first, the second evolved second, and so on). Each stage increases our ability to take control over our emotions, and the final stage involving the myelinated vagus nerve allows us to engage in social communication and behaviors. The oldest and first stage is comprised of the umyelinated vagus nerve, which arises from the dorsal motor nucleus of the vagus and allows "immobilization behaviors" such as feigning death or passive avoidance when we encounter danger. The second intermediate stage involves the sympathetic-adrenal system arising from the spinal cord, which allows for production of "mobilization behaviors" or active avoidance during dangerous situations. The third and phylogenetically newest stage is comprised of the myelinated vagus nerve arising from the nucleus ambiguus, which allows for social communication, self-calming behaviors, and inhibition of the sympathetic-adrenal influences (Porges, 2007). The vagal brake produced by this most recent phylogenetic stage of neural control of the heart allows us to suppress the older systems and control our arousal levels so we can effectively communicate and interact with others.

There are ways to assess how well this last neural control is working. The respiratory sinus arrhythmia (RSA) is a rhythmic pattern that occurs naturally in the heart at about the frequency of normal breathing. Measurements of RSA provide a sensitive index of myelinated vagal input to the heart (Porges, 2007). Low baseline levels of RSA and problems with RSA modulation/suppression have been shown to be risk indicators for problems with social and emotional

regulation and in some cases associated with psychiatric disorders (Porges, 2007). As it turns out, children with ASD are less able to suppress RSA when compared with controls (Bauman & Kemper, 2005). Lack of or decreased vagal input would cause this decreased ability to control heart rate and thus suppress RSA, leading to the behavioral problems mentioned. Interestingly, it has been shown that stimulation of the vagal nerve decreases ASD-like symptoms such as ritualistic behaviors, poor communication and social skills, and compulsion (Bauman & Kemper, 2005). Clearly, vagal input from the brainstem may play some role in symptomology of ASD.

The vagus nerve was not the only portion of the brainstem Porges (2007) mentioned. In his polyvagal theory, he also described the social engagement system, which implicates the brainstem as a major connecting point between higher-order input from the cortex and several cranial nerves (including the vagus) that allow for the social communication described in the third phylogenetic level above. The other cranial nerves are involved in somatomotor functions such as eyelid opening, facial expression production, production of prosody and intonation during speech, head turning for social gestures and orientation, and even controlling middle ear muscles to extract human voice frequencies (high) from more common background noises (low) (Porges, 2007). All of these functions are integrated so we can communicate with others, and myelinated vagal input suppresses phylogenetically old arousal circuits so that we can do so.

Thus, it follows that dysregulation of the vagus nerve and/or social engagement system, central in the brainstem, would be implicated in ASD.

Failure to parse human voice from the background, hyperacusis, problems with maintaining eye contact or socially appropriate body language, and/or understanding the nuances of incoming language are all related to ASD and all involve these cranial nerves and appropriate brainstem functioning. Dysregulation of the brainstem is yet again implicated in ASD.

Genetics and Myelin

ABR led us to the brainstem hypothesis of ASD. Lack of RSA suppression and the polyvagal theory and social engagement system in patients with ASD further supports the role of the brainstem, albeit in multiple sensory pathways other than just auditory. In addition to this research, scientists have pursued the role of genetics in ASD. Studies have shown much higher concordance rates in monozygotic (92%) over dizygotic twins (10%), and the heritability of ASD is estimated at about 90% (Bauman & Kemper, 2005). Relatives of people diagnosed with ASD seem to be more likely to be at risk as well; some studies estimate that siblings of patients with ASD have a risk of about 3%, which is 50 times higher than the regular population risk (Trottier, 1999). Apparently, some studies have shown that certain personality characteristics aggregate in families with autism, such as language abnormalities and other psychiatric disorders, perhaps indicating some underlying genetic susceptibility (Trottier, 1999). Clearly, genetics plays a crucial role.

