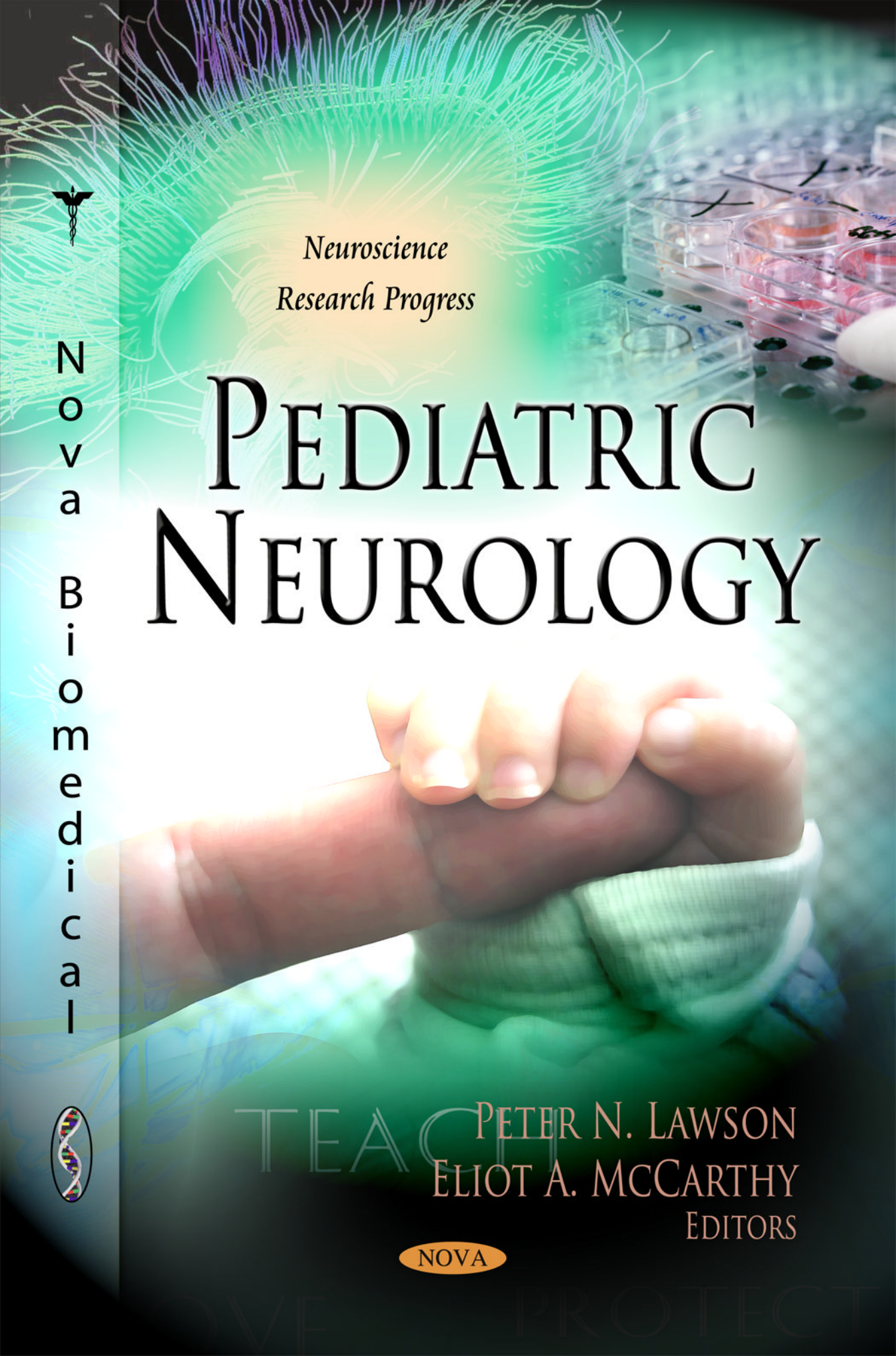
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PETER N. LAWSON
AND
ELIOT A. MCCARTHY
EDITORS

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CONTENTS

PREFACE

This book present current research from across the globe in the study of pediatric neurology. Topics discussed include the application of MLS BAER in pediatric, mainly neonatal, neurology to detect or diagnose brainstem and auditory abnormalities; childhood epilepsy and cognition; early predictors for autism spectrum disorders; acute severe hypoxia and chronic sublethal hypoxia on the neonatal brainstem and clinical neurophysiology in preterm infants.

Chapter 1 - In the last three decades, non-invasively electrophysiological examination of the functional integrity of the brainstem in pediatric, particularly neonatal, neurology has focused on the brainstem auditory evoked response (BAER) or potential (BAEP). This response reflects electrophysiological activity of a large number of neurons in the brainstem auditory pathway following acoustic stimulation. The non-invasive and objective nature of the BAER has led it to a wide use in pediatric neurology, in addition to audiology. It has been used to assess the functional integrity and development of the brainstem and the auditory system, and detect neuropathology that involves the brainstem auditory pathway in a wide range of pediatric diseases. Nevertheless, conventional BAER (i.e. the BAER recorded and analysed using conventional averaging techniques) has some limitation in detection of neuropathology, and false-negative results are not uncommon. For infants with a normal BAER the possibility of brainstem damage cannot be ruled out.

More recently, a relatively new technique — the maximum length sequence (MLS) has been introduced to record and analyze the BAER. This technique can exert a more stressful physiological/temporal challenge to brainstem auditory neurons, thus potentially improving detection of some neuropathology which may not be shown by conventional BAER. Recent studies have shown that MLS BAER improves the detection of neuropathology that affects the auditory brainstem in a range of pediatric problems. It is particularly valuable in detection of some early or subtle degree of neuropathology that cannot be detected by conventional BAER and other examination and investigations. This article reviews the application of MLS BAER in pediatric, mainly neonatal, neurology to detect or diagnose brainstem and auditory abnormalities or impairment, including perinatal hypoxia-ischemia or asphyxia, preterm birth, low Apgar score, chronic lung disease, hyperbilirubinemia, intrauterine growth restriction, neonatal necrotizing enterocolitis.

Chapter 2 - Infancy, preschool and school age periods are characterized by peak hippocampal and cortical regional development, as well as maximal white matter growth or myelinogenesis, dendritogenesis, and synaptogenesis. Occurrence of epilepsy during these

periods might result in impairment in spatial learning, memory processes and other aspects of cognition.Several variables are associated with cognitive impairment in epilepsy which includes: maternal-, seizure- and medication-related variables. High doses of antiepileptic drugs [AEDs] and polypharmacy are significant risks for cognitive impairment. In utero exposure to AEDs may cause defects in neuronal proliferation and migration and increase apoptosis. Cognitive impairment during childhood period even if trivial may adversely affect the child's psychosocial functioning by interference with educational skills and learning tasks.

Chapter 3 - Autism spectrum disorder (ASD) is a group of devastating developmental conditions whose prevalence was reported as increasing over the last decades. This may be related to changes in diagnostic criteria, comorbidity with other developmental disabilities or a true increase in cases. Diagnosis rests essentially on behavioral presentation and developmental history. Difficulties in communication and reciprocal social behavior are the core characteristics of ASDs. Motor and behavioral stereotypies, though prevalent, are not specific to ASDs and are often not observed before the age of 2. The etiology and pathophysiological mechanisms of ASD remain largely unknown, although environmental toxins and genetic factors have been implicated. Early diagnosis of ASD is of utmost importance because early intervention is especially effective in the experience of many professionals although not evidence based. Diagnosis for ASD is commonly made at approximately 3 years or older. There have been significant advances in our knowledge of the early signs of ASD through the use of retrospective videotape analysis, parental report and screening studies. However, there has been a lack of prospective methods to study early features in children who go on to develop ASD. There remains little research on the prospective identification of these children in a community-based sample before 18 months. Recently however some studies were able to identify early neurological signs and developmental predictors, which differed according to the age at assessment and allowed rather accurate identification of children with ASDs. By recruiting younger siblings of children with ASD, who are at much higher risk for developing ASD, some authors could demonstrate a prolonged latency to disengage visual attention from two competing stimuli and a delayed expressive and receptive language during the first year of life. A characteristic pattern of early temperament, with marked passivity and decreased activity level at 6 months, followed by extreme distress reactions, a tendency to fixate on particular objects in the environment, and decreased expression of positive affect by 12 months was aloso quite specifically recognized. By examining early medical and behavioral characteristics of NICU children later diagnosed with ASD, some authors showed that ASD neonates showed persistent neurobehavioral abnormalities and higher incidences of visual asymmetric visual tracking and upper limb muscle tone deficits. At 4 months, children with an eventual diagnosis of ASD specifically showed a continued visual preference for higher amounts of stimulation, behaving more like newborns. Looking at early social attention and communication skills with adapted scales in children before the age of 18 months in very large community-based settings, authors were able to identify children at “risk “ for ASD with a positive predictive value around 80 %. In this review, we review recent advances and discuss the validity of organizing early detection program for ASD in the context of a daily medical practice with the questions and hurdles raised by this approoach.

Chapter 4 - Perinatal asphyxia and neonatal chronic lung disease (CLD) are two major problems in newborn infants, often leading to neurodevelopmental deficits or disabilities later in life. Both problems are associated with hypoxia, but the nature of the hypoxia in the two

problems is different. The hypoxia after perinatal asphyxia is often acute, severe or lethal, and associated with ischaemia of the brain. In contrast, the hypoxia in neonatal CLD is chronic or prolonged and sublethal. Such differences may exert differential effects on the functional integrity and development of the neonatal brain, leading to different neuropathological changes and neurodevelopmental outcomes.

In recent years, some investigators have studied the functional integrity of the neonatal auditory brainstem in infants after perinatal asphyxia and neonatal CLD and have found differences in the effects of acute severe hypoxia and chronic sublethal hypoxia on the neonatal brainstem. In infants after perinatal asphyxia, neural conduction and synaptic function are impaired in both peripheral and central regions of the brainstem, although the impairment is slightly more severe in the more central than the more peripheral regions. In infants with neonatal CLD, however, neural conduction and synaptic function are impaired predominantly in the more central regions of the brainstem, whereas the more peripheral regions are relatively intact. These findings indicate that perinatal asphyxia affects both the central and peripheral regions of the brainstem, while neonatal CLD affects predominantly the central regions, without appreciable effect on the peripheral regions. This difference may be, at least partly, related to the different nature of hypoxia in the two clinical problems. These findings shed light on the pathophysiology underlying neurological impairment and developmental deficits in neonatal CLD, related to chronic sublethal hypoxia, and after perinatal asphyxia, related to acute lethal hypoxia and the associated ischemia. The knowledge obtained from these studies also provides valuable information for studying and implementing neuroprotective interventions or therapies for the two neonatal problems. The interventions should target more central regions of the brain for infants with CLD, but target both peripheral and central regions of the brain for infants after perinatal asphyxia.

Recent studies have also found that in infants with perinatal asphyxia, the electrophysiological activity in the neonatal brainstem is significantly depressed, suggesting major neuronal injury and/or neuronal death after severe hypoxia-ischemia. For these infants there is a need to intervene with radical neuroprotective measures (e.g. brain cooling) as early as possible to reduce further neuronal injury and death and rescue severely injured neurons. In infants with CLD, however, there was no noticeable depression of electrophysiological activity in the neonatal brainstem, suggesting no severe neuronal injury and/or neuronal death. It appears that for infants with CLD there is no need to implement radical treatments, and well regulated supplemental oxygen may remain the most valuable therapy, along with other therapeutic adjuncts.

Chapter 5 - The sensory evoked potentials in the visual auditory and somatosensory modality reflect the activity of the corresponding sensory pathways ascending to cerebral cortex and its' activation following sensory input. By contrast the event related potentials generated within specific neuropsychological paradigms reflect cognitive processing of the stimuli. It has been shown that during the first 20-45 weeks of gestation the development of the complex cortical and subcortical networks is modulated by sensory driven development of the talamo-cortical afferents and their connections with the developing cortical plate. The cortical responses recorded before the 36 gestational weeks are negative and in opposite polarity with respect to those elicited in infants born at term. It could be hypothesized that the above pattern of neurophysiological development could reflect the transient organization of immature cortex in the period of coexistence of subplate and cortical plate.

Event related potentials evoked by auditory stimulation using the oddball paradigm in the newborn is known to elicit obligate responses to sensory inputs as well as endogenous components similar to that reported in older children and adults. These responses may serve as an early index of developmental problems in the auditory cortex, in infants born pre-term.

The aim of this publication is to review the emerging evidence that evoked potential techniques may index the above maturation processes, thus providing a unique window on the brain at work during the early phases of development, in normal and pathological conditions.

Chapter 6 - Rett Syndrome (RTT) is a disorder caused by mutations in the methyl-CpG-binding protein 2 gene (MECP2) located at Xq28 that predominantly affects females. MECP2 mutations account for as many as 96% of cases with the classical features of RTT. RTT is characterized by a progressive loss of cognitive and motor skills, communication disorder, and deceleration of head growth. RTT syndrome is characterized by a period of apparently normal prenatal, perinatal and psychomotor development for the first 6 to 18 months, followed by a period of loss of previously developed language skills and purposeful hand use. Seizures, intermittent hyperventilation, ataxia and stereotypical hand movements develop over time.

The marked impairment in expressive and receptive language in patients with RTT has led to a number of studies investigating the peripheral and central auditory status in patients with RTT. These studies have included measures of the peripheral and central auditory status of patients with RTT. Procedures utilized have included tympanometry, otoacoustic emissions, the auditory brainstem response, middle latency response, long latency response, frequency following response, and long latency auditory evoked potentials. In the literature on RTT, there are conflicting reports as to the presence of abnormalities in the interpeak latency intervals of the ABR. The majority of studies have reported no abnormalities in the ABR interpeak latency intervals in RTT. It has also been reported that the interpeak latency intervals do not change over time in RTT, suggesting that RTT is not characterized by degenerative changes over time. However, several studies have reported prolongation in the I-V or the III-V interpeak latency intervals in RTT and an increased rate of ABR abnormalities has been found in patients with RTT syndrome with seizures requiring use of anticonvulsants. The use of sedation may also impact on the ABR in RTT. Evoked potential and other studies have shown that while the majority of patients with RTT have normal peripheral auditory function, hearing loss is present in some patients with RTT. Abnormal or absent middle latency responses and in the late vertex response have been reported and suggest the possibility of central auditory processing disorder in RTT. Atypical developmental patterns in auditory evoked potential responses mediated at brainstem levels (FFR) as well as at cortical levels using a passive oddball task have been reported. These studies will be systematically reviewed in the present chapter. The objective will be to consider the implications of auditory dysfunction as reflected in audiological and evoked potential studies, for the overall speech and language disorder characteristic of individuals with RTT.

Chapter 7 - Even in this age of modern medicine, managing pediatric epilepsy still poses a challenge. The seizures seen in childhood are grouped in different types of epilepsy syndromes. The epilepsy syndromes are classified on the basis of age of onset, family history, and type of epilepsy, progressive nature of the disease, EEG abnormalities, precipitating factors, family history, neuropsychological features, underlying genetic abnormalities and prognosis. Once the diagnosis is established, the next step is management. Pharmacological management of pediatric epilepsy is still the main cornerstone. The pharmacological

treatment differs in terms of acute versus chronic seizure management. Despite the availability of new anticonvulsants, there is still a portion of pediatric population which remains intractable. These patients may be evaluated for epilepsy surgery or brain neurostimulation at a specialized epilepsy center. Data have shown that effective treatment of epilepsy has improved quality of life and cognitive outcomes in children. This chapter will provide a in-depth review of various aspects of pediatric epilepsy and recent advances in diagnosis and management of this condition.

Chapter 8 - We assessed the cytokine and chemokine profile of cerebrospinal fluid (CSF) in patients with West syndrome (WS) to elucidate whether an immunological processes are concerned with the pathophysiology of WS. We analyzed CSF levels of twelve patients with WS, influenza-associated encephalopathy (IE), as a representative disease with high cytokines storms and twelve controls (cont). All samples of CSF were obtained the first 24 hours after the tonic spasms with informed consent. Seventeen items were measured using the Bio-Plex Multiplex Cytokine Assay, Interleukin (IL)-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, IL-13, IL-17, G-CSF, GM-CSF, INF-γ, MCP-1, MIP-1β and TNF-α.

Chapter 9 - In order to investigate the mechanism of seizure spread in patients with focal epilepsy, the interictal and ictal epileptiform activities were analyzed with special reference to multiple independent spike foci, and examined individual seizure semiology by video-EEG monitoring. The ictal EEG was recorded in 18 (9 in focal epilepsies, and 9 in generalized epilepsies and epilepsies undetermined whether focal or generalize) of the 77 epilepsy patients. Nine patients with focal epilepsies include two cases with temporal lobe epilepsy (TLE), 5 with frontal lobe epilepsy (FLE), and one each with parietal lobe epilepsy (PLE) and occipital lobe epilepsy (OLE). Four patients had underlying disorders, including gray matter heterotopia, periventricular leukomalacia (PVL), viral encephalitis and West syndrome (WS). Interictal EEG recordings verified multiple independent spikes in three patients, and their ictal EEGs resulted in generalized epileptiform discharges after onset. Two cases had past histories of profound brain insult such as PVL and viral encephalitis before the appearance of multiple independent spike foci. We suggest that these etiological backgrounds are closely associated with the multiple cortical excitability producing multiple independent spike foci, resulting in generalized epileptiform discharges.

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Chapter 1

MAXIMUM LENGTH SEQUENCE TECHNIQUE IMPROVES DETECTION OF NEUROPATHOLOGY INVOLVING INFANT BRAINSTEM

Ze Dong Jiang*
Department of Paediatrics, University of Oxford,
John Radcliffe Hospital, Oxford, United Kingdom

ABSTRACT

In the last three decades, non-invasively electrophysiological examination of the functional integrity of the brainstem in pediatric, particularly neonatal, neurology has focused on the brainstem auditory evoked response (BAER) or potential (BAEP). This response reflects electrophysiological activity of a large number of neurons in the brainstem auditory pathway following acoustic stimulation. The non-invasive and objective nature of the BAER has led it to a wide use in pediatric neurology, in addition to audiology. It has been used to assess the functional integrity and development of the brainstem and the auditory system, and detect neuropathology that involves the brainstem auditory pathway in a wide range of pediatric diseases. Nevertheless, conventional BAER (i.e. the BAER recorded and analysed using conventional averaging techniques) has some limitation in detection of neuropathology, and false-negative results are not uncommon. For infants with a normal BAER the possibility of brainstem damage cannot be ruled out.

More recently, a relatively new technique — the maximum length sequence (MLS) has been introduced to record and analyze the BAER. This technique can exert a more stressful physiological/temporal challenge to brainstem auditory neurons, thus potentially improving detection of some neuropathology which may not be shown by conventional BAER. Recent studies have shown that MLS BAER improves the detection of neuropathology that affects the auditory brainstem in a range of pediatric problems. It is particularly valuable in detection of some early or subtle degree of neuropathology that cannot be detected by conventional BAER and other examination and investigations. This

* Correspondence address: Department of Paediatrics, John Radcliffe Hospital, Headington, Oxford OX3 9DU, UK. E-mail address: zedong.jiang@paediatrics.ox.ac.uk.

article reviews the application of MLS BAER in pediatric, mainly neonatal, neurology to detect or diagnose brainstem and auditory abnormalities or impairment, including perinatal hypoxia-ischemia or asphyxia, preterm birth, low Apgar score, chronic lung disease, hyperbilirubinemia, intrauterine growth restriction, neonatal necrotizing enterocolitis.

INTRODUCTION

Since the discovery of brainstem auditory evoked response (BAER) in late 1960s and early 1970s, this response or, as some investigators called, brainstem auditory evoked potential (BAEP) or auditory brainstem response (ABR), soon became a major non-invasively electrophysiological tool to assess auditory function. Over the last three decades, the BAER has been the focus of non-invasively electrophysiological examination of the functional integrity of the brainstem and the auditory system in pediatric, particularly neonatal, audiology and neurology.

The BAER reflects electrophysiological activity of a large number of neurons in the brainstem auditory pathway following acoustic stimulation. It is the smallest of evoked potentials, and is detected by averaging the electroencephalogram immediately after each of several thousand acoustic, typically click, stimuli. The various deflections, which occur in the first 10-12 ms after the stimuli in infants and children, in BAER waveform represent neural activity at different levels of the brainstem auditory pathway that is anatomically close to many other pontomedullary structures in the brainstem.

The BAER consists of a sequence of seven positive waves (I, II, III, IV, V, VI, and VII) occurring in the first 10 ms after acoustic stimuli. These represent neural activity at different levels of the brainstem auditory pathway. It has been documented that BAER waves I and II are generated in the extracranial and intracranial portions of the VIIIth nerve, respectively. Subsequent waves III–VII are generated in auditory centres at gradually higher levels of the auditory pathway, with partially overlapping contributions to individual waves. Wave III is derived from the cochlear nucleus; wave IV is generated in the superior olivary complex; and wave V, together with the negative potential that follows it, is generated in the region of the lateral lemniscus and possibly inferior colliculus. The exact origins for the last two waves VI and VII remain open to speculation, although they possibly originate from the inferior colliculus and/or slightly higher levels of auditory structures.

The BAER has been well documented to change with neurological maturation and vary with the functional integrity of the auditory brainstem. The maturational phases of the BAER overlap or parallel the critical period of brainstem myelination, axonal sprouting, formation of central synaptic connections, improvement of synaptic efficiency, increase in axonal diameter and development of central dendritic properties (Moore et al., 1997; Ponton et al., 1996). The BAER also changes with chemical alterations of myelination or synaptic function. Study of the response enables us to assess the functional integrity and maturation of the auditory pathway, and provides useful information concerning functional status of the brain in clinical conditions that affect this pathway. This response has been used to assess peripheral auditory function, but also the functional integrity and development of the brain, specifically the auditory brainstem, in general in clinical conditions that affect the brainstem auditory pathway. As a non-invasive objective test, the BAER is generally unaffected by subject's

consciousness, sedatives, general anaesthetics, or anticonvulsants. This advantage makes it particularly suitable for very young or sick patients who cannot fully cooperate with a test. In pediatrics, particularly neonatology, the BAER has been used as a powerful diagnostic tool in clinical audiology and neurology to study and detect auditory and neurologic abnormalities in various clinical situations that may involve the brainstem auditory pathway, typically perinatal hypoxia-ischemia (Jiang, 2008,2010; Musiek et al., 2007; Wilkinson and Jiang, 2006).

Since the discovery of the BAER, this response has been recorded and analyzed using conventional averaging techniques. Nevertheless, conventional BAER has been found to have some limitation in detection of auditory abnormalities and diagnosis of neuropathology. It has only modest associations with neurological status and false negative results are not uncommon. Infants with neurological impairment may not show any apparent abnormalities in the BAER. Thus, for infants with a normal BAER the possibility of, particularly early or subtle, neuropathology or brainstem damage cannot be ruled out.

More recently, a relatively new technique — the maximum length sequence (MLS) has been introduced to record and analysis the BAER. This technique can be used with auditory evoked responses at all latencies (Picton et al., 1992). However, it is most effective for the responses of shorter latencies, including middle-latency responses and, especially, the BAER. By demonstrating different refractory periods for different parts of the response, the MLS technique helps delineate the component structure of the evoked responses, and disentangles the BAER from overlapping middle-latency responses.

Over the last decade, we have used the MLS technique to study the BAER to assess the functional integrity of the neonatal auditory brainstem in infants with various perinatal problems (Jiang, 2008,2010; Jiang et al., 1999, 2000, 2003, 2005a, 2006a,2007a,2008,2009a-c,2010; Wilkinson and Jiang, 2006 ; Wilkinson et al., 2007; Yin et al., 2008). The results show that the MLS BAER is a valuable technique to detect brainstem auditory abnormality and brain damage or neuropathology that involve the brainstem auditory pathway in some perinatal problems and enhance the diagnostic value of the BAER. In particular, MLS BAER can detect some early or subtle neuropathology that cannot be detected by conventional examination and investigations. This article reviews the application of MLS BAER in pediatric, mainly neonatal, neurology to detect or diagnose brainstem and auditory abnormalities or impairment in some clinical problems. These include perinatal hypoxia-ischemia or asphyxia, preterm birth, low Apgar score, chronic lung disease, hyperbilirubinemia, intrauterine growth restriction, neonatal necrotizing enterocolitis, and so forth.