Many studies have been conducted to reveal suspected chromosome and gene involvement. It is likely that a constellation of genes are involved in ASD rather than a single mutation or change. Among a number of candidate genes

identified on chromosome 7q are WNT2, RELN, EN2, and HOXA1 (Bauman & Kemper, 2005). Wnt proteins are involved early in central nervous system development. RELN codes for reelin, which is also involved in development and possibly in the initiation of myelin synthesis. EN2 is a homeobox gene expressed in the cerebellum, a structure with several observed abnormalities in ASD patients. Mice lacking certain Hox genes have shown anomalies in the brainstem and caused malformed ears and hearing deficits (Bauman & Kemper, 2005). Chromosome 15 has also been a source of focus, as duplications inherited from the mother here appear to be the most common chromosomal abnormalities described for ASD (Bauman & Kemper, 2005). UBE3A is a candidate gene on chromosome 15 expressed in the cerebellum and hippocampus that may be involved in stereotyped behavior and/or seizures (Bauman & Kemper, 2005). Many other genes have been reported to possibly play some role in ASD development, almost on every chromosome. Due to the multitude of genes, lack of very concrete evidence for all, and the limited scope of this paper, we will be focusing on only a couple of genes that have been identified in relation to the myelination defects described in Chapter 3.

Prolonged conduction time observed in ABR led to the idea that myelination deficits in the brainstem and throughout the cortex could be involved in ASD. Bursts of white matter overgrowth early in development followed by a decrease in white matter (Herbert et al., 2003) and observed increases in myelin basic protein in sera of ASD patients supported this idea (Singh et al., 1993). Recently, genetic factors have been implicated that further this support.

CNTN5 and CNTN6 are genes that encode contactin, which are neural cell adhesion molecules that promote neural growth in sensory-motor pathways (Mercati et al., 2017). CNTN5 is involved in developing glutamatergic neurons in the auditory regions of the brainstem, including the inferior colliculus. In fact, CNTN5 knockout mice showed prolonged latencies in ABR (Mercati et al., 2017). CNTN6 is more involved in the myelin pathway. Upon interaction with NOTCH1, oligodendrocytes are produced from progenitor cells. CNTN6 is also expressed in the inferior colliculus and the cerebellum, where they promote synaptogenesis and neural growth (Mercati et al., 2017). Mercati et al. (2017) used large genetic databases to search the frequencies of copy-number variants (CNVs) and single-nucleotide variants (SNVs) affecting CNTN5 and CNTN6 in subjects with ASD. Both CNVs and SNVs of the CNTN6 gene appeared much more frequently in the ASD group, with the most consistent being CNVs. Mutations in the CNTN6 gene may therefore represent risk factors for ASD (Mercati et al., 2017).

Mutations in CNTN6 may present in a number of ways. They are heavily involved in development. For example, one variant observed in this study corresponded to a location producing a critical amino acid for neogenin, a receptor for the molecule netrin, which acts as a repulsive guidance molecule during axon growth in nervous system development (Mercati et al., 2017). Some variants, as stated above, interact with the Notch signaling pathway, which leads to production of oligodendrocytes, the producers of myelin (Mercati et al., 2017). Dysfunction of these pathways could lead to problems in axonal guidance and placement in the brain or perhaps myelin overgrowth or maturation problems.

CNTN6 and CNTN5 are particularly involved in development of sensory-motor pathways, abnormalities of which could account for symptoms in ASD. Interestingly, subjects carrying variants of these genes were more likely to have hyperacusis (described earlier as hypersensitivity to certain sounds and frequencies, characteristic of some ASD patients) (81%), than subjects with ASD lacking the gene variants (66%) (Mercati et al., 2017). Subjects with these variant genes also showed more negative responses to incoming sensory stimuli (56%) than ASD subjects without (30%) (Mercati et al., 2017). It may be that defects in these genes cause abnormalities during development in the brainstem, leading to hyper-responsiveness to auditory stimuli observed in these patients.

Abnormalities in myelin development may be caused by a number of factors. So far, we have noted the increase of antibodies against myelin basic protein in sera of ASD patients (Singh et al., 1991) and variants of the CNTN6 gene, which may cause problems in oligodendrocyte production. It has also been observed that white matter growth rapidly occurs early in development and is followed by decline in ASD individuals, contrary to normal development (Herbert et al., 2003). Taking a look at the myelin development pathway, we can see that several different steps could be interrupted besides changes in myelin's creator, oligodendrocytes. Myelin is comprised of several lipids and proteins, and enzymes involved in synthesis and breakdown. Once myelin has wrapped loosely around an axon, it must become "compacted." This process involves adjustment of lipid to protein ratio and loss of fluid molecules trapped between the layers (Bauman & Kemper, 2005). Glycolipids are among the lipids included

in the lipid to protein ratio, and cerebroside appears to be a particularly important glycolipid. Cerebrosides play a role in maintaining compaction, adhesion, and stability in membrane structure (Bauman & Kemper, 2005). Thus, a decrease in cerebrosides might lead to an inability to effectively "compact" myelin and leave it loosely wrapped around axons, reducing its function as an insulator for increased axonal conduction time. Interestingly, there seems to be a decrease in expression of genes producing cerebrosides in subjects with autism. In one study, the total amount of cerebroside was reduced by 11% in ASD brains compared to controls (Bauman & Kemper, 2005).