MLS BAER – A NOVEL APPROACH TO ASSESS INFANT AUDITORY NEUROPHYSIOLOGY

Limitations of Conventional BAER

A stimulus condition that is optimal for eliciting the most easily identifiable BAER waveform may not be optimal for demonstrating pathology. Conventionally used slow repetition rates (between 10 and 21/sec) of acoustic stimuli can elicit well defined BAER

waveform, but may not be the optimal rates for detecting neuropathology. Presentation of rapid rates of acoustic stimulation while recording the BAER is of interest for evaluating the efficacy of central synaptic transmission and increasing the sensitivity of the BAER in detection of, particularly early or subtle degree of, neuropathology of the brain that involve the brainstem auditory pathway.

Some previous studies of conventional BAER in pediatric audiology and neurology suggested that the stimulus rates of greater than 91/sec could be more effective in detecting neurologic impairment of the brain (Jiang et al., 2001,2002,2004, Wilkinson and Jiang, 2006). However, increasing stimulus rate is limited by the need to prevent responses from overlapping one another. Conventional evoked potential instruments, or averagers, use stimuli separated by fixed interstimulus intervals (time intervals between the stimuli). The lower limit of these fixed intervals is determined by the duration of the electrophysiological response. Conventional stimulation and recording techniques require that the brainwave response to one stimulus be completed before delivery of the next stimulus. Stimulation before response completion results in overlapping waveforms, which are difficult to interpret. In the case of the BAER, response components last for 10 to 12 ms after stimulus onset, and this imposes a limit of 10 to 12 ms on the interpulse interval, corresponding to a rate of 100–80/sec. Refractory periods for auditory neurons are <10 ms. Thus, with conventional averaging techniques, the maximum repetition rate for click stimuli to elicit the BAER is up to 100/sec, which limits the study of adaptive or recovery processes of auditory neurons and the diagnosis of neuropathological conditions that involve the brainstem auditory pathway.

The MLS Technique and Some Advantages

One feasible method to circumvent the rate limitation imposed by conventional averaging is to use pseudorandom pulse trains which are binary sequences, called MLS, as acoustic stimuli. The MLS is a specially constructed pseudorandom binary sequence that can be used to control the presentation of sensory stimuli (Eysholdt et al., 1982; Picton et al., 1992). Unlike the uniformly spaced stimuli used in conventional BAER testing, the MLS technique uses patterned stimulus presentation. Different patterned sequences of stimuli are created by omitting a portion (e.g., 50%) of the stimuli in a pseudorandom manner. Mathematically, a MLS is a quasi-random binary sequence represented by a train of +1 second and –1 second. In its audiological application, it may be presented as +1 second and 0 seconds or as clicks and silences. This stimulus consists of distinct pulses of uniform polarity and amplitude, occurring at pseudorandom time intervals. Each pulse sequence is actually a series of pulses. Therefore, the accepted value and the number entered in the sweep count represent the number of sequences, not the number of discrete pulses as in conventional BAER testing. When there are 50% gaps in the MLS stimulus patterns, the actual repetition rate fluctuates over time and the average rates are actually one half of the rates presented. The nature of the stimuli and the newly developed processing technique make it unnecessary to wait for the response of each pulse to be completed before application of a new pulse. Therefore, pulses can be delivered at maximal rates of up to 1000 clicks per second or even higher. The patterned sequences of stimuli are generated by the averaging computer, and this information is then used to perform on-line deconvolution (separation, alignment, and averaging) of overlapping individual responses. As in conventional BAER recording, each waveform of the

response is filtered and the waveforms are averaged. By mathematically cross-correlating the collected data with a recovery sequence, the final MLS BAER is obtained for analysis. Figure 1 is a diagram showing differences in data acquisition and processing between conventional BAER testing and MLS BAER testing.

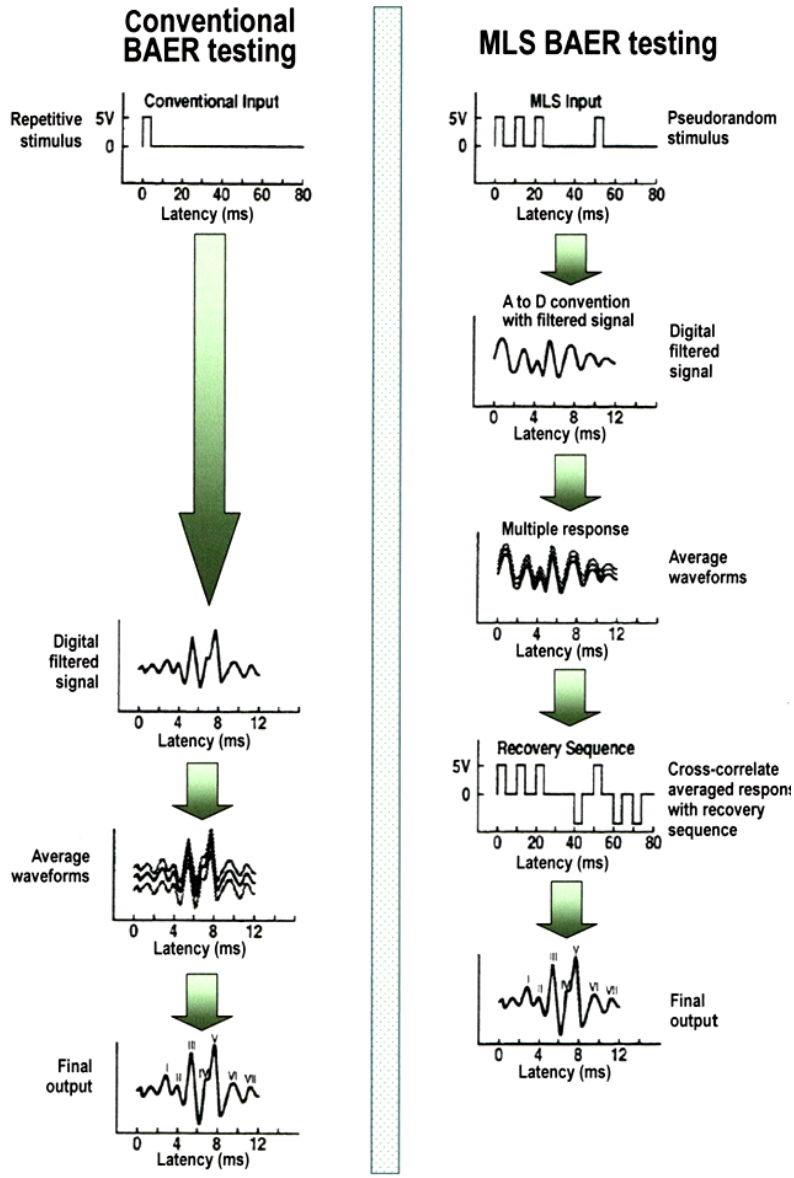

Figure 1. Diagram showing differences in data acquisition and processing between conventional BAER testing and MLS BAER testing.

Study of the MLS can gain new insights into functional properties of the brainstem and the auditory pathway, and enables us to have a more thorough description of the electrophysiological behaviour of the auditory brainstem and its development. The MLS BAER can provide novel information about neural processing that cannot be offered by conventional BAER, e.g. temporal interactions (the effect of prior stimulation on the response to current stimulation) of the auditory system that cannot be analysed in conventional BAER. By examining latency- and amplitude-rate functions at rates exceeding those used in conventional BAER and characterising the temporal interactions, the MLS and cross-correlation techniques allow us to investigate temporal processing of auditory information in the brainstem.

The MLS technique allows presentation of stimuli at much higher rates than is possible with conventional methods, because this technique permits the overlap of responses to successive stimuli (Eysholdt et al., 1982; Picton et al., 1992). Thus, this technique can be used to characterise adaptation in the auditory system at rates approaching the absolute refractory period of auditory neurones. The higher rates provide a much stronger temporal/physiological challenge to auditory neurones, and permit a more exhaustive sampling of physiological recovery or "fatigue" than is possible with conventional stimulation. This enables the MLS BAER to have a potential to detect some early or subtle neuropathology that may not be shown by conventional BAER, improving the sensitivity of the BAER in detection of neuropathology that affects the brainstem auditory pathway.

The BAER, either conventional BAER or MLS BAER, can be elicited with various repetition rates of acoustic stimuli, and changes with different rates. The BAER obtained at relatively slow repetition rates of click stimuli reflects general function of the brainstem auditory pathway, primarily related to nerve conduction velocity associated with axonal diameter and myelination. The BAER can also be obtained at high repetition rates. Changes in the BAER with increasing stimulus presentation rate, called stimulus rate-dependent changes, primarily reflect neural processes concerning the efficacy of central synaptic transmission, as well as neural synchronisation and metabolic status, of auditory neurons in the brainstem following the presentation of a temporal/physiological challenge (Jiang, 2008; Jiang et al., 2000; Wilkinson et al., 2007). Thus, the rate-dependent BAER changes provides a valuable means to assess synaptic function, specifically synaptic efficacy. By presenting a temporal/physiological challenge to auditory neurons the high-rate stimulation, so called "stimulus challenge or stress test", can also detect certain cerebral pathology or neural alteration/dysfunction that may not be demonstrated by relatively lower stimulus rates in conventional BAER, thereby improving the diagnostic value of the BAER. Patients with some CNS pathology may manifest an abnormality only at high stimulus rates. Therefore, this method, apart from its use for assessment of synaptic efficacy, provides a valuable means to enhance the detection of neural dysfunction by presenting a physiological challenge to brainstem auditory neurones. This method also potentially provides a tool to monitor dynamic changes in cerebral function following medical intervention or treatments and assess the response of the brain to therapeutic or neuroprotective measures, such as brain cooling for neonates after perinatal hypoxia-ischemia.

There are some other advantages of the MLS technique. Since this technique allows evoked responses to overlap in time and thus interpulse interval can be extremely short, the MLS BAER could reduce data collection time. This can potentially increase the amount of data obtained per session in the clinic or increase the number of patients on whom reliable information can be

collected. Another distinct advantage of the MLS technique is the ability to perform simultaneous collection of left and right ear data, which is called binaural MLS BAER (Lasky et al., 1993,1995). This ability can not only save the recording time per patient, but also make it more possible to complete the test within a relatively short time; as with babies who may wake up too soon or be too restless to accomplish conventional test. In the neonatal intensive care nursery this advantage could significantly enhance the response to background noise ratios, improving response detection. Therefore, compared with conventional BAER test, the MLS BAER test can be more efficacious in detecting neuropathology that affects the brainstem auditory pathway.

The MLS technique has been mainly used to study the BAER, as well as middle latency auditory evoked potentials (e.g. Bell et al., 2001,2002,2006; Bohórquez and Ozdamar 2006,2008; Burkard, 1991; Chan et al., 1992; Dzulkarnain et al., 2008; Eysholdt and Schreiner, 1982; Jirsa, 2001; Lasky, 1997; Lasky et al., 1992,1993,1995,1998; Lavoie et al., 2010; Lina-Granada et al., 1994; Musiek and Lee, 1997; Nagle and Musiek, 2009; Picton et al., 1992; Thornton and Slaven, 1993; Wang et al., 2006; Wilkinson and Jiang, 2006; Wilkinson et al., 2007). Some investigators have also used the MLS technique to study acoustic emission (de Boer et al., 2007; Lineton et al, 2008; Picton et al., 1993).

Over the last decade, in order to assess the applicability and effectiveness of the MLS BAER in pediatric, particularly neonatal, auditory neurology in detection of neuropathology in the brain in neonatal neurology, the author's team has studied MLS BAER in infants with a range of clinical problems or conditions (Jiang, 2008,2010; Jiang et al., 1999,2000,2003,2005a,2006a,2007a,2008,2009a-c,2010; Wilkinson and Jiang, 2006 ; Wilkinson et al., 2007; Yin et al., 2008). We found that the MLS BAER can be reliably recorded in subjects of various ages. The response has a good reproducibility, with a relatively smaller variability, compare with conventional BAER. Therefore, the MLS technique is feasible for clinical application. So far, we have studied MLS BAER in over one thousand preterm and term infants with or without major perinatal problems or complications, as well as in animal models with experimental hypoxia-ischemia or chronic sublethal hypoxia. The results have demonstrated that MLS BAER can improve the detection of neuropathology that affects the auditory brainstem in neonates with some perinatal problems or complications, particularly some early or subtle neuropathology that is difficult to detect with conventional examination and investigations.

Recording of MLS BAER

Like conventional BAER, MLS BAER can be reliably recorded in a quiet room, the ward and the intensive care unit. The procedures are generally similar to those of recording, measurement and analysis of conventional BAER, although there are some differences. The equipment we have used in the last decade for MLS BAER study is mainly a Spirit 2000 evoked potential system (Nicolet Biomedical, Madison, WI, USA).

Preparation, Equipment and Acoustic Stimuli

Before the recording, the subject is requested to lie supine in a bed or, for young infants, a cot/bassinet. The auditory meatus is inspected and cleaned of any vernix or wax. After skin preparation, three gold- or silver-plated disk electrodes are placed, respectively, on the middle

forehead (positive), the left or right (ipsilateral) earlobe or mastoid (negative), and the other side (contralateral) earlobe or mastoid (ground). Compared with mastoid placement, earlobe placement increases the amplitude of BAER wave I by about 30 percent Interelectrode impedances should be <5 kΩ, which is maintained during the recording. Acoustic stimuli are delivered to the ipsilateral ear through a TDH 39 or 49 earphone. The earphone is placed comfortably but closely over the ear to avoid sound leakage. For very young infants, a great care is needed for the placement to avoid collapsing the ear canals. While recording, the earphone should remain in place over the ear, with the centre over the ear cannel, and avoid any slippage. Displacement of the earphone will inevitably reduce the intensity of acoustic stimuli to reach the eardrum, resulting in misleading results.

The acoustic stimuli used to elicit the MLS BAER are usually clicks, generated by 100-μs rectangular electric pulses and delivered monaurally through a TDH 39 earphone. The clicks are presented at various repetition rates, defined by the shortest time interval between adjacent stimuli, usually at four rates, 91, 227, 455, and 910/sec, equivalent to a minimum interpulse interval (the duration of the sequence) of 11.1, 4.4, 2.2, and 1.1 ms, respectively.

The intensity levels of the clicks used in MLS BAER recording is slightly different from those used in conventional BAER recording (Jiang et al., 2000). For the purpose of neurologic assessment, the intensity of stimulation used to elicit conventional BAER is usually between 70 and 80 dB nHL. The repetition rates used to elicit the MLS BAER are much faster than those used in conventional BAER. Because of temporal integration, the perception of loudness of the stimulus trains increases as repetition rate is increased. An intensity of 80 dB nHL or higher is intolerable for subjects with a normal hearing. Even 70 dB nHL tends to be too loud for some subjects, particularly when the test takes a long time. Therefore, to avoid irritating the subject owing to too loud acoustic stimuli, an intensity level of 60 dB nHL is a proper level for routine use in recording MLS BAER for subjects who have a normal hearing threshold, i.e. ≤20 dB normal hearing level (nHL).

For subjects who have a hearing problem and/or an increased hearing threshold (>20 dB nHL), click intensity levels higher than 60 dB nHL, e.g. 70-80, or even up to 90 (for severe hearing problems only) dB nHL should be used, as appropriate, to obtain a well-defined MLS BAER waveform. It is often difficult to obtained well-defined MLS BAER waveforms in infants who had major peripheral hearing problems or a significant elevation in hearing threshold (e.g. >40 dB nHL). Such infants should be excluded from MLS BAER study to avoid any significant effect of peripheral auditory problems on MLS BAER waveforms and, in turn, the inaccuracy in measurements of MLS BAER components due to poor-defined waveforms.

In pediatric neurology, MLS BAER is superior to conventional BAER mainly in neurological assessment of the brainstem auditory function, although it does not appear to have any significant advantages over conventional BAER in assessment of peripheral auditory function. For neurologic assessment, to obtain well-defined MLS BAER waveform and cancel any effects of elevation of hearing threshold on MLS BAER measurements, MLS BAER recordings and data analysis should be carried out at a hearing level 40 dB or slightly higher above BAER threshold of each subject. This also allows comparison of MLS BAER data between different groups of subjects at a similar or the same hearing level. In this chapter, the MLS BAER data described are all obtained with clicks presented at the intensity level of 40 dB or slightly higher than 40 dB above BAER threshold of each subject.

Recording

Like in conventional BAER, the recording of MLS BAER is preferred to commence after the subject falls asleep naturally, often after a feeding for young infants. First, conventional BAER is recorded to obtain the threshold, defined as the lowest intensity of clicks that produced visible, replicable wave V.

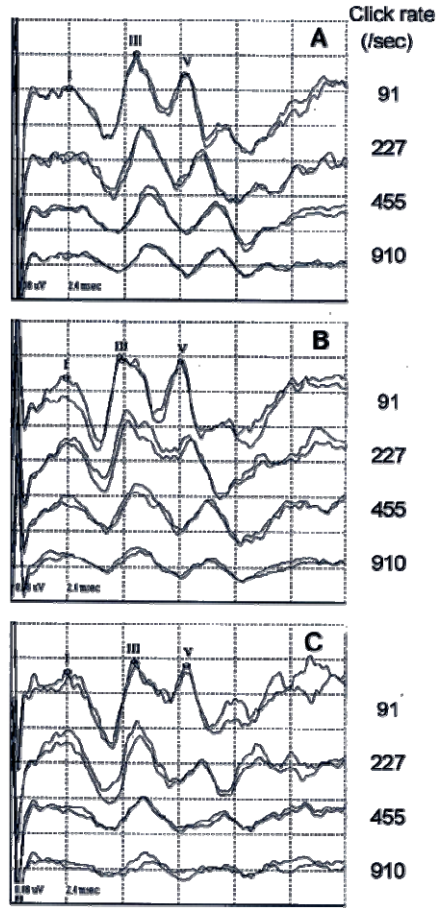

Figure 2. Sample recordings of MLS BAER from a normal term infant (A), a low-risk preterm infant (B), and a high-risk preterm infant (C), recorded at term (click intensity ≥ 40 dB above BAER threshold). Compared with the infants (A) and (B), the infant (C) shows a significant increase in wave V latency, I–V and particularly III–V intervals, and a significant reduction in wave V amplitude at 455 and 910/sec clicks, but not at lower rates.

Then, MLS BAER recording is started with clicks at a hearing level 40 dB or slightly higher above BAER threshold of each subject, usually 60 dB nHL for subjects with a normal hearing. Higher intensities can be used for subjects who have a BAER threshold >20 dB nHL. The clicks are usually presented at 91, 227, 455, and 910/sec in the first run and in a reverse sequence in the second run. The brain responses evoked by the clicks were amplified and filtered (usually at 100 to 3000 Hz). An automatic rejection system is used to automatically exclude the sweeps that exceed (e.g. 91% of) the sensitivity parameter setting (e.g. of 51 μV), usually produced by artifacts. Whenever there are excessive muscle artifacts on the

monitoring oscilloscope, sampling should be discontinued manually until the artifacts reduce significantly. Each run includes brain responses to, usually, 1500 trains of clicks. At least two runs are needed in response to each stimulus condition to obtain duplicate recordings and examine reproducibility of the recordings. Sweep duration (e.g. 24 ms) should be longer than that used in conventional BAER (e.g. 12 ms) because, as a result of much higher repetition rate used, the latencies of MLS BAER wave components are longer than those in conventional BAER.

Waveform and Analysis of MLS BAER Components

The earliest time at which MLS BAER can be recorded is 28-29 weeks of postconceptional age in most infants. All the Jewett waves I to V can be identified in MLS BAER waveform. Compared with those in conventional BAER waveform, waves III and V are often better defined in MLS BAER waveform, although waves II and IV are often less-well defined, particularly in young infants. Figure 2 shows sample MLS BAER recordings with clicks presented at 91, 227, 455 and 910/sec from a normal term infant (A), and, for comparison, a low-risk preterm infant (B) and a high-risk preterm infant (C). No apparent waveform alteration occurs at the click repetition rate up to 455/sec in MLS BAER, while the alteration often occurs at the click rates higher than 71/sec in conventional BAER. When click rate is increased to 910/sec, however, some alteration may occur in the elicited MLS BAER waveform, and, in turn, result in some difficulty in accurate and reliable identification and measurement of waves I and V.

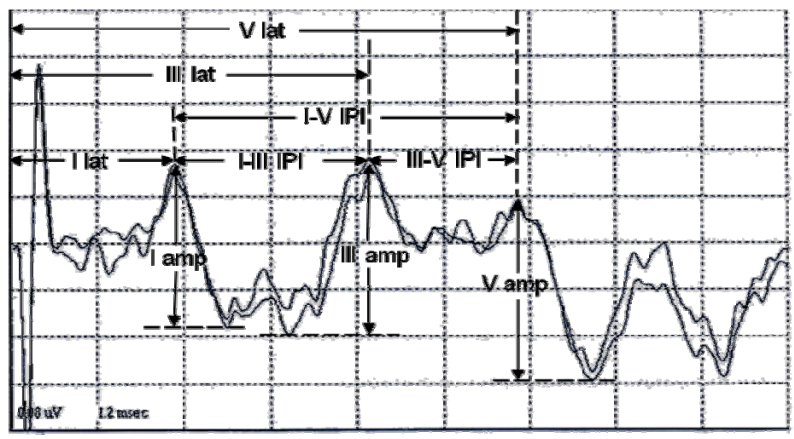

Figure 3. Schematic measurement of various BAER components. ‘lat’ refers to latency, ‘IPI’ to interpeak interval, and ‘amp’ to amplitude.

Schematic analysis of various components in MLS BAER waveform, including wave latencies and amplitudes, and interpeak intervals, is illustrated in Fig. 3. The basic analysis is the same as in conventional BAER. The latency of a MLS BAER wave is the time interval between the onset of the stimulus presentation and the appearance of a wave peak in the waveform. In other words, the latency is an absolute measure calculated from the stimulus onset to the peak of an MLS BAER wave component. There are 3 major wave latencies, i.e.

wave I, wave III and wave V latencies. Interpeak interval is the relative measure calculated as the time between the peaks of two different MLS BAER waves. As in conventional BAER, there are 3 interpeak intervals, including I-V, I-III and III-V intervals, and an interval ratio of III-V and I-III intervals (i.e. III-V/I-III interval ratio) (Jiang, 2008; Wilkinson and Jiang, 2006).

The amplitude of a MLS BAER wave is the measurement of the voltage difference between the peak and the preceding or following trough of a wave. The amplitude of wave I is measured from the positive peak of wave I to the negative trough immediately after the peak. However, in very young infants the down slope of wave I is often significantly affected by presence or absence of wave II, or the location of wave II if it is present. This can produce considerable variation in the amplitude of wave I, and in turn affect the reliability of diagnostic value of V/I amplitude ratio (Jiang et al., 2008). To minimize such a variation the amplitude of wave I in very young infants is proposed to be measured from its peak to the lowest trough between waves I and III, which is more consistent and reliable. Because the trough after wave III is considerably variable, it is not reliable to use the trough to measure the amplitude of wave III. Instead, the amplitude is measured from the lowest trough between waves I and III to the peak of wave III (Lasky, 1997; Jiang et al., 2001,2008). The amplitude of wave V is measured from the positive peak of wave V to the negative trough immediately after the peak. With the 3 wave amplitudes, relative amplitude ratios (i.e. V/I and V/III amplitude ratios) are then calculated.

Measurements of each MLS BAER variable from two replicated recordings to each stimulus condition are averaged for data analyses. The following MLS BAER data described are all obtained at the intensity level of 40 dB or slightly higher than 40 dB above BAER threshold of each subject we studied.

Changes in MLS BAER Components with Click Intensity and Repetition Rate

As the intensity level of click stimuli is increased, the latencies of MLS BAER waves are decreased and the amplitudes are increased (Jiang et al., 1999). Conversely, with the decrease in click intensity, the latencies of MLS BAER waves are increased and the amplitudes are decreased. All wave latencies shifted by roughly the same amount with varying click intensity. As a result, interpeak intervals are almost unchanged at different click intensity levels (Jiang et al., 1999). These changes in MLS BAER variables with varying click intensity are also generally similar to those in conventional BAER.

There is a general trend that with the increase in repetition rate of clicks all MLS BAER wave latencies and interpeak intervals are progressively increased and all wave amplitudes are progressively reduced (Jiang, 2008,2010; Jiang et al., 1999; Jiang et al., 2000,2003,2005a,2008,2009a-c,2010; Lasky, 1997; Lasky et al., 1997). The latencies of waves I, III and V are all correlated positively and significantly with click rate (Jiang, 2008; Jiang et al., 1999; Jiang et al., 2000,2003,2005,2009c,2010). Similarly, the interpeak intervals of I-III, III-V and I-V are all correlated positively and significantly with click rate. The same is true of the III-V/I-III interval ratio. In contrast, all amplitudes of waves I, III and V are correlated negatively and significantly with click rate (Jiang et al., 2008, 2009a,c). The V/I

and V/III amplitude ratios both vary slightly with the change in click rate, but neither is correlated significantly with the rate. The slopes of latency-rate functions for all MLS BAER wave components are progressively steeper for the later waves. These click rate-dependent changes are generally similar to those in conventional BAER.

In any MLS BAER recordings, as click rate is increased from 91/s to 455/s, there is a progressive increase in wave latencies and interpeak intervals, i.e. MLS BAER wave latencies and intervals change linearly with varying click rate. However, as the rate is increased from 455/s to 910/s, wave latencies and interpeak intervals do not show any further notable increase. The measurements of all wave latencies and interpeak intervals obtained at 910/s are almost the same as, or slightly longer or shorter than, those obtained at 455/s, although wave amplitudes are smaller than those at 455/s. The measurements of wave latencies and intervals at 910/s are often slightly longer or shorter than those at 455/s in neonates, though often slightly longer than at 455/s in older infants. In other words, the measurements of MLS BAER wave latencies and intervals reach a plateau from the rate 455/s. Further increase in click rate to 910/s cannot linearly or significantly increase wave latencies and intervals, i.e. MLS BAER wave latencies and intervals no longer change linearly with the increase in click rate. In infants with clinical problems that may involve in the brainstem auditory pathway, MLS BAER abnormalities are generally increased with the increase in click rate. However, the abnormalities at the very high click rates 455/s and 910/s are often similar. That is, when click rate is increased to 455/s, further increasing the rate may not significantly further improve the detection of brainstem auditory abnormalities.

MLS BAER IN NORMAL TERM INFANTS

Term Neonates

All wave latencies and interpeak intervals in MLS BAER in term infants are significantly longer than those in children and adults, and all wave amplitudes are smaller than those in adults (Jiang et al., 1999; Lasky, 1997; Lasky et al., 1997). This is true of all click rates. Tables 1 and 2 present means and standard deviations (SD) of wave latency and interpeak intervals, and wave amplitudes, respectively, in MLS BAER in normal term infants (37-42 weeks postconceptional age) and, for comparison, healthy young adults at various click rates (91-910/sec), with a click intensity $\geq$ 40 dB above the BAER threshold of each subject. For the purpose of comparison, measurements of conventional BAER at 21/sec in these infants are also presented. These data are obtained from the author's Neonatal Auditory Laboratory at the University of Oxford.

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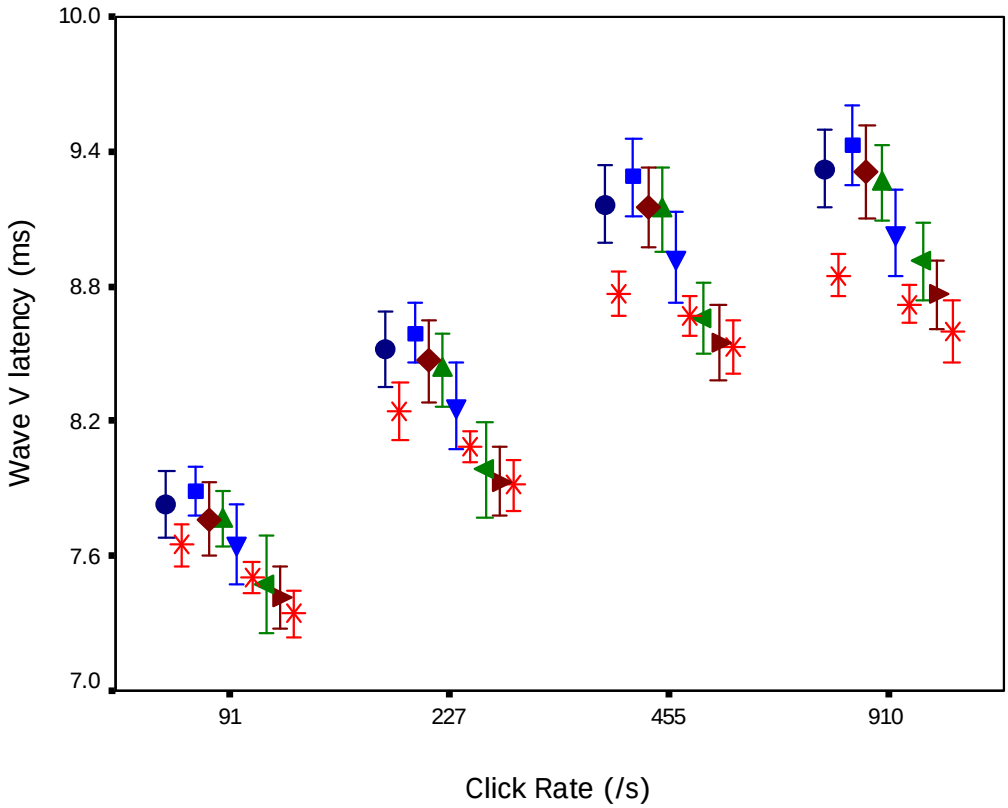

Figure 4. Means and standard errors of wave V latency at different repetition rates of clicks (≥40 dB above BAER threshold) during the first month after birth in normal term infants and term infants after hypoxia-ischemia. The symbols in sequence from left to right at each click rate represent the data of infants after hypoxia-ischemia on day 1, normal term infants on days 1-3, infants after hypoxia-ischemia on days 3, 5, 7 and 10, normal term infants on days 10-15, infants after hypoxia-ischemia on days 15 and 30, and normal term infants on day 30.

Postnatal Development

Changes in wave V latency, I-V interval and wave V amplitude, which are mainly related to central neural function of the brainstem auditory pathway, during the first month after birth in normal term infants, are shown by the measurements presented, respectively, in Figures 4-6. For the purpose of comparison, the measurements in term infants after hypoxia-ischemia are also presented. As the day after birth is increased during the first month, all wave latencies and interpeak intervals in MLS BAER are decreased rapidly (Jiang, 2008), while all wave amplitudes are increased rapidly (Jiang et al., 2008). Such age-related changes are true of all click rates 91-910/sec, and are generally similar to those in conventional BAER at 21/sec.

Table 1. Means and standard deviations (SD) of wave latencies and interpeak intervals in MLS BAER (click rates 91-910/sec) and conventional BAER (21/sec) in normal term infants and adults (click intensity ¤ 40 dB above BAER threshold)

BAER variables	Subjects	21/sec mean ± SD	91/sec mean ± SD	227/sec mean ± SD	455/sec mean ± SD	910/sec mean ± SD
I (ms)	Term	2.32 ± 0.17	2.43 ± 0.14	2.58 ± 0.15	2.70 ± 0.16	2.75 ± 0.15
	Adult	2.12 ± 0.13	2.22 ± 0.19	2.39 ± 0.22	2.54 ± 0.20	2.66 ± 0.12
III (ms)	Term	5.06 ± 0.21	5.28 ± 0.20	5.62 ± 0.23	5.88 ± 0.23	5.93 ± 0.21
	Adult	4.25 ± 0.14	4.44 ± 0.24	4.78 ± 0.26	5.12 ± 0.23	5.34 ± 0.21
V (ms)	Term	7.27 ± 0.22	7.60 ± 0.18	8.18 ± 0.23	8.74 ± 0.22	8.80 ± 0.23
	Adult	6.09 ± 0.21	6.29 ± 0.24	6.77 ± 0.27	7.29 ± 0.27	7.67 ± 0.26
I-III (ms)	Term	2.76 ± 0.14	2.85 ± 0.14	3.05 ± 0.17	3.18 ± 0.19	3.19 ± 0.18
	Adult	2.12 ± 0.16	2.19 ± 0.14	2.38 ± 0.16	2.55 ± 0.20	2.67 ± 0.22
III-V (ms)	Term	2.22 ± 0.12	2.33 ± 0.14	2.58 ± 0.15	2.85 ± 0.17	2.87 ± 0.15
	Adult	1.84 ± 0.14	1.85 ± 0.15	2.00 ± 0.16	2.19 ± 0.10	2.28 ± 0.14
I-V (ms)	Term	4.99 ± 0.15	5.18 ± 0.16	5.62 ± 0.18	6.03 ± 0.19	6.05 ± 0.19
	Adult	3.97 ± 0.24	4.08 ± 0.24	4.37 ± 0.21	4.74 ± 0.23	4.97 ± 0.26
III-V/I-II ratio	Term	0.801 ± 0.065	0.819 ± 0.072	0.850 ± 0.082	0.901 ± 0.087	0.902 ± 0.076
	Adult	0.871 ± 0.087	0.852 ± 0.082	0.838 ± 0.087	0.863 ± 0.077	0.876 ± 0.092

Table 2. Means and standard deviations (SD) of wave amplitudes of MLS BAER in MLS BAER (click rates 91-910/sec) and conventional BAER (21/sec) in normal term infants and adults (click intensity ¤ 40 dB above BAER threshold)

BAER variables	Subjects	21/sec mean ± SD	91/sec mean ± SD	227/sec mean ± SD	455/sec mean ± SD	910/sec mean ± SD
I (μV)	Term	0.175 ± 0.056	0.127 ± 0.039	0.083 ± 0.030	0.051 ± 0.017	0.034 ± 0.012
	Adult	0.141 ± 0.059	0.078 ± 0.035	0.066 ± 0.029	0.044 ± 0.016	0.039 ± 0.030
III (μV)	Term	0.214 ± 0.060	0.189 ± 0.052	0.140 ± 0.039	0.087 ± 0.027	0.056 ± 0.018
	Adult	0.170 ± 0.055	0.111 ± 0.044	0.091 ± 0.031	0.069 ± 0.027	0.043 ± 0.020
V (μV)	Term	0.204 ± 0.060	0.160 ± 0.053	0.107 ± 0.040	0.079 ± 0.028	0.050 ± 0.014
	Adult	0.341 ± 0.113	0.304 ± 0.090	0.210 ± 0.075	0.133 ± 0.052	0.093 ± 0.047
V/I ratio	Term	1.400 ± 0.860	1.428 ± 0.536	1.439 ± 0.751	1.708 ± 0.814	1.706 ± 1.211
	Adult	2.791 ± 1.095	4.656 ± 2.330	3.972 ± 1.540	3.799 ± 1.500	3.915 ± 1.425
V/III ratio	Term	1.046 ± 0.541	0.940 ± 0.419	0.799 ± 0.290	0.911 ± 0.312	0.926 ± 0.354
	Adult	2.075 ± 0.748	2.922 ± 1.191	2.620 ± 0.924	1.995 ± 0.681	2.655 ± 1.452

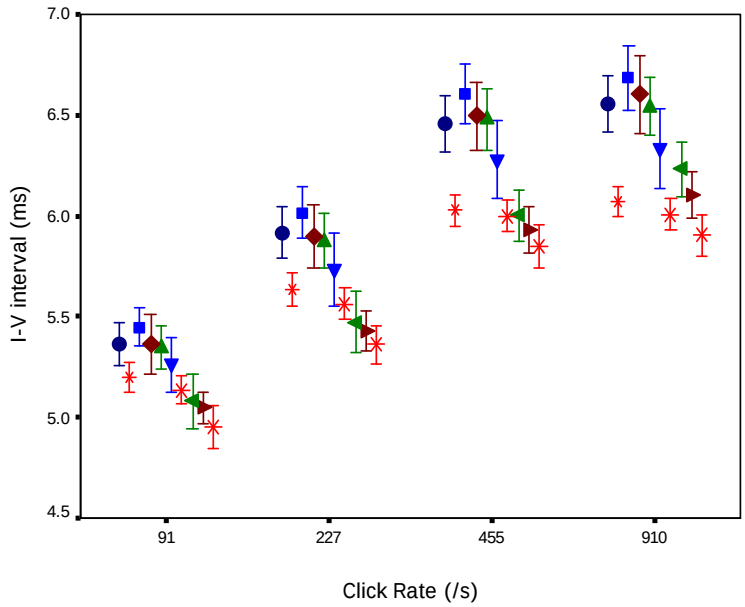

Figure 5. Means and standard errors of I-V interval at different rates of clicks (≥40 dB above BAER threshold) during the first month after birth in normal term infants and term infants after hypoxia-ischemia. The symbols in sequence from left to right at each click rate represent the data of infants after hypoxia-ischemia on day 1, normal term infants on days 1-3, infants after hypoxia-ischemia on days 3, 5, 7 and 10, normal term infants on days 10-15, infants after hypoxia-ischemia on days 15 and 30, and normal term infants on day 30.

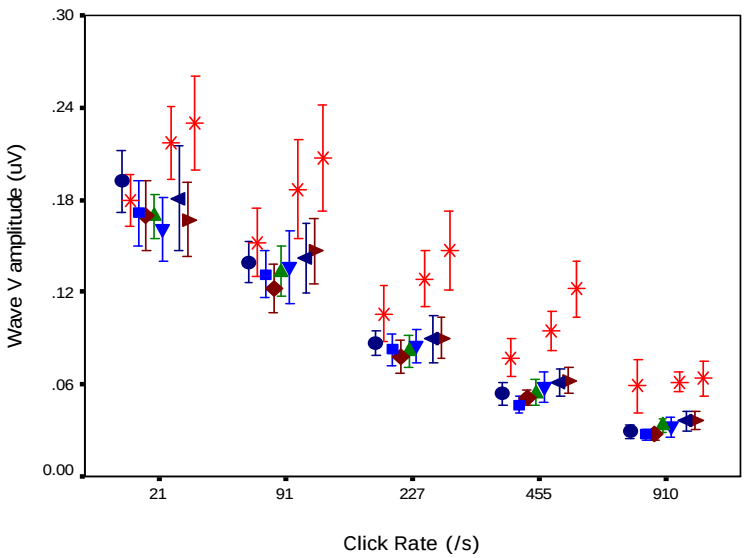

Figure 6. Means and standard errors of wave V amplitude at different repetition rates of click stimuli (≥40 dB above BAER threshold) during the first month after birth in normal term infants and term infants after hypoxia-ischemia. The symbols in sequence from left to right at each click rate represent the data of infants after hypoxia-ischemia on day 1, normal term infants on days 1–3, infants after hypoxia-ischemia on days 3, 5, 7 and 10, normal term infants on days 10–15, infants after hypoxia-ischemia on days 15 and 30, and normal term infants on day 30.

During the postnatal period, all latencies of MLS BAER waves I through to V and all I-III, III-V and I-V interpeak intervals are decreased and all wave amplitudes are generally increased as a function of advancing age. Such age-related changes occur more significantly at earlier than at later postnatal period, with the greatest changes occurring within first the 6 months of age. Most wave latencies and interpeak intervals approach adult equivalences at the ages between 1 and 3 years. The amplitudes of waves I and III are increased with the increase in postnatal age during the first 6-9 months, and then tend to be decreased with the further increase in age. However, wave V amplitude is increased continuously with the increase in postnatal age up to 2-3 years we studied. By 2-3 years, the amplitude is larger than in adults at all click rates. Figures 7-9 show age-related changes in the measurements of wave V latency, I-V interval and wave V amplitude, respectively, from birth through to 2 years old in normal term infants, and, for comparison, normal young adults. These developmental changes in MLS BAER variables are true of all click rates between 91 and 910/sec, and are generally similar to those in conventional BAER variables at 21/sec. So far, there is a lack of reported studies of postnatal development of MLS BAER. The above observations are obtained from the author's laboratory, and to be published.

In addition, we have carried out studies of MLS BAER in some children of various ages and young adults, with normal hearing and neurological status. These studies are to be completed.

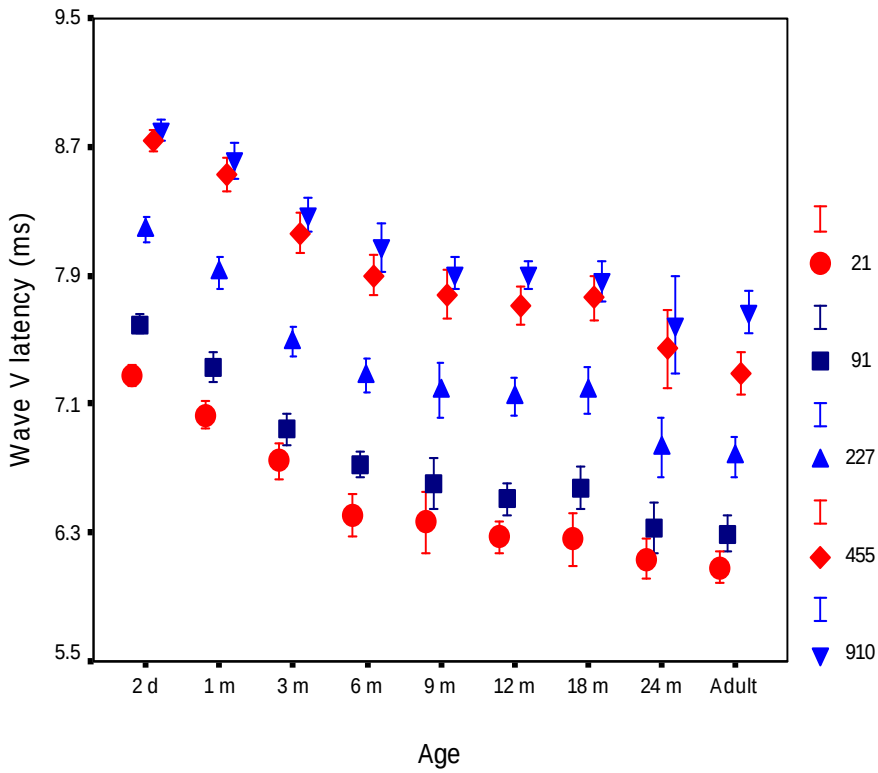

Figure 7. Postnatal development of wave V latency, presented in means and standard errors, at various repetition rates of clicks (≥40 dB above BAER threshold) in normal term infants. The latency is decreased with the increase in postnatal age, particularly during the first 6 months of age.

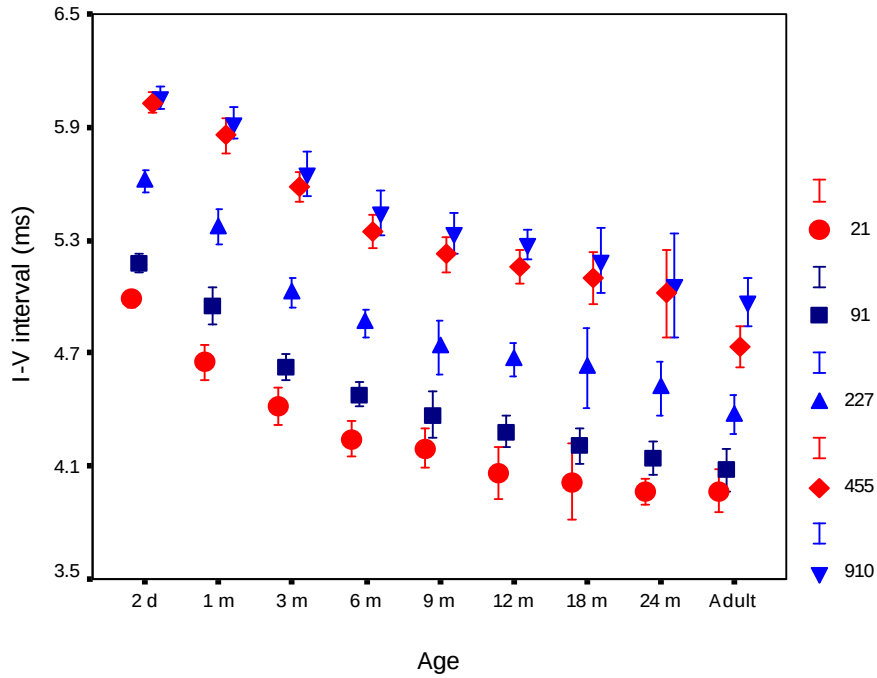

Figure 8. Postnatal development of wave I-V interpeak interval, presented in means and standard errors, at various repetition rates of clicks (≥40 dB above BAER threshold) in normal term infants. The interval is decreased with the increase in postnatal age, particularly during the first 12 months of age.

MLS BAER IN DETECTION OF BRAINSTEM AUDITORY PATHOLOGY IN INFANTS

Preterm Infants

Low-risk Preterm Infants

In general, the younger the gestational age, the longer are the wave latencies and interpeak intervals, and the lower are the wave amplitudes (Jiang et al., 2005a,2006a; Lasky, 1997; Lasky et al., 1997; Li et al., 2011; Weber and Roush, 1992,1995). Such age-related changes in MLS BAER variables in low-risk preterm infants, i.e. those who have no major perinatal problems or complications that may affect the brainstem auditory pathway, are true of all click rates, and are generally similar to those in conventional BAER.

At term equivalent age (37-42 weeks postconceptional age), MLS BAER variables in low-risk preterm infants are generally similar to those in age-matched normal term infants (Jiang et al., 2005a,2006a; Li et al., 2011; Weber and Roush, 1992,1995; Wilkinson et al., 2007; Yin et al., 1998). However, at high-rate click stimulation (455 and 910/sec), low-risk, mainly very, preterm infants often show some slight differences from those in age-matched normal term infants in MLS BAER later components that mainly reflect central auditory function. These include wave V latency, III-V and I-V intervals, and III-V/I-III interval ratio (Jiang et al., 2005a,2006a; Li et al., 2011; Wilkinson et al., 2007).

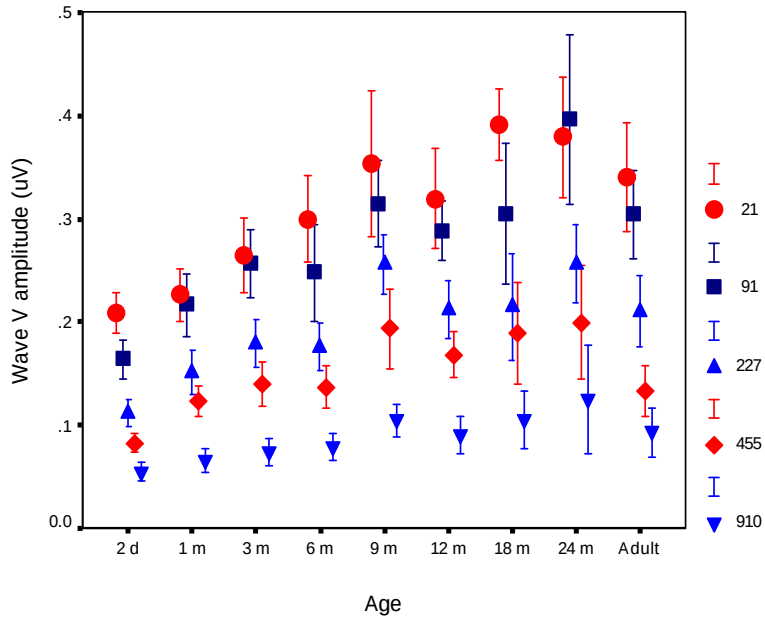

Figure 9. Postnatal development of wave V amplitude, presented in means and standard errors, at various repetition rates of clicks (≥40 dB above BAER threshold) in normal term infants. The amplitude is increased with the increase in postnatal age. At 24 months, the amplitude is larger than in adults at all click rates.