Interestingly, cerebrosides also serve as precursors for the ganglioside GM4. Gangliosides are glycolipids also involved in maintaining cell membrane structure and adhesion, and contribute to myelin compaction (Bauman & Kemper, 2005). GM4 expression has been shown to be decreased by 13% to 38% in subjects with ASD (Bauman & Kemper, 2005). Thus, abnormalities in cerebroside expression would affect functioning of cerebrosides and GM4 in myelin compaction. Disruption of myelin compaction would be another way to account for increased conduction times observed in ABR and the brainstem hypothesis of ASD.

The final gene mentioned here that may be involved in myelin changes is RELN, which encodes for Reelin. RELN is located on chromosome 7q, and was mentioned earlier as a candidate gene for ASD. Reelin is an extracellular protein involved in cytoarchitecture of the central nervous system. Among its other functions here, Reelin binds to beta integrins involved in initiating the synthesis of

myelin by oligodendrocytes (Bauman & Kemper, 2005). Thus, dysfunction in Reelin expression may cause problems in myelin development.

Many genes have been implicated as "candidates" for ASD involvement. The genetic factors mentioned that are most heavily involved in myelination that have been related to ASD include CNTN5 and CNTN6, glycolipid expression, and RELN. They each affect myelination differently: through disrupting oligodendrocyte development and axonal guidance, myelin compaction, and initiation of myelin synthesis, respectively. Each provide avenues through which myelination disruption might occur, accounting for increased conduction times observed in ABR, white matter volume changes, and symptomology seen in patients with ASD.

Anatomy: The Cerebellum & Olive

It is not surprising that with the complex etiology of ASD, many brain structures have been studied in pursuit of abnormalities consistent in ASD groups. Increased conduction times seen in ABR led us to observe white matter abnormalities due to myelin maturation dysfunctions. Excessive overgrowth of white matter was observed in the cerebral cortex of young ASD patients, followed by a decline in growth as compared to controls (Herbert et al., 2003). Small, less compact microcolumns of neurons have also been observed in the prefrontal and temporal cortices of groups with ASD (Bauman & Kemper, 2005). Changes in limbic structures have also been observed: studies have shown reduced dendritic arborization of the CA4 and CA1 regions of the hippocampus and smaller, more densely packed neurons in various nuclei of the amygdala. However, the most

consistent neuroanatomic abnormalities reported thus far are those seen in the cerebellum and brainstem, which provides further support for ABR data and the brainstem hypothesis of ASD.

The cerebellum is often associated with movement, coordination, and generally “lower-order” functioning. However, as indicated by the massive peduncles relaying afferent and efferent information to and from the cerebellum, it is obvious that its input is imperative in many brain functions. Studies suggest that beyond its role in coordination of motor activity, the cerebellum modulates an abundance of central nervous system functioning, including higher-order cortical functions such as attention-shifting control, mental imagery, some aspects of language processing, anticipatory planning, and cognitive processing (Bauman & Kemper, 2005). Bauman and Kemper (2005) additionally suggest that it plays a role in emotion and motivation and the integration of sensory and motor information. Marco, Hinkley, Hill, and Nagarajan (2011) also suggest that abnormalities in cerebellar structure may cause the sensory integration problems observed in patients with ASD.

One of the most commonly referenced neuroanatomical difference in ASD groups versus the normal population is a decrease in cerebellar size, particularly in the vermis, located medially. Trottier at al. (1999), Herbert et al. (2003), Bauman and Kemper (2005), Kwon et al. (2007), Marco et al. (2011), and Mercati et al. (2017) all reference cerebellar size changes. Interestingly, it has been observed that cerebellar nuclei cells are enlarged and normal in number in young ASD patients (ages 5-13). However, adult patients show a significant

decrease in these nuclei cells, which become smaller and more pale. Perhaps a degenerative process is involved, as neuronal swelling followed later by neuronal loss and atrophy shortly after experimental axonal transections has been observed by Bauman & Kemper (2005).