In late preterm infants (gestation 33-36 weeks), at term age the latencies and amplitudes of MLS BAER waves I, III and V are essentially similar to those in normal term controls at all click rates (Li et al., 2011). This is also the case for I-V and I-III intervals. Nevertheless, III-V interval, reflecting functional status of the more central regions of the auditory brainstem (Jiang, 2008; Jiang et al., 2009d), tends to be increased at higher click rates, although it is similar to that in normal term infants at the lower rates. All these wave latencies and amplitudes, and interpeak intervals are correlated significantly with click rates. There are no significant differences in the slopes of these MLS BAER variables-rate functions between the later preterm infants and term infants. Thus, low-risk late preterm infants only have a mild delay in neural conduction in the more central part of the auditory brainstem, suggested by the increase in III-V interval at high-rate stimulation, and late preterm birth does not have any significant effect on early development of the auditory brainstem.

In very preterm infants (gestation 32 weeks and less), MLS BAER wave latencies and interpeak intervals are longer, and wave amplitudes are smaller than those in late preterm infants (Jiang et al., 2005a,2006a). At term equivalent age, the latencies of waves I and III in very preterm infants are similar to those in age-matched normal term infants at all click rates (91-910/sec), although wave V latency is slightly increased at some click rates. I-V interval is increased slightly at some click rates. However, III-V interval and III-V/I-III interval ratio are often increased significantly, mainly at 455 and 910/sec. On the other hand, I-III interval tends to be decreased. The amplitudes of waves I, III and V amplitudes, and V/I and V/III amplitude ratios do not show any significant differences from those in age-matched term infants. Compared with late preterm infants, very preterm infants show similar differences in MLS BAER variables at term age, although the differences are less significant than those

when compared with normal term infants. Apparently, very preterm infants have a certain degree of abnormality in MLS BAER later components that mainly reflect central auditory function. These abnormalities indicate that development of the brainstem or the central auditory system in low-risk very preterm infants is slightly delayed, which can be shown by MLS BAER at high-rate stimulation. The slight shortening in I-III interval, reflecting functional status of the more peripheral portion of the brainstem auditory pathway (Jiang et al., 2009d), is probably related to the fact that the very preterm birth exposes the infants to a sound environment *ex utero* much earlier than term infants, resulting in an acceleration in maturation or myelination in the peripheral neural pathway of the auditory system.

Taken together, at term equivalent age, there are no major differences in the latencies of MLS BAER waves I, III and V in low-risk preterm infants from those in age-matched normal term infants at all click rates. This is typically seen in wave V latency (Figure 10). However, there are a mild degree of abnormalities in III-V and I-V intervals. At lower click repetition rates, both the low-risk late and very preterm infants demonstrate similar MLS BAER variables to those of age-matched normal term infants, except for a moderate increase in III-V interval. This is basically similar to what is found in conventional BAER (Jiang et al., 2002,2008; Jiang and Wilkinson, 2008). At high-rate stimulation (455 and 910/sec), however, I-V and III-V intervals, and III-V/I-III interval ratio in low-risk preterm infants show some abnormalities, suggesting a certain degree of functional abnormality in the central, specifically brainstem, auditory pathway. These findings suggest that high-rate stimulation offered by the MLS technique enhances the detection of a subtle degree of BAER abnormality that may escape from detection by presenting relatively low-rate stimulation. This is similar to the finding in infants with a transient low Apgar score (Jiang et al., 2007a).

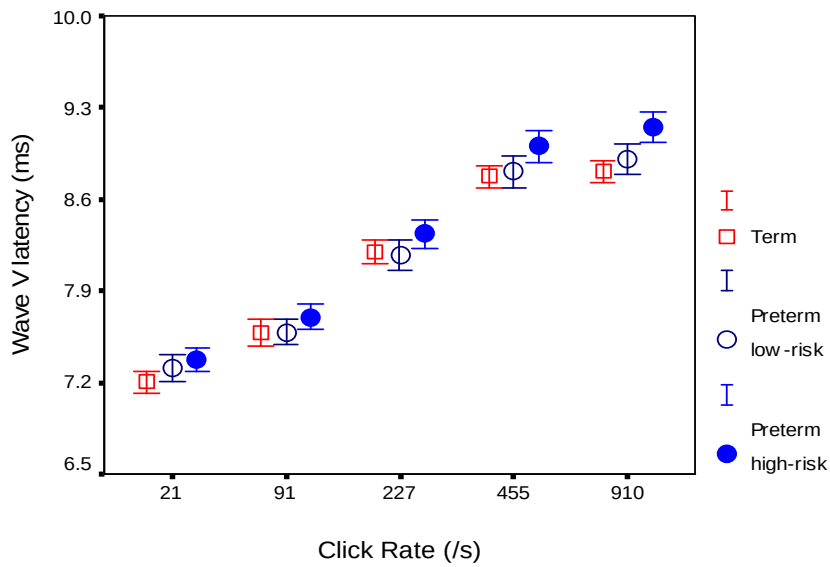

Figure 10. Means and standard errors of wave V latency at different click rates (≥40 dB above BAER threshold) in normal term infants, low-risk preterm infants, and high-risk preterm infants, recorded at term. There is no major difference in wave V latency between low-risk preterm infants and normal term infants at all click rates. However, the latency in high-risk preterm infants is significantly longer than in both normal term infants and low-risk preterm infants, particularly at 455 and 910/sec clicks.

High-risk Preterm Infants

Preterm infants are often associated with various perinatal problems or complications that may involve the brainstem auditory pathway, resulting in functional abnormality of the auditory brainstem. Prompt detection of the abnormality is of importance for management of such high-risk preterm infants. So far, however, there is a lack of published reports on detailed comparison between the high- and low-risk preterm infants during the preterm period. The author has observed that compared with age-matched low-risk preterm infants, high-risk infants often show some abnormalities in MLS BAER variables, suggesting brainstem auditory abnormality.

When they reach term (37-42 weeks postconceptional age), high-risk preterm infants still show some abnormalities in MLS BAER (Jiang et al., 2005a,2009b,c). Sample recordings of MLS BAER from a preterm high-risk infant, and, for comparison, a normal term infant and a low-risk preterm infant, recorded at term, are illustrated in Figure 2. Measurements of wave V latency at different click rates in a group of high-risk preterm infants, and, for comparison, a group of normal term infants and a group of low-risk preterm infants, recorded at term, are presented in Figure 10. Compared with normal term infants, wave V latency and I–V interval in high-risk preterm infants are often increased significantly at higher rates (455 and 910/sec), though not at lower rates (91 and 227/sec). III–V interval and III–V/I–III interval ratio are increased at almost all click rates. However, I–III interval does not show any significant difference from that in normal term infants. The amplitudes of MLS BAER waves I, III and V are reduced, particularly at 455 and 910/sec (Jiang et al., 2005a). The slopes of wave V latency-, I-V and III-V interval- and III-V/I-III interval ratio-rate functions are all increased significantly. These findings indicate that the auditory brainstem, mainly the more central regions, in high-risk preterm infants is impaired, which is more evident at high-rate click stimulation.

Compared with low-risk preterm infants at term age, high-risk preterm infants also show a significant increase in wave V latency, I–V and III–V intervals, and III–V/I–III interval ratio, mainly at 455 and 910/sec clicks. This is not only seen in very preterm infants, but, though less significant, also seen in later preterm infants. It appears that after cancelling the mild effect of preterm birth on MLS BAER, there are still abnormalities in MLS BAER in high-risk preterm infants. Obviously, some perinatal problems or complications that are associated with high-risk preterm infants damage the immature auditory brainstem, leading to, mainly, central auditory abnormality in high-risk preterm infants.

Perinatal Hypoxia-ischemia

Term Infants

Sample MLS BAER recordings in newborn infants after severe perinatal hypoxia-ischemia within the first a few days after birth are shown in Figure 11. During the first week after birth, term neonates after perinatal hypoxia-ischemia demonstrate some abnormalities in conventional BAER (click rate 21/sec), e.g. an increase in latencies and interpeak intervals. In MLS BAER, there are more abnormalities in these infants (Jiang et al., 2000,20032009a,2010). Wave V latency and III–V and I–V intervals are increased significantly at all click rates 91-910/sec. I–III interval is also increased at 455 and 910/sec.

Wave V amplitude is reduced significantly at all the click rates (Jiang et al., 2008). Both amplitude ratio V/I and amplitude ratio V/III are significantly decreased at the click rates 455 and 910/sec. These MLS BAER abnormalities indicate that term neonates after perinatal hypoxia-ischemia have major, acute impairment in the auditory brainstem, which is slightly more severe in the more central, or rostral, part of the brainstem than in the more peripheral, or caudal, part of the brainstem.

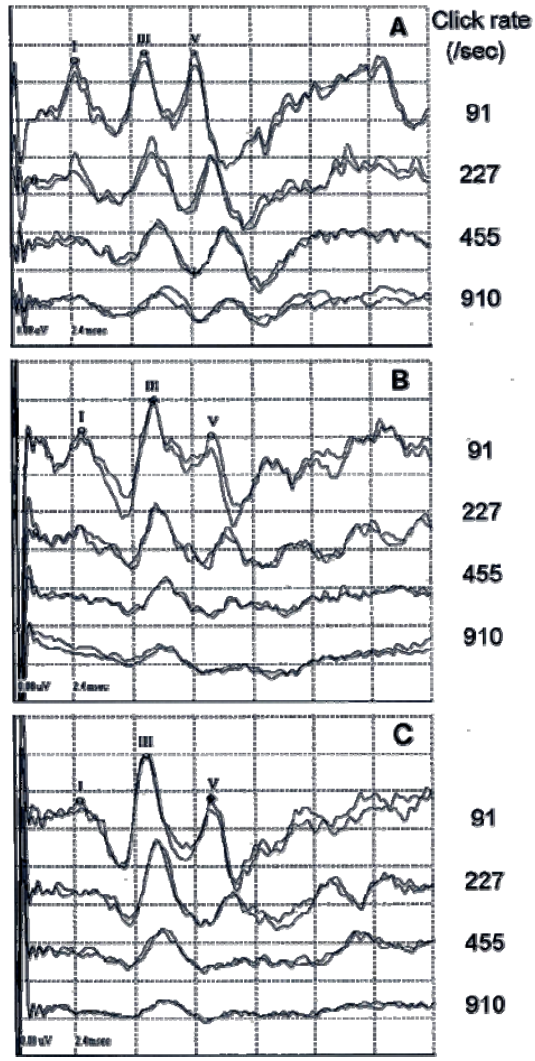

Figure 11. Sample MLS BAER recordings made from a normal term infant (A) and two term infants after severe perinatal hypoxia-ischemia (B and C) on day 3 after birth (click ≥ 40 dB above BAER threshold). Compared with normal infant A, wave V latency and III–V and I–V intervals in infants B and C after severe perinatal hypoxia-ischemia are increased significantly at all click rates, particularly at 455 and 910/sec. I-III interval is also increased at high click rates. The amplitudes of waves I, III and V in infant B after hypoxia-ischemia are reduced, particularly for wave V at higher rates. In infant C after hypoxia-ischemia, all wave amplitudes are normal at 91/sec clicks, but, with increasing click rate, the amplitudes of wave I and particularly wave V are reduced remarkably.

In most neonates after perinatal hypoxia-ischemia, the MLS BAER abnormalities are more significant at higher than at lower rates of click stimuli. The slopes of wave V latency-rate function, and I-V, I-III and III-V interval-rate functions are increased significantly. The slope of wave V amplitude-rate function is also increased. These increased slopes reflect a decreased ability of brainstem auditory neurones to recover in time to transmit the next stimulus-evoked response or a decreased efficacy of synaptic transmission in the central nervous system, particularly in the rostral brainstem or the more central part of the brain after perinatal hypoxia-ischemia.

There are characteristic dynamic changes in the MLS BAER during the first month after birth in term infants after hypoxia-ischemia, which reflects a characteristic time course of pathophysiological changes in the brainstem during the acute phase or period of perinatal hypoxic-ischemic brain damage (Jiang, 2008; Jiang et al., 2003). On the first day after birth, wave III and V latencies, and I-V and III-V interpeak intervals, which mainly reflect central neural function, are increased significantly at all click rates 91-910/sec, particularly higher rates. The abnormalities reach a peak on the third day after birth. Thereafter, the increased wave latencies and intervals recover progressively, although wave V latency and interpeak intervals remain abnormal on days 7 and 10. These MLS BAER variables approach normal values on day 15. By the end of the first month, the MLS BAER abnormalities almost completely recover, although III–V and I–V intervals are still increased slightly. These MLS BAER changes have a good correlation with the stage of hypoxic–ischemic encephalopathy, and have been further confirmed in our more recent studies in a larger population (Jiang, 2008; Figures 4 and 5).

The dynamic changes in MLS BAER variables demonstrate a general trend of electrophysiological changes in the impaired auditory brainstem during the neonatal period in infants after perinatal hypoxia-ischemia, although there are individual variations. Perinatal hypoxic-ischemic brainstem damage progresses after birth, reaches a peak on the third day, and tends to recover progressively thereafter. The first week, particularly the first three days, is a critical period of hypoxic-ischemic brainstem damage. Two weeks after birth, the damage recovers significantly and largely returns to normal. By one month, the damage almost completely recovers. These findings provide new insights into the pathophysiological process of hypoxic-ischemic brain damage in human neonates. The knowledge obtained may have important implications for the management of infants after hypoxia ischemia and for the study and implementation of timely neuroprotective and/or therapeutic interventions.

This characteristic time course could be used as a reference to monitor cerebral function, assess the responses of the brain to neuroprotective and/or therapeutic interventions, and help to judge the value of interventions. Early intervention during the first three days could prevent or reduce deterioration of the damage. The time course also provides a time frame in which recording of the MLS BAER is most valuable for general clinic application in infants after perinatal hypoxia-ischemia. The first recording can be made in the first day after birth for the early detection of hypoxic-ischemic brain damage. The second recording can be made at around day 3 for any progress or deterioration of the damage. The third recording can be made on day 7 to assess any significant recovery. The fourth recording can be made on day 15 or slightly later for any normalization.

In contrast to the above dynamic changes in wave latencies and interpeak intervals, the amplitudes in MLS BAER wave components in infants after perinatal hypoxia-ischemia demonstrate a quite different profile of dynamic changes during the first month after birth

(Jiang et al., 2008). This is typically shown by the changes in wave V amplitude, as illustrated by the measurement of wave V amplitude in Figure 6. On day 1 the amplitudes of waves I, III and V are all reduced significantly, especially at higher rates. On day 3 these amplitudes are reduced further. Thereafter, the reduction persists. From day 10 the reduced amplitudes are increased slightly. By the end of the first month, all amplitudes remain reduced. During the first month, the reduction of wave amplitudes is more significant for the later MLS BAER components than for the earlier ones, and occurs more significantly at higher than at lower click rates. The amplitude reduction in MLS BAER is more significant than in conventional BAER. The persistent reduction in MLS BAER wave amplitudes during the whole period of the first month suggests that there is sustained depression of brainstem auditory electrophysiology, reflecting severe neuronal damage of the auditory brainstem, after perinatal hypoxia-ischemia (Jiang et al., 2008).

The sustained depression of electrophysiological activity in the auditory brainstem, and the reported extended cascade of biochemical and histopathological events and delayed cell death after perinatal hypoxia-ischemia could create the potential for therapeutic intervention aimed at preventing further brain damage and rescuing the impaired neurons. It may be possible to intervene before delayed cell death or deterioration of neuronal impairment occurs, and so reduces the impairment. The progression and deterioration of the abnormalities in MLS BAER wave latencies and amplitudes and interpeak intervals during the first 3 days after birth indicate that early intervention during the first hours after birth might prevent or reduce the deterioration. The persistent reduction in wave amplitudes during the first month, which is comparable with some other observations that abnormal cerebral energy metabolism and cell death can persist for weeks or months, and suggests that delayed treatment might still be effective in rescuing the damaged brain cells. There is evidence that in newborn animals with experimental cerebral hypoxia–ischemia delayed treatment such as hypothermia can improve neuroprotection and long-term behavioural outcome. This has also been demonstrated in our preliminary BAER study of newborn piglets treated with hyperthermia after experimental hypoxia–ischemia (Jiang et al., 2006c).

The above MLS BAER findings show clearly that after perinatal hypoxia-ischemia the time profile of changes in wave amplitudes of the MLS BAER is quite different from those in wave latencies and intervals. The amplitude reduction is much more persistent than the increase in wave latencies and intervals. In particular, the recovery of the reduced wave amplitudes is much slower than that of the increased wave latencies and intervals. It appears that the reduction in wave amplitude and the increase in wave latencies and intervals after perinatal hypoxia-ischemia involve somewhat different mechanisms and reflect somewhat different pathophysiology following hypoxic–ischemic insult. Thus, MLS BAER wave amplitude and latency (and interpeak interval) might afford different windows into the underlying pathophysiology of perinatal hypoxia-ischemia. The increase in interpeak intervals, which is more related to impairment in nerve conduction, mainly reflects acute hypoxic–ischemic damage to the brain, whereas the reduction in wave amplitudes, which is more related to impairment in brainstem neuron, mainly reflects prolonged or sustained hypoxic–ischemic damage to the brain.

Preterm Infants

During the first a few days after birth, preterm infants after hypoxia–ischemia demonstrate significantly abnormalities in MLS BAER components that mainly reflect central

auditory function. Later, the abnormalities recover with the improvement of clinical conditions of the infants. This process is generally similar to those in term infants after perinatal hypoxia-ischemia, although there are slight differences (Jiang, 2008; Jiang et al., 2000,2003,2008).

At term equivalent age, the MLS BAER abnormalities found during the preterm period in preterm infants after hypoxia–ischemia have largely recovered, but there remain some abnormalities (Jiang et al., 2005a,2009b). Figure 12 presents the measurements of I-V interpeak interval at different click rates at term age in preterm infants after perinatal hypoxia-ischemia. Compared with healthy (i.e. low-risk) preterm infants, preterm infants after perinatal hypoxia-ischemia show a significant increase in I-V interval at 455 and 910/sec of clicks. III-V interval and III-V/I-III interval ratio are also increased significantly at the two very high rates. The slope of III-V interval-rate function is significantly steeper than in the healthy preterm infants. Compared with normal term controls, the preterm infants after hypoxia-ischemia demonstrate similar, but slightly more significant, abnormalities in MLS BAER. The differences between preterm infants after hypoxia-ischemia and healthy preterm and term infants are generally increased with increasing click rate. These findings demonstrate that MLS BAER central components are abnormal, which occurs predominately at very high click rates in preterm infants after hypoxia-ischemia, and click rate-dependent change in the more central regions of the auditory brainstem is also abnormal. These MLS BAER abnormalities indicate that the functional integrity of the brainstem, mainly in the more central regions, is impaired in preterm infants after perinatal hypoxia-ischemia.

After term there are also abnormalities in the amplitudes of MLS BAER wave components in preterm infants after hypoxia–ischemia (Jiang et al., 2009c). Figure 13 presents the measurements of wave V amplitude at different click rates at term age in preterm infants after perinatal hypoxia-ischemia. Compared with age-matched healthy preterm infants, the preterm infants after hypoxia–ischemia do not show any major abnormality in wave I and III amplitudes at any click rates. However, wave V amplitude is reduced significantly at 227-910/sec, though not significantly at the lowest rate 91/sec. V/I amplitude ratio is slightly reduced at 455 and 910/sec. Compared with normal term infants, waves III amplitude in the preterm infants after hypoxia–ischemia tend to be reduced at all click rates. Wave V amplitude is significantly reduced at very high click rates 455 and 910/sec. V/I amplitude ratio is slightly decreased. The reduction of wave amplitudes generally becomes more significant with the increase in click rate. The slope wave V amplitude-rate function is significantly steeper than in both healthy preterm and term infants. Apparently, at term the major abnormality in MLS BAER wave amplitude variables in preterm infants after hypoxia–ischemia is a significant reduction in wave V amplitude, suggesting that brainstem auditory electrophysiology is depressed.

Taken together, at term age there remain some abnormalities in MLS BAER wave latencies and amplitudes, and interpeak intervals in preterm infants after perinatal hypoxia-ischemia. This implies that hypoxic-ischemic damage to the preterm brainstem is unlikely to completely recover within a relatively short period, which is of clinical implication regarding postnatal management of preterm infants after perinatal hypoxia-ischemia. After term, the MLS BAER abnormalities largely recover, and return to normal in most infants during the postnatal period, with only very few infants who still have a certain degree of MLS BAER abnormalities, typically amplitude reduction of wave V.

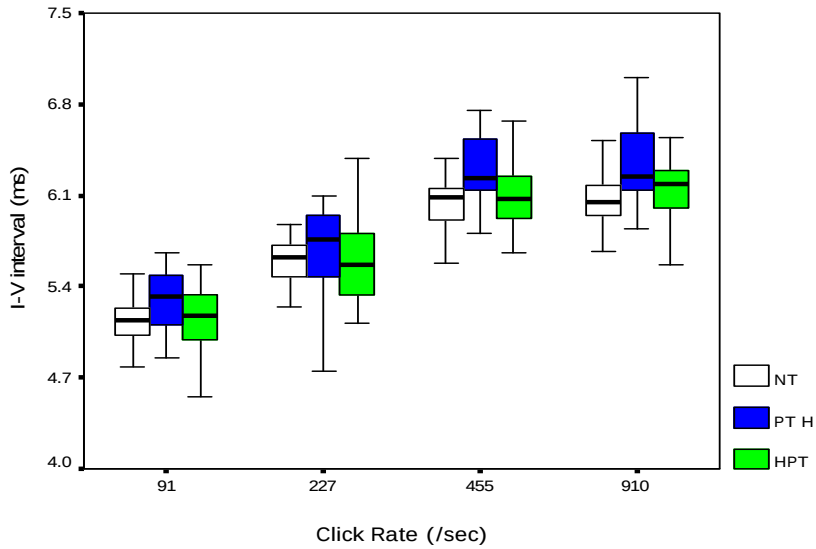

Figure 12. Boxplots of I-V interpeak interval (bold line across the box, median; box, 25th and 75th centile; extensions, the largest and smallest values) at different repetition rates of clicks (≥40 dB above BAER threshold) at term age in normal term infant, preterm infants after perinatal hypoxia-ischemia and healthy preterm. NT, normal term infants; PT HI, preterm infants after perinatal hypoxia-ischemia at term; PT, preterm infants without perinatal HI at term (the same for Figure 13). I-V interval in preterm infants after perinatal hypoxia-ischemia is increased significantly at 455 and 910/sec of clicks, compared with that in normal term infants and healthy preterm infants.

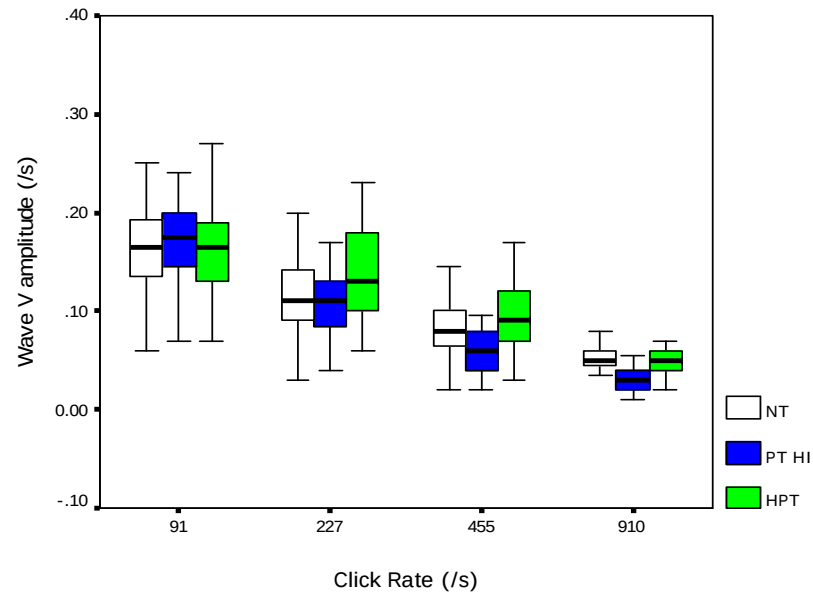

Figure 13. Boxplot of wave V amplitude (bold line across the box, median; box, 25th and 75th centile; extensions, the largest and smallest values) at different rates of clicks (≥40 dB above BAER threshold) at term age in normal term infant, preterm infants after HI and healthy preterm infants. NT, normal term infants; PT HI, preterm infants after perinatal HI at term; PT, preterm infants without perinatal HI at term. Wave V amplitude in preterm infants after perinatal hypoxia-ischemia is decreased significantly at 455 and 910/sec of clicks, compared with that in normal term infants and healthy preterm infants.