Although evidence for cerebellar size decrease is increasingly reported, the most consistent neuroanatomical change observed in ASD brains regardless of cognitive ability, sex, and age are decreases in Purkinje cell number and density (Bauman & Kemper, 2005). Purkinje cells are unique to the cerebellum, integrating inputs from their massive dendritic arborizations to function as the sole output from the cerebellum. They receive input from many cells, but most importantly for this study, they receive input from climbing fibers of the olive of the brainstem. These inferior olivary climbing fibers synapse onto Purkinje cells of the cerebellum in a zone called the "lamina dissecans," which disappears at about 29-30 weeks gestation in the developing embryo. Interestingly, retrograde olivary cell loss is also observed in ASD patients along with Purkinje cell loss. It is thought that whatever is causing the decrease in Purkinje cell number and retrograde olivary cell loss occurs during the developmental period at or before the disappearance of the lamina dissecans, when these cells have been in contact (Bauman & Kemper, 2005). The cerebellum and brainstem have a close relationship in the normally functioning brain. Perhaps disruption of this connection and failure of the cerebellum to properly integrate the multiple sensory inputs coming in from the brainstem contribute to abnormalities observed in ABR and thus the symptoms observed in ASD.

Clearly, the olive of the brainstem has also been implicated in ASD research. In fact, both the inferior and superior sections seem to play a role. The inferior olive is the location of the retrograde neuron loss as described above (Bauman & Kemper, 2005). Similar to the developmental issues observed in cerebellar nuclei cells, olivary neurons appear to be enlarged in size in younger ASD patients (less than 13 years old) and appear to be smaller and paler in color in adult ASD patients (Bauman & Kemper, 2005). Perhaps some degenerative process is at work here as well. Interestingly, neurons of the inferior olive have been shown to be clustered more peripherally in brains of subjects with ASD. The brainstem has also observed to be shortened between the inferior olive and the trapezoid body (Bauman & Kemper, 2005).

There is also evidence showing a decrease in the number of neurons in the superior olive and facial nucleus (Bauman & Kemper, 2005). These changes may corroborate the brainstem hypothesis based on information from ABR data and the polyvagal theory, respectively. For example, Kallstrand et al. (2010) proposed the involvement of the medial olivocochlear system (MOC), which is located in the superior olive, to be dysfunctional in ASD. They describe the MOC as being involved in filtering ascending auditory inputs and integrating feedback from higher-order auditory nuclei and corticies (Kallstrand et al., 2010). Dysfunction of this system, as evidenced by wave III latencies mentioned in Chapter 3 and neural loss in the superior olive, support this idea.

Neural loss in the facial nucleus may also support the polyvagal theory. The social engagement system integrates visceral and sensorimotor input from

cranial nerves, the brainstem, and higher-order cortices to produce behaviors appropriate for social communication (Porges, 2007). The facial nucleus is involved in muscle control over pharyngeal and laryngeal muscles needed for production of speech, particularly prosody and intonation that allows for conveying emotions beyond the basic messages of speech. It also controls muscles of the face to produce facial expressions in social contexts. Dysfunction of the ability to filter and understand incoming auditory information such as language, coupled with dysfunction of the ability to communicate back using facial expressions and different tones of speech contribute to symptoms seen in ASD. Yet again, brainstem abnormalities may be implicated in the development of ASD.

Converging Evidence for the Brainstem Hypothesis

Bauman and Kemper (2005) said, "...there is no region but the brainstem for which so many lines of evidence indicate a role in autism." The ABR served a useful tool in leading researchers toward the brainstem's heavy involvement in ASD. Here, we have explored some lines of evidence corroborating these theories. The polyvagal theory and respiratory sinus arrhythmia measurement in patients with ASD point toward dysfunction of the center of the social engagement system—the brainstem. Genetic variants observed in ASD populations may contribute to problems in the development of myelin in a number of ways. Finally, neuroanatomical changes such as decreased Purkinje cell and olivary neuron numbers seem to be consistently observed in ASD brains.

All of these lines of evidence point us back toward involvement of the brainstem and potential indicators for risk of ASD. ABR has brought us here; what now?