In addition, we have performed a MLS BAER study in newborn piglets with experimental hypoxia-ischemia. The dynamic changes in MLS BAER during the first two weeks after experimental hypoxia-ischemia are essentially similar to those observed in our human neonates, although there are differences. We have also used MLS BAER as a major tool to assess the effect of hyperthermia treatment on hypoxic-ischemic brain damage in newborn pigs. The preliminary results suggest that hyperthermia is a valuable neuroprotective measure for hypoxic-ischemic brain damage, which is essentially consistent with many reported studies in both animal models and human infant after hypoxia-ischemia.

Low Apgar Score

In neonatal audiology, a low Apgar score is regarded as one of the perinatal indicators associated with peripheral auditory impairment in infants. Newborn infants who have a depressed Apgar score are more likely to develop peripheral auditory impairment when they grow up. MLS BAER studies have revealed that infants with a prolonged low Apgar score and clinical signs of hypoxic-ischemic encephalopathy (HIE) often show major abnormalities that indicate brain damage and central auditory impairment (Jiang, 2008; Jiang et al., 2000,2003,2008,2009a-c,2010). However, it is less known if infants who have a transient or short term low Apgar score but no HIE have any central auditory abnormality or impairment.

Recent studies revealed that term newborn infants who have a transient low Apgar score but no clinical signs of HIE do not show any apparent abnormality in conventional BAER (Jiang et al., 2005b), but show mild abnormality in MLS BAER (Jiang et al., 2007a). On the first one or two days after birth, wave V latency and I-V interval in MLS BAER in these infants are increased at 455 and 910/sec clicks, though not at lower rates. On day 3, the abnormality tends to recover. Click rate-dependent change in I-V interval is slightly more significant than in the normal controls during the first 3 days. It appears that auditory function in the brainstem is sub-optimal during the first 3 days of life in infants who have a transient low Apgar score but no clinical signs of HIE. After the first 3 days of life, the abnormality returns to normal. Apparently, the recovery of the functional abnormality in the auditory brainstem in these infants is much sooner than that in infants with both a low Apgar score and clinical signs of HIE, i.e. perinatal hypoxia-ischemia (Jiang, 2008; Jiang et al., 2003). The short-term outcome is generally favourable in infants with a transient low Apgar score.

In some infants, a low Apgar score is likely to be caused by or related to short-term perinatal hypoxia which was not severe enough to result in clinical signs of encephalopathy. The mild, transient MLS BAER abnormality in infants with a low Apgar score alone suggest that transient less severe hypoxia may cause sub-optimal function in the auditory brainstem or sub-clinical auditory abnormality during the first three days of life in newborn infants. In other words, brainstem auditory function is sub-optimal during the first few days, though recovers soon after, in neonates with transient low Apgar scores. The finding that the abnormality is revealed only at very high-rate stimulation implies that MLS BAER elicited at very high rates of clicks can detect some subtle or early abnormality that cannot be demonstrated by conventional BAER, enhancing the detection of sub-optimal or sub-clinical auditory abnormality or brain damage.

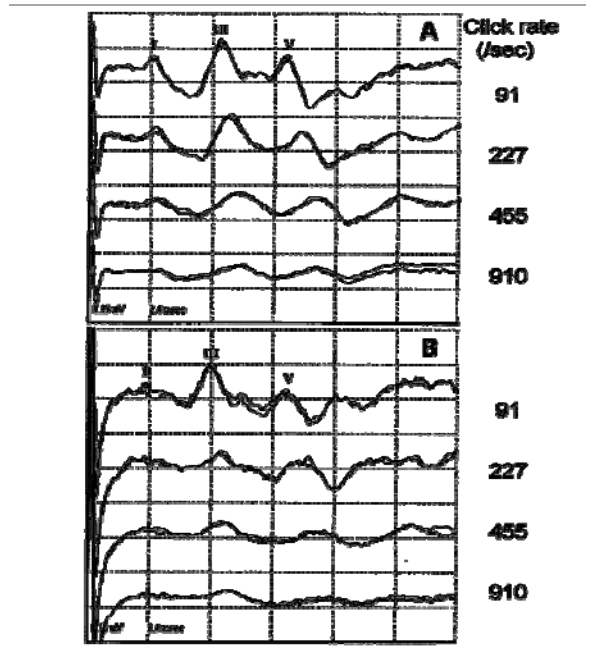

Figure 14. Sample recordings of MLS BAER from a normal term infant (A, gestation 39 weeks) and a BPD infant (B, gestation 26 weeks) at 40 weeks postconceptional age (clicks ≥ 40 dB above BAER threshold)e. Compared with the term infant, the BPD infant shows an increase in wave V latency, I-V and particularly III-V interpeak intervals. The increase in III-V interval is most significant at 455 and 910/sec.

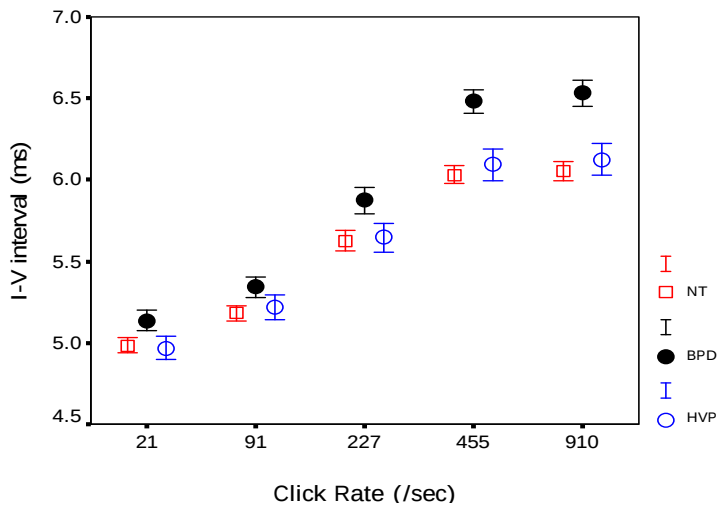

Figure 15. Means and standard errors of I-V interval, recorded with 21-910/sec clicks (≥40 dB above BAER threshold), at term in normal term infants (NT), healthy very preterm infants without BPD (HVP) and very preterm infants with BPD. The interval increases with increasing click rate, and correlates significantly and positively with click rate (all P <0.01), which is true of all the three groups of infants. Analysis of variance shows that the interval in BPD is significantly longer than in HVP and NT at all click rates (P <0.01-P <0.0001). The differences increase with increasing click rate. In regression analysis, the slope of I-V interval-rate function is significantly steeper in the infants with BPD than in the NT and HVP groups (P <0.01 and 0.01).

Neonatal Chronic Lung Disease

Neonatal chronic lung disease (CLD), particularly bronchopulmonary dysplasia (BPD) — a severe type of CLD, is a major lung disease in very preterm or very low birthweight infants (Jeng et al., 2008). It is one of the greatest risk factors of neurologic impairment and developmental deficits in infants. The underlying pathophysiological processes remain poorly understood. In the literature, only very few reported studies are available regarding conventional BAER in neonates with CLD, mainly for detecting peripheral auditory impairment (Jiang et al., 2006b,2007b). More recently, detailed studies of MLS BAER have been carried out in infants with CLD or BPD (Jiang et al., 2009a,2010; Wilkinson et al., 2007). Compared with MLS BAER in age-matched normal or healthy infants, there are major abnormalities in MLS BAER in infants with CLD or BPD. The abnormalities are shown clearly from 36 weeks postconceptional age at which time the diagnosis of CLD or BPD is made. Figure 14 shows sample recordings of MLS BAER from a BPD infant (B, gestation 26 weeks), and, for comparison, a normal term infant (A, gestation 39 weeks) at 40 weeks postconceptional age. The infant shows an increase in wave V latency, I-V and particularly III-V interpeak intervals.

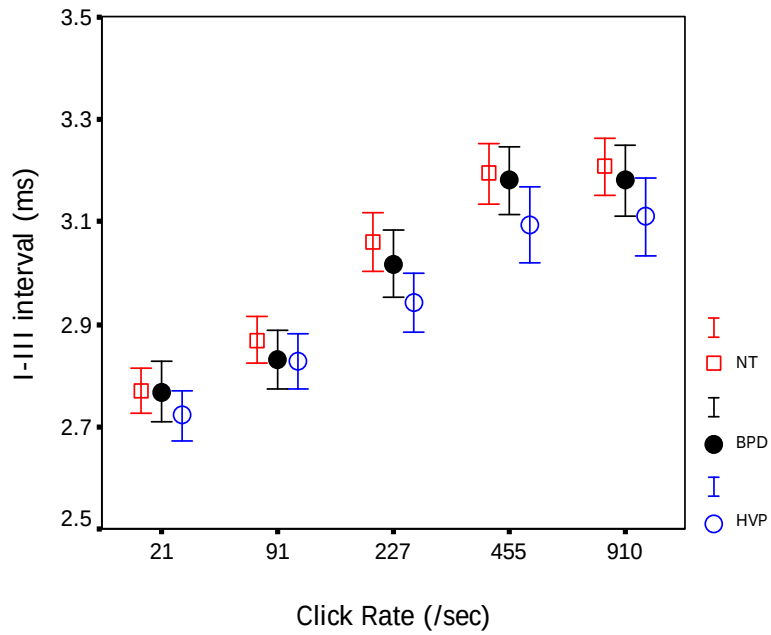

Figure 16. Means and standard errors of I-III interval , recorded with 21-910/sec clicks (≥40 dB above BAER threshold), at term in normal term infants (NT), healthy very preterm infants without BPD (HVP) and very preterm infants with BPD. The interval increases with increasing click rate, and correlates significantly and positively with click rate (all $P < 0.01$), which is true of all the three groups of infants. Analysis of variance shows that the interval in BPD is similar to that in NT at all click rates and HVP at lower rates (21 and 91/sec), but longer than HVP at higher rates (227-910/sec, all $P < 0.05$). In regression analysis, the slope of I-V interval-rate function in BPD is similar to that in NT and HVP, without any significant differences.

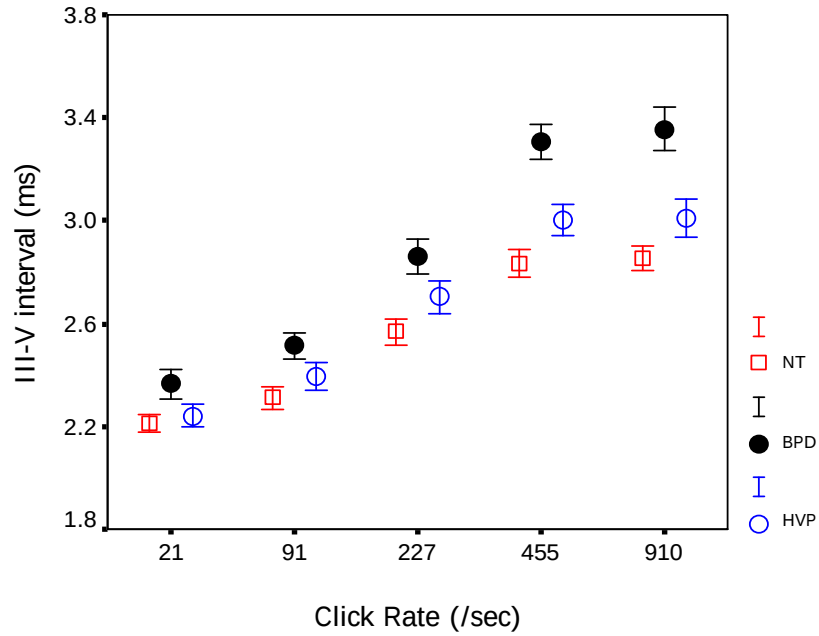

Figure 17. Means and standard errors of III-V interval, recorded with 21-910/sec clicks (≥40 dB above BAER threshold), at term in normal term infants (NT), healthy very preterm infants without BPD (HVP) and very preterm infants with BPD. The interval increases with increasing click rate, and correlates significantly and positively with click rate (all P <0.01), which is true of all the three groups of infants. Analysis of variance shows that the interval in BPD is significantly longer than in HVP and NT at all click rates (P <0.01-P <0.0001). The differences increase with increasing click rate. In regression analysis, the slope of III-V interval-rate function is significantly steeper in the infants with BPD than in the HVP and, in particular, NT infants (P <0.01 and 0.001).

Figures 15-18 present the measurements of MLS BAER I-V, I-III, III-V intervals and III-V/I-III interval ratio, respectively, at various click repetition rates at term ages in infants with BPD. Compared with normal term controls, infants with neonatal CLD or BPD do not show any major abnormalities in wave I and III latencies and I–III interval at all click rates. However, wave V latency in these infants is increased significantly at all 91-910/sec clicks. Similarly, I–V and particularly III–V intervals, and III–V/I–III interval ratio are increased significantly at all click rates. The increase in III-V interval is most significant at 455 and 910/sec. Compared with age-matched healthy very preterm controls, MLS BAER variables that mainly reflect central auditory function in infants with CLD or BPD are also increased significantly at all click rates, although the differences between the two groups of infants are slightly smaller than those between infants with CLD or BPD and normal term infants. Regression analysis revealed that the slopes of the wave V latency-rate function, I–V and III–V interval-rate functions, and III–V/I–III interval ratio-rate function in infants with CLD or BPD tend to be steeper than those in term and healthy very preterm infants. Nevertheless, there are no major abnormalities in the amplitudes of MLS BAER wave components.

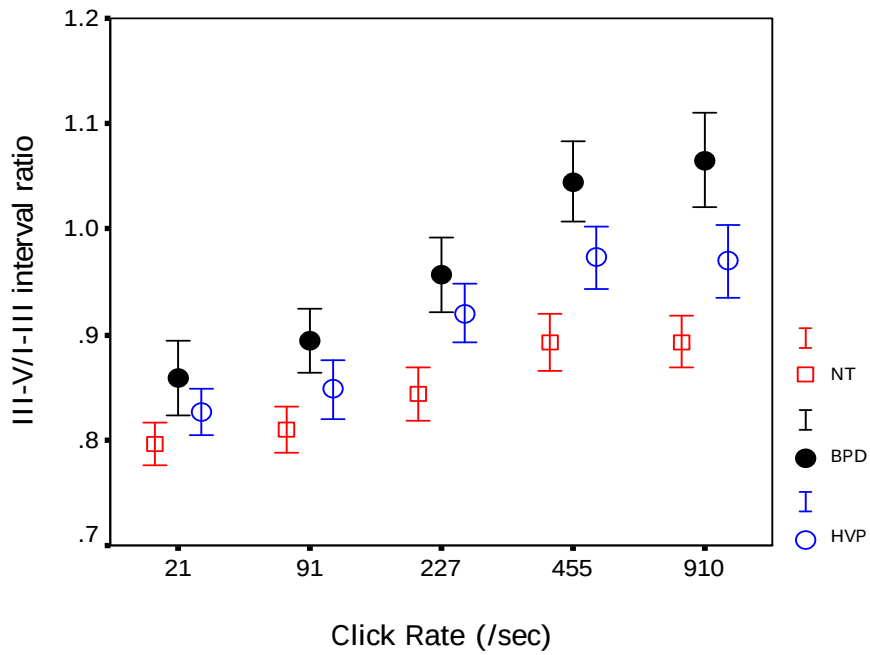

Figure 18. Means and standard errors of III-V/I-III interval ratio, recorded with 21-910/sec clicks (≥40 dB above BAER threshold), at term in normal term infants (NT), healthy very preterm infants without BPD (HVP) and very preterm infants with BPD. The interval increases with increasing click rate, and correlates significantly and positively with click rate (all P <0.01), which is true of all the three groups of infants. Analysis of variance shows that the interval in BPD is significantly longer than in HVP (P <0.05-P <0.0001) and NT at all click rates (P <0.01-P <0.0001). The differences increase with increasing click rate. In regression analysis, the slope of the interval ratio-rate function is significantly steeper in the infants with BPD than in the HVP and, in particular, NT infants (P <0.01 and 0.001).

These MLS BAER abnormalities in CLD or BPD indicate that the fundamental abnormality is the significant increase in III-V interval that reflect functional status of the more central regions of the auditory brainstem (Jiang et al., 2009d). Therefore, the MLS BAER abnormalities suggest that CLD or BPD exerts a major damage to myelination and synaptic function of the more central regions of the immature brainstem (Jiang et al., 2010; Wilkison et al., 2007). On the other hand, peripheral neural function was relatively intact. Neonatal CLD or BPD does not appear to exert any major damage to neuronal function, reflected by the generally normal amplitudes of MLS BAER wave components (Jiang et al., 2009a). Such characteristic changes are, at least partly, related to the nature of hypoxia in neonatal CLD or BPD. The hypoxia is chronic or prolonged, and sublethal. This is in contrast to that in perinatal hypoxia-ischemia, where the hypoxia is often acute, severe or lethal, and associated with ischemia of the brain. These findings provide new insights into the pathophysiology underlying neurological impairment and developmental deficits in neonatal CLD or BPD, and into pathophysiological differences between neonatal CLD or BPD and perinatal hypoxia-ischemia. The knowledge obtained from MLS BAER studies also provides valuable information for studying and implementing neuroprotective interventions or therapies for infants with CLD or BPD. The interventions or therapies should target more central regions of the brain.

In the BAER, the I-V interpeak interval is the most important and widely used variable to reflect functional status of the auditory brainstem (Wilkinson and Jiang, 2006). The MLS BAER findings in infants with neonatal CLD or BPD indicate that analysis of I–III and III–V intervals and III–V/I–III interval ratio offers more detailed information about brainstem function than analysis of I–V interval only (Jiang et al., 2009a,2010; Wilkinson et al., 2007). The I–V interval, the sum of I–III and III–V intervals, do not show any difference between infants with CLD and those after perinatal hypoxia-ischemia at any click rates in both conventional BAER and MLS BAER. It seems that brainstem function might be impaired similarly in neonatal CLD and perinatal hypoxia-ischemia, and that the two clinical situations might exert a similar effect on the functional integrity of the brainstem. However, I–III and III–V intervals, the two sub-components of I–V interval, demonstrate major differences between infants with CLD and infants after hypoxia-ischemia at all click rates (Jiang et al., 2010). The differences are shown particularly obviously by the analysis of III–V/I–III interval ratio. Apparently, a same or similar change in I–V interval in different clinical problems does not exclude a possible difference in its sub-components I–III and III–V intervals. The two small intervals and their ratio III–V/I–III are valuable BAER variables in differentiating neural function in the more peripheral regions from the more central regions of the brainstem, which cannot be done by analysing the I–V interval only. Therefore, although the I–V interval is the most commonly used BAER variable reflecting brainstem function, in some situations I–III and III–V intervals and III–V/I–III interval ratio can uncover and/or differentiate some abnormalities that cannot be shown by analysing I–V interval only, and thus enhance the detection of brainstem auditory pathology.

These MLS BAER abnormalities last for several weeks in most infants, with a few for months, and then recover. During the postnatal period, III-V interval is often slightly increased, compared with age-matched normal or healthy infants.

Other Perinatal Problems

The MLS BAER in infants with other perinatal problems or complications is currently under investigation, and there is a lack of reported studies. The following observations are obtained from the author's laboratory, and to be completed for report.

Hyperbilirubinemia

Hyperbilirubinemia is a most common condition requiring clinical evaluation and treatment in newborn infants. The brainstem auditory pathway is sensitive to bilirubin neurotoxicity. For years, the BAER has been used as an important tool to study and assess bilirubin neurotoxicity to the brain and the auditory system. A considerable body of previous reports has described changes in conventional BAER in infants with hyperbilirubinemia. Although some investigators did not find any abnormalities, most others had abnormal findings (Ahlfors and Parker, 2008; Jiang et al., 2007c,2009e; Okumura et al., 2009; Smith et al., 2004). More recently, we have studied MLS BAER in infants with hyperbilirubinemia for a better understanding of bilirubin neurotoxicity to the immature brain, which is to be published.

In conventional BAER, infants who have no apparent peripheral hearing problems after hyperbilirubinemia demonstrates a basically normal wave I latency and a slight increase in

the latencies of waves III and V. In MLS BAER however, although wave I latency in infants with hyperbilirubinemia is also basically normal, wave III latency tends to be increased, and wave V latency is significantly increased at all click rates, particularly at the very high rates 455 and 910/sec. In conventional BAER, I-V and I-III intervals in infants with hyperbilirubinemia are both slightly increased, while III-V interval does not show any apparent abnormality. In MLS BAER, however, I-V interval in these infants is significantly increased at all click rates 91-910/sec. The two smaller intervals (i.e. I-III and III-V) are both increased, and, in turn, III-V/I-III interval ratio does not show any significant changes at any click rates. Apparently, the functional impairment in the auditory brainstem due to hyperbilirubinemia is demonstrated more prominently in MLS BAER than in conventional BAER. This is particularly for the more central regions of the brainstem, as reflected by the increase in III-V interval in MLS BAER but a basically normal III-V interval in conventional BAER.

The amplitudes of MLS BAER waves III and V are reduced significantly in infants with hyperbilirubinemia, although wave I amplitude is only slightly reduced. V/I amplitude ratio is decreased. These abnormalities are true of all click rates, though tend to be more significant at higher than at lower click rates. This major reduction in wave amplitudes indicates that brainstem auditory electrophysiology is suppressed by hyperbilirubinemia, and there is neuronal damage due to bilirubin neurotoxicity.

Shortly (several hours or days) after prompt treatment with phototherapy and/or exchange transfusion, the increased wave latencies and interpeak intervals and reduced wave amplitudes in MLS BAER components in infants with hyperbilirubinemia recover quickly, and return to normal or near normal in most infants. There are only few infants whose MLS BAER abnormalities persist for several days or weeks. This is similar to what is found in visual evoked potential studies (Chen et al., 2006). It appears that bilirubin neurotoxicity to the evoked potentials is largely transient. Following prompt treatment, the impaired auditory function due to bilirubin neurotoxicity recovers quickly. In other words, the toxic effect of hyperbilirubinemia on the brainstem and auditory system is largely transient, provided that prompt treatment is initiated.

Intrauterine Growth Retardation

As a failure of the fetus to achieve intrinsic growth potential, intrauterine growth retardation (IUGR) is an important prenatal risk factor for perinatal morbidity and neurodevelopmental deficits (de Bie et al., 2010; Walker and Marlow, 2008). It is the single most important condition affecting the viable fetus (Gardosi, 2011). IUGR is a common condition, and affects about 10% to 15% of the general maternity population. It may occur up to one-third of some maternity population (Carreno et al., 2011). IUGR often accompanies intrauterine undernutrition and chronic hypoxia due to placental insufficiency, adversely affecting neural function and development of the brain (Vedmedovska et al., 2011). Despite its importance and relatively high prevalence, early detection of neurodevelopmental deficits, which is important for any early interventions, in infants with IUGR is relatively poor. In the studies of conventional BAER, some previous investigators reported a shortened I-V interpeak interval, suggesting precocious neural maturation following IUGR (e.g. Henderson-Smart et al., 1991). On the other hand, some others reported an increased I-V interval, suggesting delayed neural maturation, in IUGR infants (Sarda et al., 1992). The remaining did not find any appreciable abnormalities.