CHAPTER FIVE

Future Directions

We have described the ABR and how its use has evolved and infiltrated many scientific fields since the 1970's, when it was first used as a research tool in understanding the auditory pathway and as a diagnostic tool for hearing impairment. Specifically, we have explored the use of the ABR as a tool for ASD research, showing increased conduction times and wave and inter-peak latencies in this population. We discovered how ABR led to the brainstem hypothesis, and found other lines of genetic and anatomical evidence supporting it.

Though the ABR has been useful in ASD research, our understanding of this neurodevelopmental disorder is far from complete. There are still inconsistencies in anatomical, genetic, and ABR data that have yet to be reviewed. Likely, further research will more clearly define sub-populations and gender differences within the highly heterogeneous autism spectrum. Until then, the ABR should continue to contribute to our understanding. There are three ways in which the ABR will continue to be useful to researchers and clinicians involved in ASD: it may be important for testing for hearing impairment in recently diagnosed ASD patients who may be at a higher risk, it may be used as a potential indicator of risk for ASD in infants, and it may also continue to be used as a research tool for discovering gender differences and sub-populations.

ABR Testing for Hearing Impairments in ASD

Individuals with ASD may be more at risk for hearing impairments. ABR recordings have historically been used to test for hearing impairment in infants. Researchers began recording ABR in patients with ASD initially to test for hearing or auditory impairment of some kind based on the language and communication difficulties observed in these subjects. They have found that hearing impairment is very likely not the problem and that prolonged latencies in later (III-V) waves were common and reflective of brainstem abnormalities rather than sensory problems (Skoff et al., 1980).

However, many studies have reported a slightly higher incidence in hearing impairment (3.5%) than the normal population (0.1-0.2%) (Rosenhall et al.,1999). More recently, Beers et al. (2014) concluded that insufficient evidence exists to prove that ASD puts subjects at any more of a risk for hearing impairment than normal. It would be useful for future studies to address this question and to conclude with greater certainty if there is an increased risk of hearing loss for ASD patients.

ABR as a Predictor for ASD

In Chapter 3, we mention one study in which prolonged wave V latencies in infants were able to predict whether subjects would develop ASD with 78% validity (Miron et al., 2015). This was observed in a fairly large population (n = 70). If in fact the ABR wave V latency could be a predictor for ASD, then the ABR test should be conducted on any infant with predisposing genetic factors,

such as ASD in the family. This type of testing could help families prepare for behavioral interventions to improve and foster communication and social skills early on. This would also prevent many patients and their families from years of incomplete knowledge and misdiagnoses.

Of course, more ABR data is needed to confirm its ability to predict ASD. Large-scale studies involving hundreds to thousands of infants would be needed. It would also be useful to break down these subjects into the three subtypes of ASD recognized by the *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition* (DSM-IV): Asperger disorder, autistic disorder, and pervasive developmental disorder-not otherwise specified (PDD-NOS) (Christensen et al., 2016). It is likely that some subtypes of ASD will produce slightly different ABR results. Additionally, results should be separated by gender and compared in order to see if ABR abnormalities present differently. With more evidence, the ABR could become a very useful predictor for ASD.

ABR to Continue ASD Research

Though much has been learned about ABR in patients with ASD, there have been few studies that separate out the five sub-populations mentioned above. Kallstrand et al. (2010) compared patients with Asperger's to subjects with schizophrenia, ADHD, and controls, and discovered ABR abnormalities (shortened peak III latency) specific to the Asperger's group and contrasting with the other sub-populations. Studies like these that compare autism spectrum disorders with other neurodevelopmental disorders are helpful. However, based

on the fact that ABR data is not always consistent, future studies should also compare recordings between the three DSM-IV ASD subpopulations.

Few studies testing ABR in subjects with ASD have found or reported gender differences in recordings. According to a 2012 CDC report, prevalence of ASD is higher in boys (23.6 per 1,000) than girls (5.3 per 1,000). However, recently there has been some debate over whether girls have been under-diagnosed. Lai, Lombardo, Auyeung, Chakrabarti, and Baron-Cohen (2015) explain that females often present with fewer observable social and communication problems. This may reflect the increased pressure women and girls feel to conform to societal and peer norms, so much so that some are able to adapt enough that ASD symptoms are not as easily identified. This leads to the question of whether there are neurobiological differences between males and females. A recent study by Lai et al. (2013) found significant differences in MRI scans between 30 male and 30 female adult ASD patients, particularly between white matter and grey matter volume. Future studies should take these results into consideration. As stated above, few ABR studies have compared male and female ASD subjects to look for differences. Finding differences in brainstem abnormalities may help us understand functional differences in gender. For example, if females with ASD seem to generally have better social and communication skills, could it be that sensory information processing in the brainstem is less affected? If so, could white matter differences observed in Lai et al.'s study (2013) account for such discrepancies? These are the kinds of questions still in need of answers.