Our recent MLS BAER studies (to be published) have revealed that most IUGR infants who are born at term and late preterm have slight differences from age-matched normal infants. However, IUGR infants who are born at very preterm often show MLS BAER abnormalities. Compared with normal infants, very preterm IUGR infants have an increase in wave V latency, and I-V and, particularly III-V, intervals at most click rates between 91 and 910/sec. In contrast, wave III latency is decreased at high click rates, and I-III interval is decreased at almost all rates, which is more significant at higher than at lower rates. The significant increase in III-V interval and the significant decrease in I-III interval result in a significant increase in III-V/I-III interval ratio. The slopes of wave III latency- and I-III interval-rate function in IUGR infants are decreased, while the slope of III-V/I-III interval ratio is increased. Compared with very preterm infants without IUGR, very preterm infants with IUGR show similar, though less significant, abnormalities in MLS BAER. There are no major changes in wave amplitudes. These findings suggest that in very preterm IUGR infants there is a delay in central maturation of the neonatal brainstem but an acceleration in peripheral maturation in the auditory brainstem. These interest findings shed lights on neurodevelopmental deficits in very preterm IUGR infants.

Neonatal necrotizing enterocolitis

Necrotizing enterocolitis (NEC) is a gastrointestinal disease that involves infection and inflammation that causes destruction of the bowel. It mostly affects preterm infants. Although it affects only 1 in 2,000 to 4,000 births, or between 1 and 5% of neonatal intensive care unit admissions, NEC is the most common and serious gastrointestinal disorder among hospitalized preterm infants, and a major cause of morbidity and death in neonates (Henry and Moss, 2008). Whether NEC affects infants' brain, neural function and neurodevelopment remains far from clear. Some follow-up studies showed that infants who survive NEC have delayed psychomotor development, although no significant abnormality in mental development (Yeh et al., 2004). It seems that infants who survive NEC are at risk of neurodevelopmental impairment.

We have recently studied MLS BAER in preterm infants with NEC. Compared with age-matched low-risk preterm infants, infants with NEC showed a significant increase in wave V latency, I-V and III-V interpeak intervals and III-V/I-III interval ratio. These abnormalities occurred at almost all click rates, but most significantly at very high click rates 455 and 910/s. On the other hand, wave I and III latency and I-III interval did not show any abnormalities. At term age, the NEC infants demonstrated a similar, but less significant, change in MLS BAER. Apparently, MLS BAER variables that mainly reflect central auditory function are affected by neonatal NEC. These preliminary results suggest that more central part of the auditory pathway is functionally impaired in NEC, and the brainstem auditory impairment partially, not completely, recovers with the improvement of clinical conditions at term age. These unique findings, which have never been reported before, are of great significance, though further studies in larger patient population are warranted.

Others

We have also carried out MLS BAER studies in infants with perinatal problems or complications other than those described above. The preliminary results have shown that MLS BAER abnormalities may also occur in some other perinatal problems or complications. Nevertheless, more detailed studies are needed before any conclusion can be made.

CONCLUSIONS

Over the last decade, the MLS technique has been introduced to pediatric, mainly neonatal, neurology to study the BAER in an effort to assess the functional integrity of the developing auditory brainstem and, more importantly, to examine clinical applicability of this technique in detecting neuropathology that involves the auditory brainstem in infants with various clinical problems. The results so far show that the MLS BAER is a valid tool to detect auditory abnormality and brain damage or neuropathology, and the MLS technique enhances the diagnostic value of the BAER for some neuropathology. In particular, MLS BAER can detect some early or subtle neuropathology that cannot be shown by conventional examination and investigations. Apparently, MLS BAER is a promising tool for clinical application in pediatric neurology. In addition to the clinical problems described in this review, there is a lack of reported studies of the use of MLS BAER in other pediatric problems where the diagnostic value of MLS BAER remains to be assessed. Thus, there is a need to study MLS BAER in large populations and wider ranges of pediatric problems to have a full evaluation of its clinical value.

For detection of neuropathology that involves the brainstem auditory pathway, MLS BAER abnormalities are better detected with high-rate stimulation, typically 455 and 910/sec, which cannot be achieved in conventional BAER. The 455/sec appears to be the best repetition rate of the four rates (91, 227, 455 and 910/sec) we used. The rates 91 and 227/sec can elicit well-formed MLS BAER waveform, and could, to a certain degree, enhance the detection of neuropathology by the BAER. However, the two relatively lower rates may be not “stressful” enough for the brainstem auditory neuron as not to significantly enhance the detection. The highest rate 910/sec is very “stressful”, but some alteration may occur in the elicited waveform, which may result in a difficulty in accurate and reliable identification and measurement of waves I and V. At the rate 455/sec, the MLS BAER waveform elicited is well-formed, without any appreciable alteration. There is usually no difficulty in accurate and reliable identification and measurement of all BAER waves. This high rate appears to be “stressful” enough for the brainstem auditory neuron as to significantly enhance the detection of neuropathology by the BAER in infants.

Apparently, compared with conventional BAER, MLS BAER is a better method to detect brain damage and central auditory impairment in some clinical problems, and the MLS technique improves the diagnostic value of BAER. However, MLS BAER is not necessarily used for all neuropathology. For the simplicity of clinical application, conventional BAER is useful for detection of most neuropathology, particularly for severe one which is relatively easier to be detected. For some neuropathology, particularly early or subtle one, that may not be detected with conventional BAER, MLS BAER is recommended to enhance the detection.

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Chapter 2

CHILDHOOD EPILEPSY AND COGNITION

Sherifa A. Hamed*
Department of Neurology and Psychiatry, Assiut University Hospital, Assiut, Egypt

ABSTRACT

Infancy, preschool and school age periods are characterized by peak hippocampal and cortical regional development, as well as maximal white matter growth or myelinogenesis, dendritogenesis, and synaptogenesis. Occurrence of epilepsy during these periods might result in impairment in spatial learning, memory processes and other aspects of cognition.Several variables are associated with cognitive impairment in epilepsy which includes: maternal-, seizure- and medication-related variables. High doses of antiepileptic drugs [AEDs] and polypharmacy are significant risks for cognitive impairment. In utero exposure to AEDs may cause defects in neuronal proliferation and migration and increase apoptosis. Cognitive impairment during childhood period even if trivial may adversely affect the child's psychosocial functioning by interference with educational skills and learning tasks.

Keywords: Childhood epilepsy; antiepileptic drugs; cognition; long-term potentiation.

A. INTRODUCTION

It is estimated that 0.5-1% of all children have epilepsy [1].The prevalence of epilepsy in the pediatric population is 4-6 per 1,000 children.When categorized by age, the overall average annual incidence of epilepsy is73-150 per 100,000 in children <1 year of age,with this value decreasing by 50% in children 1-4 years, 72-86 per 100,000 in children <9 years and 40 per 100,000 in patients 10-14 years of age [2].Epilepsy has numerous causes and clinical manifestations. Importantly, seizures are only one aspect of epilepsy, as cognitive and

* Corresponding author: Sherifa Ahmad Hamed, MBBch., MSc., M.D. Consultant Neurologist; Associate Professor, Department of Neurology and Psychiatry, Assiut University Hospital, Assiut, Egypt, P.O.Box 71516. Telephone: +2 088 2371820; Fax: +2 088 2333327,+2 088 2332278; Email: hamed_sherifa@yahoo.com.

psychiatric comorbidities induced by seizures or antiepileptic drugs (AEDs) may also have a deleterious impact on child's life [3-4].

Normally, biological development and organization of the brain in the human is very rapid in utero and starts to slow down in the second year of postnatal life [5]. Thus, seizures are most prevalent during the neonatal and infantile periods, a time when the brain is developing [6]. The period of infancy is characterized by peak hippocampal and cortical regional development, as well as myelinogenesis, dendritogenesis, and synaptogenesis in the brain [7]. Nearly gross organization is complete by 2 or 3 years of age, but maturation may continue through adolescence and beyond [8]. Therefore, cognitive impairment even if trivial may adversely affect the child's psychosocial functioning by interfering with educational skills and learning tasks.

Cognitive deficits may occur in the presence or absence of clinically manifest seizures, convulsive or non-convulsive status epilepticus (SE) that occur during awakening or during sleep, and may occur due to focal or generalized electroencephalographic (EEG) epileptic discharges without epileptic symptomatology [9]. Cognitive deficits associated with epilepsy and EEG epileptic discharges may be transient [10-11], persistent [12] or progressive [13-14].

Several variables are associated with cognitive impairment in epilepsy which include: maternal-related, seizure-related and medication-related variables.The neonates of mothers with epilepsy have an increased risk for low IQ, congenital malformations, hemorrhagic disease, and hypocalcemia. Lower IQ was observed in children whose mothers had more than four convulsions during the pregnancy independent of the effect of in utero AEDs exposure [15]. Maternal grand mal seizures associated with apnea can cause transient hypoxia and acidosis in the fetus [16]. The occurrence of early [before the age of 5 years], frequent, severe and prolonged seizures are also important risk factors for intellectual deterioration with epilepsy [17-19].Status epilepticus (SE), severe epileptic syndromes, symptomatic and cryptogenic epilepsies, tend to show early, marked and progressive intellectual decline [20-23]. Epilepsy syndromes associated with cognitive or behavioral impairment include a) tuberous sclerosis [24], b) Lennox–Gastaut syndrome, and c) Landau–Kleffner syndrome [25,26]. Cognition and learning processes are also affected by the intake of AEDs [27]. Decreased vigilance, memory, attention and reduced psychomotor speed are common adverse effects of AEDs that may lead to significant problems at school. These problems can sometimes arise even in the therapeutic ranges of the drugs [28]. At times, after a child has been started on an AED, the parents will call within a few days to report that their child is different, such as slow to respond, less talkative, or difficult to control, while parents of children whose AEDs are temporarily discontinued for the purpose of pre-surgical monitoring are at times amazed at how much more alert and more responsive their child has suddenly become [29]. The repetitive nature of such observations leaves no doubt in a clinician's mind that AEDs can affect cognition and behavior.

Generally, most of the AEDs have the potential for affecting the cognitive domains. AEDs produce global changes in the excitation levels in the central nervous system (CNS) and often lead to cognitive deficits. Polypharmacy and high blood levels of AEDs increase the risk of cognitive side effects. Cognitive side effects of AEDs therapy are well established for medications that were introduced before the 1990s, with the greatest cognitive impairment risk is seen to be associated with phenobarbitone (PB) and traditional benzodiazepines (BZ) [30-32]. For other AEDs, the cognitive profiles are generally comparable, with modest motor and psychomotor slowing and no consistent cognitive difference has been identified with

carbamazepine (CBZ), phenytoin (PHT), or valproate (VPA) [33, 34]. Many AEDs introduced during the 1990s (e.g., lamotrigine (LTG), gabapentin (GBP), levitracetam (LEV) and tiagabin (TGB) are thought to have fewer side effects [35-38]. However, the cognitive side effects of topiramate (TPM) are found to be slightly greater if initiated quickly and given at higher doses [39, 40]. Many authors found that discontinuation of the AED in children with epilepsy was accompanied by improvement in cognitive function [29,41,42].

The present article serves as an overview of recent studies in childhood epilepsy present in pubmed which highlighted cognitive evaluation [publications till 2010 were checked]. We also checked the reference lists of retrieved studies for additional reports, in addition to our experience in this field. In this review, we provided a brief text regarding neuronal plasticity and memory formation to facilitate the readers understanding the types and causes of cognitive deficits with epilepsy. We also discussed the mechanisms underlying cognitive impairment with antiepileptic medications. The information included in this review has important clinical and research implications and recommendations.

B. Neuronal Plasticity and Memory Formation

The term cognition refers to the highest levels of various mental processes and systems involved in receiving information, comprehending it, storing and retrieving it and using it [43]. Most neuropsychological assessment models require the independent evaluation of six specific areas of cognition: attention, perceptual functions, verbal and language functions, spatial/constructural processing abilities, memory and learning and executive functioning. Attention: it is the process that enables an individual to focus on the relevant information in a stimulus array while also inhibiting further processing of non-relevant information. It is the gateway to the rest of cognition. The frontal lobe is mainly responsible for control of attention [44-46]. Perceptual functions include activities such as awareness, recognition, discrimination, patterning and orientation. Perception is a central step in the processing of sensory attentional information [as olfaction, vision, gustation, tactile and auditory]. This information is later transformed into higher-order codes for use by the various higher-order cognitive subsystems. The sensations reach the brain's emotional center, the amygdala. In the amygdala, the brain decides the faith of the sensory information in a matter of seconds and the autobiographic and emotional meaning of a sensation is decided [47]. Memory is the set of processes that temporarily holds new information while it is being utilized or processed for other purposes (short-term memory), or that more permanently holds learned information for future reference and use (long-term memory) [48]. There are three main aspects of memory and learning: a) encoding:which means putting the memory into one’s head or knowledge base; b) storage: which means holding the memory into ones head over time; and c) retrieval: which is the act of remembering something that was previously learned or experienced [48]. The hippocampus and associated temporal lobe structures (mammillary bodies and dorsomedial nucleus of thalamus) are important in memory [49-52]. Executive functions include conceptual reasoning, problem solving, planning, flexibility in cognitive strategies and implementing cognitive plans [53].

During infancy and early childhood, the more basic elements of attention and perception undergo the most rapid development, while in later childhood and adolescence, development of higher-order linguistic, spatial and executive elements are primary [48].

The hippocampus is important not only for storing cognitive maps, but for encoding memories. The hippocampus is also involved in memory consolidation, the slow process by which memories are converted from short- to long-term memory [54, 55]. The process of consolidation may take up to a couple years. According to traditional models, information has been encoded into long-term memory if it can be accurately retrieved after an interval during which active rehearsal is prevented. Thereafter, a process of 'consolidation' renders the memory trace progressively less vulnerable to disruption [56]. This process, thought to involve a gradual reorganization of the memory trace at a neural systems level [57], memory trace continue for weeks, months or even years but it is often assumed that its efficacy can be assessed at relatively brief delays.

The amygdala works to encode recent emotional information into memory. The amygdala is also involved in memory consolidation [58]. The right half of the brain cortex stores the emotional recalls, autobiographic and time-related memories while the left half of the brain cortex stores the cognitive and factual memories [59-62].

At the physiological level, long-term synaptic plasticity [e.g. long-term potentiation [LTP] and long-term depression [LTD] is thought to be an important cellular mechanism for learning and memory [63]. LTP is defined as a persistent increase in the efficacy of synaptic connections induced by high frequency stimulation, while in LTD, a transient decrease in synaptic strength is caused by repetitive stimulation of the presynaptic neuron [64]. The induction of LTP and LTD requires the appropriate integration of GABAergic inhibitory and glutaminergic excitatory transmission [65]. Human neuropsychological studies indicates that hippocampal N-Methyl-D-Aspartate (NMDA) receptors are necessary for mediating repetition/recognition effects of limbic event-related potentials to continuous word recognition paradigms as well as for intact verbal memory performance [66]. In LTP, a train of postsynaptic potentials (PSP) continues to come in (due to the high frequency stimulation), the NMDA receptor's channel is kept open causing Na^{+}, K^{+} and Ca^{++} flow through this channel, and Mg^{++} blockade is relieved. Ca^{++} inside the postsynaptic neuron triggers Ca^{++} dependent kinases (protein kinase C and Ca^{++}/calmodulin kinase II) which induce LTP. In LTD, the slow process of building of the synaptic vesicle, causes decrease in the neurotransmitter release and decrease in post-synaptic potentiation [67].

C. Types of Memory Impairment in Epilepsy(Table 1)

1) Transient Amnesia or Transient Cognitive Impairment (TCI)

Some types of seizures are manifest by episodes of transient amnesia, It is due to intermittent clinical seizure activityor sub-clinical epileptiform discharges [generalized or focal epileptic discharges] in the medial temporal lobes [left or right-sided] that disrupt neocortical networks for encoding processes and results in loss of new information [11,68,69]. TCI was first demonstrated during 3 Hz generalized spike-and-wave discharges [68]. Sirén et al. [70] found that the duration of generalized 3-Hz spike-wave discharges and

clinical absence seizures was negatively correlated with performance on the visual memory tasks. TCI was also demonstrated in many cases of benign childhood epilepsy with centrotemporal spikes, a disorder once thought to have no adverse psychological effects [68]. In patients with benign childhood epilepsy with centrotemporal spikes, deficits in IQ were found to be significantly correlated with the frequency of EEG spikes but not with the frequency of seizures [71]. Left-sided focal spiking frequently produces errors in verbal tasks, whereas right-sided discharges are often accompanied by impairment in handling non-verbal material. Transient disruption of cognitive processing during paroxysmal epileptic activity is attributed to the following: a) the involvement of a neuronal circuitry in epileptic spiking rendering the same neurons unavailable for normal physiological processes, b) the antidromiccorticothtalmic backfiring, that would collide and annihilate any incoming information through orthodromicthalamocortical pathways, and c) the prolonged membrane hyperpolarization following paroxysmal depolarization shift mediated by recurrent postsynaptic inhibitory mechanisms which elelctrophysiologically correspond to the after-coming slow wave [72,73]. Presence of slow EEG activity in the same regions showing abundant spike wave activity has been interpreted as reflecting increased cortical inhibition mediated by hypersynchronousGABAergic inhibitory postsynaptic potentials. This increment of cortical inhibition might temporarily alter normal physiological processing of cognitive disruptions [74]. High seizure frequency disrupts the first encoding state of the memory process and specifically disrupts attention, concentration and working memory. However, in individual cognitive performance, even single seizures can generate long-term attentional slowing in the post-ictal period that exists for at least 24 hours. It was recorded that a single tonic-clonic generalized seizure may have a lasting negative effect on attention for about 30 days [75]. TCI was recorded in ~ 50% of patients with subclinical or interictal[76]. EEG paroxysms usually pass as unrecognized by standard memory tests, but are detected by sensitive methods of observation as continuous psychological testing, which show brief episodes of impaired cognitive function during such discharges.

Cognitive impairment evenif trivial may adversely affect the patient's psychosocial functioning in daily life as educational skill, learning tasks, attention, behavior, sleep and motor function [9]. Autistic features observed in some children with epilepsy have been suggested as a consequence of apparently subclinical spikes interfering with specific cerebral processes [77].

2) Accelerated Long-term Forgetting (ALF)

Some epileptic individuals learn and initially retain information normally, but forget it at an unusually rapid rate over the following days or weeks. This phenomenon is called accelerated long-term forgetting (ALF). Electrophysiological studies of the rodent hippocampus show that repeated seizure activity has a profound, deleterious effect on an important form of synaptic plasticity (LTP) which has been suggested to underlie memory formation. Repeated seizure activity has been found to incrementally cause an indiscriminate and widespread induction of LTP, consuming and reducing overall hippocampal plasticity available for information processing [78].

3) The Mechanisms of Cognitive Impairment Associated with Specific Epileptic Syndromes as Landau-KleffnerSyndrome (LKS) and Continuous Epileptic Activity during Sleep (CSWS)

Landau-Kleffner syndrome (LKS) and the syndrome of continuous spike-and-wave discharges during slow sleep (CSWS) represent a spectrum of epileptic conditions which share many common features including: 1) onset during childhood, 2) deterioration of cognitive functions that were normally acquired in the past, 3) continuous spike-and-wave discharges during slow wave sleep, 4) pharmacological reactivity, 5) improvementof the neuropsychological symptoms when the EEG abnormalities improves (spontaneously or after drugs as corticosteroids) and 6) absence of obvious structural lesion detected by computer tomography (CT) or magnetic resonance imaging (MRI) scan [25,26].

The cognitive deficits of children with CSWS are long-lasting, present during months or years, and complete recovery is unusual. The pathophysiology of cognitive deficits in CSWS and LKS is complex and different from that described with TCI as some patients with CSWS or LKS may have a completely normal awake EEG while cognitive deficits are present in the awake state when interictalepileptiform discharges are rare or absent. Recently, positron emission tomography (PET) studies using [18F]-fluorodeoxyglucose (FDG) during acute and recovery phases of CSWS in a group of children with epilepsy, showed that increased glucose metabolism at the epileptic focus was associated with hypometabolism in distant connected areas and both hypermetabolism and hypometabolism resolved at the recovery phase of CSWS [79,80]. Altered effective connectivity between focal hypermetabolism (centro-parietal regions and right fusiform gyrus) and widespread hypometabolism (prefrontal and orbitofrontal cortices, temporal lobes, left parietal cortex, precuneus and cerebellum) was found at the acute phase of CSWS and markedly regressed at recovery whether spontaneously or with corticosteroids [81]. The parietofrontal altered connectivity observed in patients with hypermetabolism is interpreted as a phenomenon of remote inhibition of the frontal lobes induced by highly epileptogenic and hypermetabolic posterior cortex [12,82].

4) Mechanisms of Epilepsy Induced Progressive Brain Damage

Many animal and human studies reported persistent and progressive cognitive decline and behavioral impairment in developing and mature brains with epilepsy.Lesions in the hippocampus, amygdala or the piriform cortex and epileptogenesis-related neuronal plasticity, reorganization, sprouting, and impairment of cellular metabolism are fundamental determinants for progressive cognitive deterioration in epilepsy [83-87]. At the molecular level, recurrent seizures result in a dramatic imbalance between the brains's increased oxidative requirements and the energetic supplies. Recurrent seizures causes increased systemic and cerebral metabolic activities with subsequent inadequacy of energy substrates, secondary defective glucose transport, an inability of these brains to use ketones for energetic sources, disruption of cortical blood flow autoregulation, impeding synthesis of brain DNA and profound disruptions in synthesis and transport of protein and lipid transport.Also brain injury from seizures results in abnormal reorganization of a variety of excitatory and inhibitory neurotransmitter/neuromodulator systems tipped towards excitation [88,89].

It was shown that seizures selectively impaired myelin accumulation out of proportion to their effect on brain growth. In epileptic rats, examination of cerebroside and proteolipid protein, relatively specific myelin lipids, was found to be reduced by about 11-13% in immature rats [90]. The net result is cascade of deleterious effects leading to consequent neuronal necrosis, alterations in neuronal proliferation and differentiation, impaired synaptogenesis and myelination and subsequent neurodevelopmental deficits. Executive functions, mainly under frontal lobe control, seem to be particularly vulnerable to epileptic EEG activity during the period of maturation [91].

Table 1. Types and mechanisms of memory impairments with epilepsy

Types	Mechanisms
Transient cognitive impairment (TCI)	Repeated epileptic activity results in disruption of the encoding state of the memory process due to involvement of a neuronal circuitry in epileptic spiking (same neurons for normal physiological processes) or due to the prolonged membrane hyperpolarization following paroxysmal depolarization shift.
Accelerated long-term forgetting (ALF)	Repeated epileptic activity results in indiscriminate and widespread induction of long-term potentiation (LTP) and this consumes and reduces the overall hippocampal plasticity available for information processing.
Progressive cognitive impairment (PCI)	Repeated epileptic activity results in lesions in the hippocampus, amygdala or the piriform cortex and epileptogenesis-related neuronal plasticity, reorganization, sprouting, and impairment of cellular metabolism

D. Selective Vulnerabilities of Immature Brains versus Mature Brains

However, despite the above and the fact that the immature brain has increased vulnerability to seizures but it appears to be more "resistant" to the damaging effect of epilepsy than does the mature brain [92,93]. This is attributed to two main reasons: 1) there is a difference in computation of the immature brains versus mature brains, and 2) there is a selective vulnerability period in immature brain before which little or no change occur as a result of epilepsy.

a) The Difference in Computation of the Immature Brains versus Mature Brains

Systematic studies indicated that neuronal computation of immature brain is different than mature brain. The vulnerability of the developing brain to generate seizures is high with increased susceptibility to develop SE compared to mature brains. This is attributed to the following [94-98]: a) NMDA receptor subunits create populations of receptors that flux more Ca^{++}, open more easily and block less frequently than mature forms, b) activation of $GABA_A$

receptors causes membrane depolarization rather than hyperpolarization. Classically, activation of $GABA_A$ or $GABA_C$ receptors allows the influx of Cl^-, following its electrochemical gradient resulting in neuronal hyperpolarization, however, early in life, there is a reversal of Cl^- flux outward rather than at the receptor-associated channel and thus depolarizing, c) the differential expression of glutamate transporters during development, and d) Na^+/K^+-ATPase is less abundant and rectifier K^+ channels are generally slower in immature brain. Both of which may serve to prolong action potentials and increase excitability (Table 2).

Table 2. Causes of physiologic hyperexcitability of immature brains

a) NMDA receptor flux more Ca^{++}, open more easily and block less frequently.
b) Activation of $GABA_A$ receptors causes reversal of Cl^- flux outward rather inward resulting in membrane depolarization rather than hyperpolarization.
c) The differential expression of glutamate transporters during development.
d) The less abundant Na^+/K^+-ATPase and slower rectifier K^+ channels prolong action potentials and increase membrane excitability.

However, the immature brain appears to be more resistant to excitotoxic effects of repeated seizures and SE-induced damage. The proposed mechanisms include (table 3): 1) preservation of GABA synthesis which declines with maturation [99], 2) increased expression of $GABA_A$ receptor-1 subunit in contrast to adults [100-101], 3) presence of mitochondrial uncoupling protein [102], 4) absence of mitochondrial oxidative stress [103], 5) absence of glia activation and cytokine production [104], 6) absence of GluR2 down-regulation or even up-regulation of GluR2 and down-regulation of GluR3 receptor subunits which are neuroprotective [105], and 7) presence of higher expression of growth factors and neurotrophins (as BDNF) [106].

In addition to the above neuroprotective mechanisms of immature brain, the nervous system of the developing brain has an important degree of plasticity, which allows functional defects caused by lesions to be compensated by acquisition of new functions by other brain areas. This is particularly apparent after surgery for localized intractable epilepsies, where functions originally performed by the ablated tissue can be progressively taken up by other cortical areas. Even hemispheric dominance can be switched by the developing nervous system if the electrophysiological activity of the left hemisphere language areas is ablated or becomes aberrant [107].

Table 3. Causes of endogenous neuroprotection in immature brains

1)	Preservation of GABA synthesis which declines with maturation
2)	Increase expression of $GABA_A$ receptor-1 subunit.
3)	Presence of mitochondrial uncoupling protein.
4)	Absence of mitochondrial oxidative stress.
5)	Absence of glia activation and cytokine production.
6)	Absence of GluR2 down-regulation or even up-regulation of GluR2 and down-regulation of GluR3 receptor subunits.
7)	Higher expression of growth factors and neurotrophins.

b) Selective Vulnerability Period in Immature Brain

It was observed that adult animals surviving SE show deficits in learning, memory, and behavior whereas young rats with SE experience fewer such deficits [108,109]. This supports the notion that cognitive and behavioral consequences are also related to the age of the animal at the time of kindling or SE. There is a selective vulnerability period in the immature brain at which little or no neuronal damage is seen before it [110-113].In animal models, Marks et al. [114] found that the degree of calcium entry into the hippocampal subfield CA-1 and subsequent damage was directly related to age. It was found that glutamate increased intracellular Ca^{++} minimally in post-natal day 1 to post-natal day 3 neurons (P1-P3), whereas in P21-P25 neurons, glutamate resulted in marked increases in intracellular Ca^{++} and caused considerable swelling, blebbing, and retraction of dendrites into the soma of the neuron. Cell loss and mossy fiber changes after seizures are also age-related, with younger animals demonstrating less sprouting than older animals after experiencing seizures of similar intensity. Also reactive synaptogenesis of the mossy fibers appears to occur only when the mossy fibers have reached the mature state after the 3rd post-natal week. Yang et al. [115] evaluated the effects on synaptic reorganization of seizures induced by kainic acid [KA] during development, using the expression of growth-associated protein-43 [GAP-43], a marker for synaptogenesis, and the Timm stain, which detects the presence of zinc in granule cell axons. Age-specific doses of KA, a glutamate agonist and potent convulsant, were used to induce seizures of similar intensity in rats varying in age from P12 to P60. Until age P25, no differences were noted in either Timm or GAP-43 staining between animals with KA seizures and control animals. In KA-treated rats aged P25 and older, Timm staining was found in the supragranular layer of the dentate gyrus. This staining increased with age at the time of KA injection. KA-treated adult rats [P60] also exhibited increased staining in the suprapyramidal layer of the CA-3 subfields, but younger rats did not. Changes in GAP-43, the marker for synaptogenesis, were delayed as compared to the Timm staining, with no differences between KA-treated animals and controls until age P35, when a band of GAP-43 immunostaining appeared in the supragranular inner molecular layer, progressively increasing in intensity and thickness over time. This study confirmed the observations of Sperber et al. [116] that the degree of sprouting after SE is age-related. By P21-P25, an adult pattern of mossy terminals has developed [117] and, by P21, the adult pattern of GAP-43 immunostaining has been achieved.

However and despite of the above noticed neuroprotective mechanisms and selective vulnerabilities of immature brains, it was found that if a seizure is sufficiently long, it can result in damage at any age [118-121]. It was found that prolonged seizures can cause synaptic reorganization with aberrant growth [sprouting] of granule cell axons (the so-called mossy fibers) in the supragranular zone of the fascia dentate [122] and the infrapyramidal region of CA-3 [123]. In the early postnatal period, the development of these axons may be particularly prone to seizure-induced changes. Seizures also activate the trk subtype of neurotrophin receptor in the mossy fiber pathway [124] and alter the expression of certain glutamate subreceptors [125].

E. Antiepileptic Medications and Cognition

Although the information on the role of fetal and postnatal exposure to AEDs is limited in humans, there is a growing body of information from animals suggesting that AEDs may have substantial effects on brain development.

In utero exposure to PB, PHT, CBZ or VPA can produce behavioral defects, which can occur at dosages lower than those required to produce somatic malformations [126,127], deficits in the hippocampal eight-arm maze, spontaneous alternations and water maze behaviors in adulthood [128], affected operant conditioning [129,130], decrements in various spatial learning tasks [131,132] as well as increased aggression and locomotor activity [133-134]. It has been observed that perinatal exposure to conventional AEDs (as PB, PHT, CBZ or VPA) causes a reduction in brain weight [130,135] and a number of behavioral deficits [seen at subteratogenic doses], including deficits in spatial learning tasks and hyperactivity in rats [136] and hyperexcitability [screeching, refusing to attend to stimuli, lack of visual orientation] [137].

In clinical studies, Dean et al. [138] investigated 261 pregnancies exposed to AEDs and 38 siblings not exposed to AEDs. Most of the mothers were receiving monotherapy [80%]. In the assessed pregnancies, congenital malformations were present in 14% of AED-exposed versus 5% of non-exposed siblings. After accounting for family history, 19% of AED exposed versus 3% of non-exposed siblings exhibited developmental delay. Overall, 31% of AEDs-exposed children had a major malformation or developmental delay. The risk was highest in the group receiving polytherapy. In a retrospective study from Denmark, Reinisch et al. [139] found that prenatal exposure to PB significantly reduced verbal IQ scores (7 IQ points or 0.5 standard deviation [SD]) in two cohorts of men born at the largest hospital in Copenhagen between 1959 and 1961 and exposed to PB during gestation via maternal medical treatment for causes other than epilepsy. In study 1: Wechsler Adult Intelligence Scale (Danish version) was used; while in study 2: Danish Military Draft Board Intelligence Test (BørgePriensPrøve) was used. Exposure that included the last trimester was the most detrimental. The study also found that the presence of low socioeconomic status and an "unwanted" pregnancy synergistically enhanced the adverse effects of PB, decreasing the verbal IQ score by approximately 20 points lower than expected in the subset that had all of these risk factors. An investigational group from Liverpool/Manchester, UK, has conducted several large, retrospective studies of cognitive outcomes in children born to women with epilepsy. Their first study assessed 594 school-age children and found that 30% of the children exposed in utero to VPA monotherapy, required special education compared with 3% to 6% for other monotherapy groups. In a follow-up study, they examined the development in one cohort of children 6 to 16 years old (n = 249) and in another cohort of children younger than 6 years old (n = 119). The authors observed that ~50% in the cohort of older children required special education [140-142]. In a prospective, observational, multicenter USA and UK study, pregnant women with epilepsy treated with a single AED (CBZ, LTG, PHT, or VPA) were enrolled between 1999 and 2004. The authors observed that children who had been exposed to VPA in utero had significantly lower IQ scores than did those exposed to other AEDs. Average IQ score was 9 points lower in children exposed to VPA than the score of those exposed to LTG, 7 points lower versus PHT, and 6 points lower versus CBZ. There was a dose-dependent association between VPA use and IQ [143]. A

prospective study from Finland also reported a greater risk for impaired cognition in children exposed in utero to VPA [144]. The study included 182 children of mothers with epilepsy (107 monotherapy, 30 polytherapy, and 45 on no AED) and 141 healthy children as controls. Overall, verbal IQ was reduced with polytherapy and VPA exposure compared with the healthy controls and CBZ monotherapy. Verbal IQ (mean, 96) for children exposed to CBZ did not differ from that of healthy controls (verbal IQ, 95). Verbal IQ was 82 for the VPA monotherapy group. In the study of Seidel et al. [145], ten children with benign rolandic epilepsy were evaluated with and without CBZ treatment. The authors observed that the children performance was better in visual-search task and recall of stories in absence of CBZ compared to that in presence of CBZ. They also observed that higher CBZ serum level was associated with slower performance on the same visual-search task.

Regarding new AEDs, no effect on cognition was observed with lamotrigine (LTG) [35]. The profound effects of topiramate (TPM) on cognitive function in children have received increased attention during the past several years. In a retrospective evaluation of 87 children receiving TPM, 41% were taken off therapy because of adverse effects, primarily cognitive dulling [39,40,146,147].In contrast, the effect of TPM on cognitive function in the immature brain was recently evaluated [148-150]. Recent experiments revealed that TPM elicits a neurotoxic effect in infant rat brain beginning at a dose of 50 mg/kg which is higher than the effective anticonvulsant doses in infant rodent seizure models [150]. In one study, TPM was given to rats with neonatal seizures induced by lithium-pilocarpine at P20 and rats were tested in the water maze for spatial learning, and their brains were examined for cell loss and sprouting of mossy fibers. The authors observed an improvement in visual-spatial performance in the water maze following TPM therapy in rats with neonatal seizures. The authors also found that neonatal rats without seizures and exposed to 4 weeks of TPM did not differ from untreated controls in water maze performance or histologic examination. The authors concluded that TPM has a rather beneficial profile, since it shows no detectable toxicity at anticonvulsant doses.

The Mechanisms of Progressive Cognitive Deficits due to Antiepileptic Medications (Table 4)

The pathophysiologic mechanisms responsible for teratogenicity and the cognitive and behavioral impairments due to AEDs remain largely unknown. However, various mechanisms have been proposed which include adverse effects on neuronal proliferation and migration and increased apoptosis [151-154].

Normally, neuroblast migration is influenced by crucially promoting signals (motility, acceleratory and stop signals) from GABA and glutamate neurotransmittors that act on several receptors subtypes ($GABA_A$, $GABA_B$ and NMDA). One can hypothesize that drugs which acts on these neurotransmitters and their receptors might affect neuronal migration. In support: Manent and colleagues [155] reported that prenatal exposure (from embryonic days 14 to 19) to vigabatrin (VGB) (200 mgkg/day), and VPA (100 mg/kg/day), doses which are similar to those used clinically, resulted in neuronal migration defect and neuronal death observed postnatally in the form of hipocampal and cortical dysplasias [156]. Also, several AEDs (i.e., clonazepam, diazepam, PB, VPA CBZ, PHT, LTG, VGB, TPM and LEV) have been shown to induce widespread neuronal apoptosis in immature animal brains at plasma

concentrations relevant for seizure control in humans [157,158]. The vulnerable developmental period to drug-induced neuroapoptosis in rodents includes the first two postnatal weeks of life and coincides with the brain growth spurt period [159]. The comparable period in humans extends from the sixth month of gestation to several years after birth. Considerable variability exists in both anatomical and behavioral outcomes for individual children exposed in utero to a specific AED, even when similar dosages are employed during pregnancy. The different interactions between teratogens and susceptible genotypes [154] may explain the individual variance.

It was shown that the administration of PB to rat pups results in significant decreases in brain weight [160], a reduction of purkinje and granule cells in the cerebellum [161] and pyramidal and granule cells in the hippocampus [162], PB has also been shown to disrupt cholinergic neurotransmission in the hippocampus [132] and DNA, RNA, protein, and cholesterol concentrations and reduced neuronal number [128,134,163]. Also chronic exposure of cultured mouse spinal cord neurons to PB resulted in reduced cell survival and decreased length and number of dendrite branches. For offspring of pregnant mice treated with PB, the reduced brain concentrations of dopamine and norepinephrine result in increase the uptake of dopamine, norepinephrine, serotonin, and GABA into synaptosomal preparations of brain tissue and thus reduce the neuronal excitability in the pyramidal neuron dendrites [152,164].

Following treatment with PHT, widespread and dose-dependent neurodegeneration and ultrastructural changes similar to those described in neurons undergoing programmed cell death was seen in infant rats within the medial septum, nucleus accumbens, thalamic and hypothalamic nuclei, subiculum, globuspallidus, piriform and entorhinal cortices, the amygdala, the frontoparietal, cingulate and retrosplenial cortices. The threshold dose for triggering an apoptotic response was 20 mg/kg, which resulted in PHT plasma concentrations ranging between 10 and 15 g/ml over 4 hours [165]. Others reported delayed migration of granule cells and altered development of Purkinje cells and cerebellar damage by PHT [166-169]. Cerebral atrophy, changes in neuronal membranes, decreased brain weight and cerebellar anomalies were observed with in utero exposure to VPA [170-172].

The apoptotic effect of some AEDs appears to result from reduced neurotrophins and protein kinases (e.g. brain derived neurotrophic factor, neurotrophins 3 and 4) as well as reduced levels of the active phosphorylated forms of extracellular signal regulated kinase (ERK1/2) and protein kinase B (AKT) [173-175]. Such changes reflect an imbalance between neuroprotective and neurodestructive mechanisms in the brain and such an imbalance will likely promote apoptotic death [176]. Tandon et al. [106] blocked the synthesis of brain-derived neurotrophic factor (BDNF) by the infusion of an 18-mer antisense oligodeoxynucleotide to BDNF in the right ventricle of immature (P19) rats using micro-osmotic pumps and then subjected the rats to SE. Rats in whom BDNF synthesis was blocked experienced more cell loss in the hippocampus after SE than did control rats that did not receive oligodeoxynucleotide. These results suggest that BDNF is involved in providing protection against seizure-induced neuronal loss in the developing brain [173]. Most mechanistic studies focused on the toxic effect of VPA. Lee et al. [177] found that VPA suppressed protein kinase C (PKC) activity in both membrane and cytosol compartments in hippocampal slices. PKC is highly enriched in the brain and plays a major role in regulating both pre- and postsynaptic aspects of neurotransmission, including neuronal excitability,

neurotransmitter release, and long-term alterations in gene expression and plasticity. PKC is critical for the induction of LTP and LTD [178].

Table 4. The mechanisms of progressive cognitive impairments of immature brains due to antiepileptic medications

1)	***Adverse effects on neuronal proliferation and migration:***through a) Interference of the promoting signals (motility, acceleratory and stop signals) from GABA and glutamate neurotransmittors that act on several receptors subtypes ($GABA_A$, $GABA_B$ and NMDA), b) Suppression of protein kinase activities, and c) Alteration of gene expression.
2)	***Increased apoptosis***caused by: a) Reduction of neurotrophins, b) Suppression of protein kinases and reduction of the levels of the active phosphorylated forms of extracellular signal regulated kinases, c) Alterations in gene expression, and d) Blockage of NMDA receptors.

The anti-proliferative effect of VPA may be relevant for its teratogenicity, as alterations of normal proliferation rate of the tissues involved with neuronal tube closure may result in an embryo with a neural tube defect [179]. It was found that at therapeutically relevant concentrations, VPA alters the expression of certain homeobox genes [180]. Concentrations of VPA within its therapeutic range inhibit histone deacetylase, which is involved in the repression of gene expression [181-183]. Inhibition of histone deacetylase can prevent cell proliferation and may be responsible for the ability of VPA to reduce proliferation of C6 glioma cells [180]. The same mechanism for neuronal apoptosis can be applied for AEDs that cause decrement in glutamate-mediated excitation by antagonizing the response mediated by NMDA or AMPA/KA subtype of glutamate receptors. There is also evidence from lab studies that blockage of NMDA receptors can increase neuronal apoptosis resulting in chronic behavioral, structural and molecular effects. In support, Harris et al. [184] studied the long-term consequences of NK-801 (0.5 mg/kg), an NMDA antagonist, in a group rats treated postnatally on day 7. The authors observed reduced volume and neuronal number within the hippocampus and altered hippocampal NMDA receptor (NR1 subunit).

E] KEY MESSAGES AND RECOMMENDATIONS(TABLE 5)

The above information raises significant clinical and research implications and recommendations:

1. Maternal Issues
   - Women with epilepsy should understand that they are exposed to a two- to three-fold increase in the risk of major malformations in their fetuses than non-epileptics; however, the message should be balanced so that they understand that the large majority of children born to them are normal. Further, it is important that they should understand the risks of seizures during pregnancy, which are substantially greater than those of AEDs.

- It is important to know that drug pharmacokinetics is altered during pregnancy, so monitoring of free levels of the drugs may be needed.
- VPA should especially be avoided in epileptic women during the child bearing period as it increases the risk of congenital malformations and cognitive impairment in the offspring.
- Folate supplementations should be given before conception (1–4 mg/day) and continued during pregnancy to avoid adverse effects to the fetus related to AEDs.
- Polypharmacy should be avoided in women with epilepsy during the child bearing period.
- Because several AEDs can interfere with vitamin K and increase the risk of perinatal hemorrhage, vitamin K should be given to the infant at birth (1 mg) and probably to the mother during the last month of pregnancy (10 mg daily).

2. Age at onset of epilepsy and duration of illness appear to be a critical determinant of the cognitive impact of epilepsy. So epilepsy should be treated as early as possible to avoid impairment of the cognitive functions of the epileptic patient. Repeated seizures also have been associated with increased intellectual impairment. An appropriate AED should be given once the epilepsy is diagnosed to avoid the accumulating detrimental effects of repeated seizures. SE should be treated as an emergency by giving BZ and AEDs to stop seizures as early as possible to avoid seizure-induced damage.

   It is also important to take into consideration the cognitive factor while choosing an AED as long-term treatment. The harmful cognitive effects of AEDs are especially important to those who require maximal cognitive efficiency for their school, or daily activities. AEDs with higher efficacy and tolerability with less or no effects on cognitive functions have to be targeted. The risks of adverse effects or toxicity from chronic medications and cognitive difficulties increase significantly with the use of combined AEDs therapy. So, the treatment of epilepsy should be started with one drug and gradually increasing the dose to the maximum dose. Polytherapy should be reserved for cases of medically intractable epilepsy.
3. Whether to treat or not to treat the first seizure in a child or an adult is still a debate? Most neurologists do not recommend treating adults or children after a single seizure if they have a normal neurologic examination, EEG and imaging studies. Even many neurologists prefer to wait before starting treatment for children and adults who have had two or three seizures if there was either a long period of time between seizures or the seizure was provoked by an injury or other specific causes. In children, the risk for recurrence after a single unprovoked seizure is rare. The risk even after a second seizure is low, even when the seizure is prolonged. However, after a single seizure, if tests (EEG or MRI) reveal any brain injury, or if specific neurologic, developmental, or epilepsy syndromes put a person at special risk of recurrence, treatment with AEDs must be initiated (should be considered).
4. Cognitive side effects of AEDs are well established for medications that were introduced before the 1990s (old, conventional or first-line AEDs), with the greatest cognitive impairment risk associated with PB and BZ. For others, the cognitive profiles are generally comparable, with modest motor and psychomotor slowing. No

consistent cognitive difference was identified with CBZ, PHT, or VPA. The cognitive profile of new AEDs or those introduced during the 1990s (e.g., GBP, LEV, TGB, TPM, etc.) showed that LTG is the least of AEDs that cause cognitive impairment, while TPM is the worst. Future long-term longitudinal studies have to be undertaken to elucidate the mechanism(s) of epilepsy-related cognitive issues, particularly in relation to newer AEDs (including mature versus immature brain).

5. The positive and negative psychotropic effects of AEDs have to be among the treatment choices for epilepsy as many epileptic patients deal with epilepsy as a stigma and not a disease. They hide their condition from others, which can result in depression, social isolation, reduced self esteem and inadequate schooling.

Table 5. Key messages and recommendations

- Women with epilepsy are at higher risk of major malformations and cognitive impairment in their fetuses. However, the risks of seizures during pregnancy are substantially greater than those of AEDs.
- Drug pharmacokinetics is altered during pregnancy, so monitoring of free levels of the drugs may be needed.
- VPA should especially be avoided in epileptic women due to increases the risk of congenital malformations and cognitive impairment in the offspring.
- Folate supplementations should be given before conception (1–4 mg/day) and continued during pregnancy to avoid adverse effects to the fetus.
- Polypharmacy should be avoided in women with epilepsy during the child bearing period.
- Vitamin K should be given to the infant at birth (1 mg) and probably to the mother during the last month of pregnancy (10 mg daily) because several AEDs can interfere with vitamin K and increase the risk of perinatal hemorrhage.
- Epilepsy should be treated as early as possible taking into consideration the cognitive factor and the positive and negative psychotropic effects of AEDs while choosing an AED as long-term treatment.

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Chapter 3

TOWARD BETTER RECOGNITION OF EARLY PREDICTORS FOR AUTISM SPECTRUM DISORDERS (ASDS)

Nicolas Deconinck[1], Marie Soncarrieu[2] and Bernard Dan[1]

[1]Department of Neurology - Hôpital Universitaire des Enfants Reine Fabiola, Université Libre de Bruxelles, Brussels, Belgium
[2]Department of Child Psychiatry - Hôpital Universitaire des Enfants Reine Fabiola, Université Libre de Bruxelles, Brussels, Belgium

ABSTRACT

Autism spectrum disorder (ASD) is a group of devastating developmental conditions whose prevalence was reported as increasing over the last decades. This may be related to changes in diagnostic criteria, comorbidity with other developmental disabilities or a true increase in cases. Diagnosis rests essentially on behavioral presentation and developmental history. Difficulties in communication and reciprocal social behavior are the core characteristics of ASDs. Motor and behavioral stereotypies, though prevalent, are not specific to ASDs and are often not observed before the age of 2. The etiology and pathophysiological mechanisms of ASD remain largely unknown, although environmental toxins and genetic factors have been implicated. Early diagnosis of ASD is of utmost importance because early intervention is especially effective in the experience of many professionals although not evidence based. Diagnosis for ASD is commonly made at approximately 3 years or older. There have been significant advances in our knowledge of the early signs of ASD through the use of retrospective videotape analysis, parental report and screening studies. However, there has been a lack of prospective methods to study early features in children who go on to develop ASD. There remains little research on the prospective identification of these children in a community-based sample before 18 months. Recently however some studies were able to identify early neurological signs and developmental predictors, which differed according to the age at assessment and allowed rather accurate identification of children with ASDs. By recruiting younger siblings of children with ASD, who are at much higher risk for developing ASD, some authors could demonstrate a prolonged latency to disengage

visual attention from two competing stimuli and a delayed expressive and receptive language during the first year of life. A characteristic pattern of early temperament, with marked passivity and decreased activity level at 6 months, followed by extreme distress reactions, a tendency to fixate on particular objects in the environment, and decreased expression of positive affect by 12 months was aloso quite specifically recognized. By examining early medical and behavioral characteristics of NICU children later diagnosed with ASD, some authors showed that ASD neonates showed persistent neurobehavioral abnormalities and higher incidences of visual asymmetric visual tracking and upper limb muscle tone deficits. At 4 months, children with an eventual diagnosis of ASD specifically showed a continued visual preference for higher amounts of stimulation, behaving more like newborns. Looking at early social attention and communication skills with adapted scales in children before the age of 18 months in very large community-based settings, authors were able to identify children at "risk " for ASD with a positive predictive value around 80 %. In this review, we review recent advances and discuss the validity of organizing early detection program for ASD in the context of a daily medical practice with the questions and hurdles raised by this approoach.