Finally, as ABR continues to be a tool used in ASD research, age groups should be very carefully considered. As noted earlier (Herbert et al., 2003), studies have pointed to developmental differences in the brain, where young ASD patients experience a rapid brain and white matter growth followed by a sharp decrease later on. Although in the end, brain sizes typically average out to be equal to a normal adult, ASD brains have undergone a different developmental path. Thus, recording ABR from a subject with ASD who is 12 years old versus a subject who is 25 years old likely will produce different results. Often studies referenced here have obtained age-matched controls. However, there is little information available on whether ABR recordings differ between age groups of patients with ASD. It would be useful to know more about how the brainstem (and thus the brainstem hypothesis) is affected over developmental periods.

In conclusion, not all of the potential questions relating ABR and ASD have been answered. Future studies should take into account the five distinct subpopulations of ASD as listed in the DSM-IV, gender differences, and age group differences. By more clearly defining these specific populations within the broad and heterogeneous "autism spectrum disorders," we hope to further understand and treat them in the most effective way possible.

BIBLIOGRAPHY

Boston, J.R. & Moller, A.R. (1985). The Origins of Brainstem Auditory-Evoked Potentials. *CRC Critical Reviews in Biomedical Engineering, 13(2)*, 98-123.

Achor, J. L. & Starr, A. (1980). Auditory Brainstem Responses in the Cat. II. Effects of Lesions. *Electroencephalography and Clinical Neurophysiology, 48*, 174-190.

Aiyer, R. G. & Parikh, B. (2009). Evaluation of Auditory Brainstem Responses for Hearing Screening of High-Risk Infants. *Indian Journal of Otolaryngology and Head & Neck Surgery, 61(1)*, 47-53.

Bauman, M. L., & Kemper, T. L. (2005). *The neurobiology of autism*. JHU Press.

Beers, A.N., McBoyle, M., Kakande, E., Dar Santos, R.C., & Kozak, F.K. (2014). Autism and peripheral hearing loss: a systematic review. *International Journal of Pediatric Otorhinolaryngology,* 78(1), 96-101.

Biacabe, B., Chevallier, J. M., Avan, P., & Bonfils, P. (2001). Functional Anatomy of Auditory Brainstem Nuclei: Application to the Anatomical Basis of Brainstem Auditory Evoked Potentials. *Auris Nasus Larynx, 28*, 85-94.

Christensen, D. L. (2016). Prevalence and characteristics of autism spectrum disorder among children aged 8 years—autism and developmental disabilities monitoring network, 11 sites, United States, 2012. *MMWR. Surveillance Summaries*, *65*.

Courchesne, E., Karns, C. M., Davis, H. R., Ziccardi, R., Carper, R. A., Tigue, Z. D., ... & Lincoln, A. J. (2001). Unusual brain growth patterns in early life in patients with autistic disorder an MRI study. *Neurology*, *57*(2), 245-254.

Dabbous, A. O. (2012). Characteristics of auditory brainstem response latencies in children with autism spectrum disorders. *Audiological Medicine*, *10*(3), 122-131.

Galambos, R. & Hecox, K. E. (1978). Clinical Applications of the Auditory Brainstem Response. *Otolaryngologic Clinics of North America, 11(3)*, 709-722.

Herbert, M. R., Ziegler, D. A., Deutsch, C. K., O'brien, L. M., Lange, N., Bakardjiev, A., Hodgson, J., Adrien, K.T., Steele, S., Makris, N., Kennedy, D., Harris, G.J., Caviness, V.J. (2003). Dissociations of cerebral cortex, subcortical and cerebral white matter volumes in autistic boys. *Brain*, *126*(5), 1182-1192.

Jewett, D. L. & Williston, J.S. (1971). Auditory-Evoked Far Fields Averaged From The Scalp of Humans. *Brain, 94,* 681-696.

Källstrand, J., Olsson, O., Nehlstedt, S. F., Sköld, M. L., & Nielzén, S. (2010). Abnormal auditory forward masking pattern in the brainstem response of individuals with Asperger syndrome. *Neuropsychiatric Disease and Treatment*, *6*, 289-296.

Klin, A. (1993). Auditory brainstem responses in autism: brainstem dysfunction or peripheral hearing loss?. *Journal of autism and developmental disorders*, *23*(1), 15-35.

Kwon, S., Kim, J., Choe, B. H., Ko, C., & Park, S. (2007). Electrophysiologic assessment of central auditory processing by auditory brainstem responses in children with autism spectrum disorders. *Journal of Korean medical science*, *22*(4), 656-659.

Lai, M. C., Lombardo, M. V., Auyeung, B., Chakrabarti, B., & Baron-Cohen, S. (2015). Sex/gender differences and autism: setting the scene for future research. *Journal of the American Academy of Child & Adolescent Psychiatry*, *54*(1), 11-24.

Lai, M. C., Lombardo, M. V., Suckling, J., Ruigrok, A. N., Chakrabarti, B., Ecker, C., ... & Baron-Cohen, S. (2013). Biological sex affects the neurobiology of autism. *Brain*, *136*(9), 2799-2815.

Marco, E. J., Hinkley, L. B., Hill, S. S., & Nagarajan, S. S. (2011). Sensory processing in autism: a review of neurophysiologic findings. *Pediatric Research, 69,* 48-54.

McClelland, R. J., Eyre, D. G., Watson, D., Calvert, G. J., & Sherrard, E. I. L. E. E. N. (1992). Central conduction time in childhood autism. *The British Journal of Psychiatry*, *160*(5), 659-663.

Mercati, O., Huguet, G., Danckaert, A., André-Leroux, G., Maruani, A., Bellinzoni, M., ... & Amsellem, F. (2017). CNTN6 mutations are risk factors for abnormal auditory sensory perception in autism spectrum disorders. *Molecular psychiatry*, *22*(4), 625-633.

Miron, O., Ari‐Even Roth, D., Gabis, L. V., Henkin, Y., Shefer, S., Dinstein, I., & Geva, R. (2015). Prolonged auditory brainstem responses in infants with autism. *Autism Research*.

Porges, S. W. (2007). The polyvagal perspective. *Biological psychology*, *74*(2), 116-143.

Rosenhall, U., Nordin, V., Brantberg, K., & Gillberg, C. (2003). Autism and auditory brain stem responses. *Ear and hearing*, *24*(3), 206-214.

Rosenhall, U., Nordin, V., Sandström, M., Ahlsen, G., & Gillberg, C. (1999). Autism and hearing loss. *Journal of autism and developmental disorders*, *29*(5), 349-357.

Roth, D. A. E., Muchnik, C., Shabtai, E., Hildesheimer, M., & Henkin, Y. (2012). Evidence for atypical auditory brainstem responses in young children with suspected autism spectrum disorders. *Developmental Medicine & Child Neurology*, *54*(1), 23-29.

Singh, V. K., Warren, R. P., Odell, J. D., Warren, W. L., & Cole, P. (1993). Antibodies to myelin basic protein in children with autistic behavior. *Brain, behavior, and immunity*, *7*(1), 97-103.

Skoff, B. F., Mirsky, A. F., & Turner, D. (1980). Prolonged brainstem transmission time in autism. *Psychiatry Research*, *2*(2), 157-166.

Stockard, J. J., & Rossiter, V. S. (1977). Clinical and pathologic correlates of brain stem auditory response abnormalities. *Neurology*, *27*(4), 316-316.

Starr, A., & Achor, L. J. (1975). Auditory brain stem responses in neurological disease. *Archives of Neurology*, *32*(11), 761-768.

Steffenburg, S. (1991). Neuropsychiatric Assessmenit of Children with Autism: A Population‐Based Study. *Developmental Medicine & Child Neurology*, *33*(6), 495-511.

Tas, A., Yagiz, R., Tas, M., Esme, M., Uzun, C., & Karasalihoglu, A. R. (2007). Evaluation of hearing in children with autism by using TEOAE and ABR. *Autism*, *11*(1), 73-79.

Trottier, G., Srivastava, L., & Walker, C. D. (1999). Etiology of infantile autism: a review of recent advances in genetic and neurobiological research. *Journal of Psychiatry and Neuroscience*, *24*(2), 103.