INTRODUCTION

Clinical Description

Autism spectrum disorders (ASDs) are among the most severe and disabling neurodevelopmental disorders affecting children. The disorder spectrum includes autistic disorder (AD), Asperger's disorder and Pervasive Developmental Disorder- Not Otherwise Specified (PPD NOS) (Barbaro et al., 2010). These conditions are characterized by patterns of both delay and deviance in multiple areas of development. Their onset is typically in the first months of life. The signs of ASDs in infancy and toddler age consistently fall within the domains of social attention and communication. Clinical "red flags" include lack of eye contact, social smiling, imitation, response to calling by name, interest and pleasure in others, emotional expression, directed vocalizations, joint attention skills (pointing to "show," following a point, monitoring others' gaze, and referencing objects/events), requesting behaviors, and gestures (e.g., waving, clapping, nodding, and shaking head). Imagination skills, such as pretend play, have also been found to be deficient in late infancy and toddler aged in many children with an ASD (see Barbaro et al., 2010).

Autism Disorder

Rutter (1978) recapping early phenomenological description of autism by Kanner, suggested that four features were essential for the diagonsis of autism: (1) onset prior to 30 months, (2) distinctive impairment in social development which did not simply reflect mental retardation, (3) distinctive impairment in communicative development which did not simply reflect mental retardation, and (4) unusual behaviors related to 'insistence on sameness', i.e. resistance to change, idiosyncrasic responses to the environment. This definition shaped the first largely consensual categorial definition of autism. (Kanner, 1943 ; Rutter, 1978 ; Volkmar, 1998).

The categorial definition of AD was stressed with publication of DSM IV and ICD 10, which allowed greater uniformity in diagnostic practice. DSM IV criteria for autistic disorder

are described in Table 1 (DSM IV, 1994). AD is associated with variable degree of mental retardation in about 75 % of the patients. However, the pattern of developmental and behavioral features differs from that seen in children with mental retardation not associated with AD in that certain sectors of development, such as social interaction and communication, are most severly affected wheras other areas, such as nonverbal cognitive abilities may be within normal limits (Volkmar, 1998). Although sensory and motor behaviors and stereotypies are seen in infants with an AD, they are also indicative of general intellectual disability with many children not showing these behaviors until the age of 3 years (Barbaro et al., 2009).

Asperger Syndrome

In Asperger syndrome patients show a marked deviance in social interaction and also present unsual behavior with a resistance to change (Asperger, 1991). There remains controversy in the differential diagnosis of Asperger syndrome and higher functioning autism. Using more stiringent definition out of the ICD-10 or DSM IV criteria, cases with Asperger syndrome would be expected to have better language skills than typically observed in autism (Klin et al., 1995). Cases of Asperger syndrome also appear to be at risk for exhibiting a particular profile of learning disability, parituclarly with non verbal learning skills, including deficits in tactile perception, psychomotor coordination, and nonverbal problem solving the face of well-developed verbal and memory skills (Rourke, 1989).

Pervasive Development Disorder not otherwise Specified (PDD-NOS)

The PDD-NOS category is essentially considered as a defintion by exclusion, which currently remains problematic. Essentially, this category is used when an individual does not meet the threshold criteria for autism disorder but has difficulties of similar quality. It is considerd as a « subthreshold category ». This is strikingly paradoxical, as this condition is probably more common than the other conditions included in the ASDs (Towbin ,1997 a, Towbin .1997 b).

Epidemiology

According to recent reports, prevalence rates of the combined ASDs are currently 1 in 160 in Australia, 1 in 100 in the United Kingdom and 1 in 91 in the United States. The prevalence of AD is 1 in 500 with a 4:1 male to female ratio (Chakbarti et al., 2005, Fombonne, Center for disease control and prevention, 2007). Strikingly, an increase of prevalence was observed in the United States, where it was reported as 1 in 1000 in the 1980s, as ~1 in 150 (centers for disease control prevention, 2009) early in this decade. The reason for this increased prevalence is still debated. Firstly, there was an increased identification rate of ASD patients with growing clinical awareness and knowledge about ASD semiology. Secondly, the diagnostic sepctrum of the condition has expanded from the typical Kanner strict definition of autism to a larger DSM IV concept of ASD, recognizing also the concept of ASDs and some subthreshold categories. These reasons explained that studies between 1967-1982 using Kanner's criteria provide prevalence's of 0.5 in 1000 children, using Rutter's criteria on a range of 1 to 2 in 1000 children and when large DSM-IV

criteria were used, the most recent epidemiological studies, prevalence ranged from 5.7 to 6.7 in 1000 children (Center for disease control and prevention, 2007; Filipek et al., 1999, Bryson et al., 2003, Barbaresi et al. , 2005).

Table 1. DSM IV criteria for Autistic Disorder

A. total of at least six items from (1), (2), and (3), with at least two from (1), and one each from (2) and (3)
   1) Qualitative impairment in social interaction, as manifested by at least two of the following :
      a) marked impairment in the use of multiple nonverbal behaviors such as eye-to-eye gaze, facial expression, body postures, and gestures to regulate social interaction
      b) failure to develop peer relationships appropriate to developmental level or markedly impaired expression of pleasure in other people's happiness
      c) lack of social or emotional reciprocity
   2) Qualitative impairments in communication as manifested by at least one of the following*
      a) delay in, or total lack of, the development of spoken language
      b) in individuals with adequate speeech, marked impairment in the ability to initiate or sustain a conversation with others
      c) stereotyped and repetitive use of language or idiosyncrasic language
      d) lack of varied, spontaneous make-believe play or social imtative play appropriate to developmental level
   3) Restricted repetitive and stereotyped patterns of behavior, interests, and activities
      a) preoccupation with one or more stereotyped and restricted patterns of interest that is abnormal either in intensity or focus
      b) failure to develop peer relationships appropriate to developmental level
      c) apparently inflexible adherence to specific, non functional routines or rituals
      d) stereotyped and repetitive motor mannerisms (e.g., hand or finger flapping or twisting, or complex whole body movement)
      e) persistent preoccupation with parts of objects

B. Delays or abnormal functioning in at least one of the following areas, with onset before 3 years old: (1) social interaction, (2) language as used in social communication, or (3) symbolic or imaginative play.

Etiology

There are two contrasting views in relation to the etiology of autism which have wide implications for neurbiological research and interpretation. Some authors are convinced that autism is not a homogenous disease, but as several different pathogenic pathways analogous to other disorders with a stable course like for example cerebral palsy. As described below, different syndromes are associated with autism and the significant psychopathology are not different from idiopathic cases. On the other hand the rate of underlying known conditions in autism are around 10 % are are typically associated with profound mental retardation.

No consistent etiological pattern has been found to date.

A) Genetics

There are strong indications of a genetic influence in autism (Folstein et al., 2001, Connors et al., 2005). Heritability of autism is well documented. Overall, five twin studies have shown that an average of 60% of monozygotic twins (MZ) are concordant for autism, while more than 90% of the monozygotic co-twins of probands with autism had significant social impairment. In contrast, dizygotic twins (DZ) have only a 10% concordance rate for autism or autism-related socia deficits (Folstein et al., 2001).

However, available data do not support a single gene model for autism. Described as a clinical syndrome, autism is accompanied by genetic heterogeneity. There is in fact, considerable evidence ythat ASDs are both aetiologically and genetically heterogenous (The autism genome project consortium, 2007; Bonnet Brilhault, 2011).

Recent insights show that a variety of genetic mechanisms may be involved in autism spectrum disorders, i.e. single gene disorders, copy number variations and polygenic mechanisms (Bonnet-Brilhault , 2011).

Considering monogenic diseases, autism was initially described as occurring in association with phenylketonuria (PKU) and fragile X syndrome. In these conditions, autism is often associated with more severe mental retardation (Bailey et al., 2001). ASD is also frequently reported in neuroectodermoses with brain malformation, such as tuberous sclerosis complex, and more rarely neurofibromatosis, which are caused by mutations in respectively *TSC1/TSC2* and *NF1* genes (Bourgeron, 2009, Volkmar, 1998). More recently mutations in the *PTEN* gene were reported in association with ASD, and macrocephaly. Interestingly, mutations in *TSC1/TSC2* and *PTEN* genes activate the mTOR/PI3K pathway which is associated with abnormal cellular/synaptic growth rate. Mutations in *NLGN3/4*, *SHANK3*, or *NRXN1* were also reported in patients with mental retardation, autism or Asperger syndrome and were shown in animal models to have a role in synaptogenesis and leading to imbalance between excitatory and inhibitory currents. (Bourgeron, 2009).

Linkage studies have identified several replicated susceptibility loci for autistic disorder, including 2q24-2q31, 7q, and 17q11-17q21 (The Autism Genome Project Consortium, 2007).

Conventional cytogenetic approaches highlight the high frequency of large chromosomal abnormalities (3%-7% of autistic patients), including the most frequently observed maternal 15q11-13 duplications (1%-3% of patients). Over the last decade, several microdeletions were shown to be associated with severe autism and mental retardation often associated with subtle dysmoprphic features making ASD part of a more polymalformation syndrome: tetrasomy 15; inversion-duplication of the 15q11-q13 region (Koochek et al., 2006) ; the 22q13 deletion syndrome or Phelan-McDermid syndrome with the disruption of the SHANK3 gene (coding for a postsynaptic protein) that is thought to be responsible for the syndrome (Peça et al., 2011)

Recent advances in microarray-based technology have increased the resolution in detecting submicroscopic deletions and duplications, referred to as copy-number variations (CNV). ASD-associated copy-number variations, which are considered to be present in individuals with ASD but not in unaffected individuals, have been extensively investigated (Sebat et al., 2007). Several independent array CGH platforms identified small CNV in autism: (Kakinuma et al., 2008; Harvard et al., 2006; Jacquemont et al., 2006; Rajcan-Separovic et al.,2007; Susan et al., 2008).

In sum, no single genetic cause has been identified that accounts for autism in all or even a significant proportion of patients. Due to this genetic heterogeneity, many unique genetic

variants are likely to result in the ASD phenotype, including single genes affecting multiple cellular processes or function in the same neurodevelopmental pathway and cytogenetic abnormalities affecting multiple contiguous genes. Although great progress has been made in autism genetics, the molecular bases of most ASDs remains enigmatic.

B) Environmental Factors

Drug / Environmental Exposure

Anedoctal reports described associations between autism and in utero exposure to valproic acid (Christianson et al. 1994), alcohol (fetal alcohol syndrome) (Harris et al.,1995) and thalidomide (thalidomide embryopathy) (Stromland et al.,1995).

There has been ongoping debate around a possible association between of ASD and te use of MMR vaccination. In a rigorous study, investigators from three institutions found no differences between children with autism and gastrointestinal disorders and control children who had gastrointestinal disorders, but not autism. There was no difference in the results of testing for measles vaccine virus in the intestine or with the timing of MMR and the onset of gastrointestinal disorders. These findings disproved the original hypothesis about measles vaccine and autism and refute an earlier study by one of the investigators (Horning et al., 2008)

Gluten-restricted diets have become increasingly popular among parents seeking treatment for children diagnosed with autism. Some of the reported response to celiac diets in children with autism may be related to amelioration of nutritional deficiency resulting from undiagnosed gluten sensitivity and consequent malabsorption. However a recent metaanalysis did not find any increased risk factor of gluten intolerance in the development of ASD (Lionetti et al., 2010).

Perinatal Risk

Higher prevalence of ASD also has been associated with obstetric and neonatal factors that result in NICU admission (Glasson et al., 2004; Johnson et al., 2010; Kurban et al. 2009; Limperopoulos et al. , 2008, Larsson et al., 2005). These include preterm birth and indicators of obstetric (eg, prenatal infection, maternal bleeding, growth restriction), birth (eg, fetal distress, hypoxia, low Apgar scores), and postnatal (eg, respiratory distress, intraventricular hemorrhage) complications. Even after controlling for other developmental disorder outcomes, Schendel and Bhasin found a twofold increased ASD risk as a result of lower birth weight and gestational age (GA) (Schendel et al., 2008).

Neuroanatomy and Neuroimaging Studies

The most recent findings about the very particular brain structure development in children with ASD (mostly during the first months of life) probably explain some of the early typical clinical features. We therefore emphasize these results in relation to the main focus of this review.

The vast majority of published studies on autism anatomy have focused on abnormalities present ten or more years after clinical onset, leaving unaddressed the question of which

structural abnormalities underlie the emergence of autistic behavior in the early stages. Although few in numbers, more recent developmental studies have revealed the phenomenon of abnormal early brain overgrowth during the first years of life in ASD. These studies argue for early malformation of neural circuits in specific higher-order cortices that mediate the social, emotional, language, and communication dysfunctions that are core features of the disorder.

Let us first summarize MRI, DTI, and postmortem evidence in the adolescent or adult brain.

Neuroanatomical Findings in Older Children or Adults with Autism

The overall picture from the postmortem literature on the older child or adult with autism starkly contrasts with what one might expect from early brain overgrowth. It is one of neuron loss, degeneration, inflammation, and reduced size of cortical minicolumns (the vertical organization of neurons in the neocortex). The largest postmortem study of autism using stereological methods for quantifying neuron numbers in 10- to 44-year-old cases found fewer neurons in the amygdala, a structure important in emotion, learning, and memory (Schumann and Amaral, 2006). The cerebellum, a structure that is important for its role in modulating a variety of cognitive and motor functions, has been consistently reported to have reduced numbers of Purkinje neurons although has yet to be confirmed with a quantitative stereological study (Bailey et al., 1998; Kemper and Bauman, 1998; Lee et al., 2002; Vargas et al., 2005). Interestingly, Bailey et al. (1998) observed astrogliosis, a sign of glial activation that may be associated with neuronal degeneration or death, in the cerebellum. Vargas (2005) reported degenerating Purkinje neurons, glial activation, and increases in pro- and anti-inflammatory molecules in the cerebellum. Neurons in the deep cerebellar nuclei, the only pathway exiting the cerebellum, were reported to be abnormally small and pale in adolescent and adult autistic cases (Kemper and Bauman, 1998). In the frontal cortex, which mediates many higher-order functions, Araghi-Niknam and Fatemi (2003) found increases in proapoptotic (pro-cell death) and decreases in antiapoptotic (anti-cell death) molecules in adult autistic cases. Vargas et al. (2005) reported glial activation and pro- and anti-inflammatory molecules in frontal cortex. Studies have also found smaller frontal and temporal cortical minicolumns in older children, adolescents, and adults with autism (Buxhoeveden et al. 2006; Casanova et al., 2002, 2006).

From MRI studies, the most recent data from Hadjikhani et al. (2006) provided a detailed cortical map showing abnormally thin cortices in multiple superior parietal, temporal, and frontal regions in adolescents with autism. Interestingly, these regions include the mirror neuron system, which has been hypothesized to be critical in autism (Hadjikhan et al., 2006; Dapretto et al., 2006; Oberman et al., 2005; Williams et al., 2006). On MRI, the amygdala in autism is reported to be either similar to or smaller than (Nacewicz et al., 2006; Pierce et al., 2004) normal adolescents and adults. The corpus callosum, which carries interhemispheric axons, has been consistently reported to have reduced size in one or another of its subregions (Alexander et al., 2007; Chung et al., 2004; Courchesnes, 2007 neurone). The cerebellar vermis, which may be involved in modulating emotion, arousal, and sensory responsiveness, has been reported to be similar to (Piven et al., 1992) or smaller than (Kaufmann et al., 2003; levitt et al., 1999) typically developing adolescents and adults. It has to be noted, to conclude, that these results were not always consistent and not all MRI studies have found abnormally

thin cortices in mature autistic subjects (Hardan et al., 2006); some have reported that cortical gray matter enlargement may persist into adolescence and adulthood (Hazlett et al., 2006).

Neuroanatomical Findings in Toddlers and Early Childhood

Head Circumference and Brain Overgrowth

During the first years of life, head circumference correlates with brain size in typically developing and children with ASD. It has therefore been used as a retrospective indicator of relative brain size in autism. At birth, head circumference in infants who later go on to develop autism is typically near normal or slightly below the normal average (Courchesne et al., 2003; Dawson et al., 2007; Dementieva et al., 2005; Dissanayake et al., 2006; Gillberg and de Souza, 2002; Hazlett et al., 2005; Lainhart et al., 1997; Mason-Brothers et al., 1990) (Figure 1). However, by 1 or 2 years of age in autism, head circumference (HC) becomes abnormally enlarged. This finding of early overgrowth has now been replicated by many independent research groups (Courchesne et al. (2003) ; Dementieva et al., 2005; Dissanayake et al., 2006; Hazlett et al., 2005; Dawson et al., 2007) (Figure 1). By the time children with autism reach 2–4 years of age, overall MRI brain volume is abnormally enlarged by about 10% relative to typically developing 2- to 4-year-olds (Carper et al., 2002; Courchesne et al., 2001; Hazlett et al., 2005). A meta-analysis of all published MRI brain size data on children, adolescents, and adults through early 2005 showed that the period of greatest brain enlargement in autism is during the toddler years and early childhood (but it is important to note that even at older ages there remains an overall 1%–3% percent greater brain volume in autistic patients) (Figure 1) (Redcay and Courchesne, 2005). Postmortem brain weight data, although not necessarily always a reliable measure, does corroborate conclusions from HC and MRI studies. In the only study to statistically analyze age-related changes in autism brain weights, Redcay and Courchesne (2005) found that brain weight was 15% greater in 3- to 5-year-old male autism cases than male control cases (1451 g versus 1259 g).

Probably a Regional Overgrowth

Research to date suggests that early overgrowth is not ubiquitous across the brain. By 2–4 years of age, some regions and structures display overgrowth while others do not (Carper and Courchesne, 2005; Carper et al., 2002). MRI research on autistic 2- to 4-year-olds implicates the frontal lobes, temporal lobes, and amygdala as sites with the most important enlargement/ overgrowth and the occipital lobe with the least across a number of studies and ages (Figure1) (Carper and Courchesne, 2005; Carper et al., 2002; Hazlett et al., 2005). This regional gradient of abnormal enlargement parallels regions whose cognitive functions may be most impaired versus those most spared. In one study, frontal and temporal sulci are abnormally shifted anterior and/or superior in older children with ASD (Levitt et al., 2003), which would be consistent with a disproportionate increase in frontal and temporal lobes. Together with these regional overgrowths, white matter abnormalities were also observed in DTI experiments in young autitic children. Especially interesting is recent evidence of premature myelination in frontal, but not posterior, white matter regions in very young autistic children (Ben Bashat et al., 2007). A DTI study of adolescents with ASD reported white matter abnormality underlying dorsal and medial prefrontal cortices, superior temporal cortex,

temporoparietal junction, and the corpus callosum (Barnea-Goraly et al., 2004). In an MRI study of older autistic children and preadolescents, cerebral white matter was subdivided into internal and external compartments, and it was reported that the outer radiate portion of white matter, particularly in frontal lobes, was prominently disturbed; it was least deviant in occipital lobes (Herbert et al., 2004). This result suggests that autism might involve abnormal increases in short-distance connectivity, especially in brain regions that mediate higher-order language, cognitive, social, and emotional functions (see also Belmonte et al., 2004; Courchesne et al., 2007; Just et al., 2004).

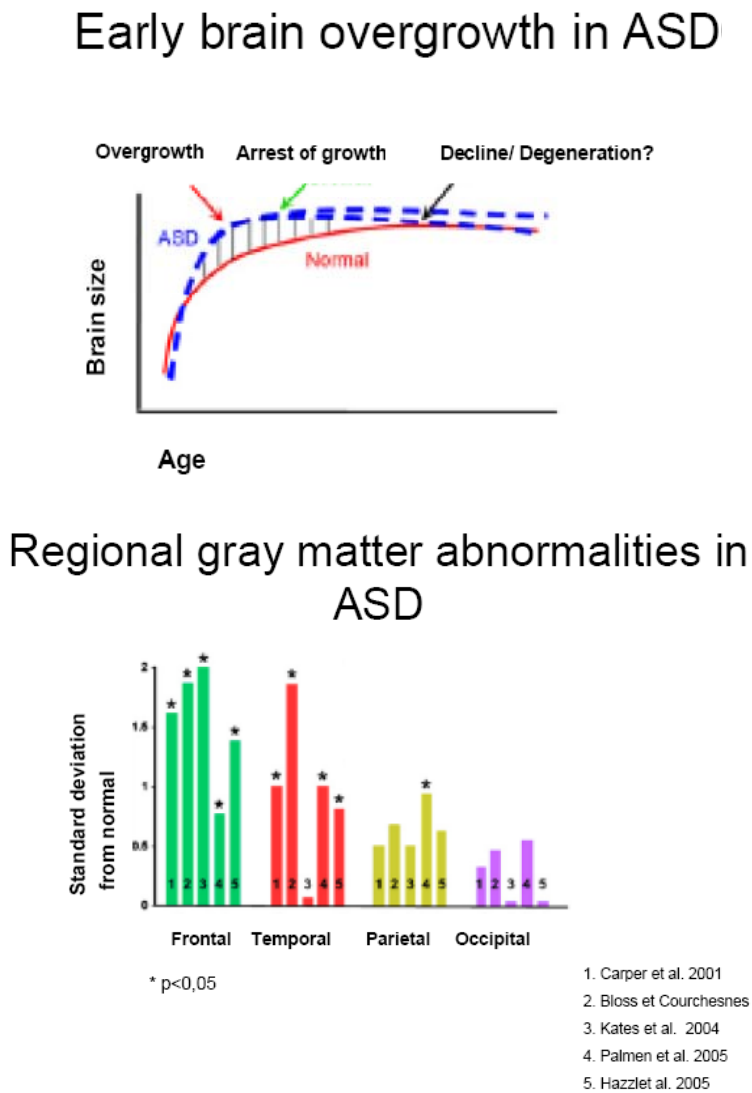

Figure 1. 1a: Early brain overgrowth followed by arrest of growth. Blue lines: ASD; red line: age-matched normal developing infants. In some regions, the arrest of growth is followed by degeneration. 1b: Regional gray matter abnormalities from studies of adolescents and children. Frontal and temporal regions are most profoundly enlarged.

Thus, MRI studies of gray and white volume, cerebral sulci, and cerebral white matter development each point to pronounced frontal and temporal lobe abnormalities. These regions mediate the higher-order social, emotional, cognitive, and language functions. Studies are needed to determine whether these regional increases reflect an abnormally expanded cortical sheet, an abnormally thickened one, or both, as this information would suggest different forms of very early defects in corticogenesis.

A neuron excess in key frontal and temporal cortical regions (Courchesnes et al., 2007) to explain cerebral regional overgrowth in autism, would produce defects in neural patterning and wiring, with exuberant local and short-distance cortical interactions impeding the function of large-scale, longdistance interactions between brain regions. Because large-scale networks underlie socio-emotional and communication functions, such alterations in brain architecture could relate to the early clinical manifestations of autism.

Until now the cellular and molecular bases of pathological overgrowth in the young autistic brain are unknown. As already discussed, in some patients ASD is associated with a neuroectodermosis, like tuberous sclerosis complex, and more rarely neurofibromatosis with *TSC1/TSC2* or *NF1* gene mutation. *PTEN* gene mutations are now well recognized as causing ASD. Interestingly, mutations in *TSC1/TSC2* and *PTEN* genes activate the mTOR/PI3K pathway which is associated with abnormal cellular/synaptic growth rate. It is currently postulated that additional still to dicover genes could also be involved in this pathway and be responsible for some other forms of ASD (Bourgeron, 2009).

Numerous abnormalities could create overgrowth in the young brain: an excess neuron numbers, excess glia, activated and enlarged glia, excess synapse numbers, excess numbers of minicolumns, excessive and premature axonal and/or dendritic growth, excess axon numbers, and excessive and/or premature myelination (see reviews by Bauman and Kemper, 2005; Palmen et al., 2004). Among the more prominent speculations has been that there are increased numbers of minicolumns in autism (Casanova et al., 2006). However, minicolumn numbers have not been stereologically quantified in either the child or adult autistic brain.

Finally, the critical question of what age-related cellular and molecular changes occur between this time of early overgrowth and later maturity 10–20 or more years later has not been studied. Studies to date have been hampered by the low availability and quality of control as well as autistic brain tissue from younger cases.

Having described our current knowledge on some of the early steps /aspects of brain development in autism, what do we know about the early clinical symptoms occurring during the same period in very young children with ASD?

EARLY SIGNS OF AUTISM: EVIDENCE FROM DIFFERENT SOURCES

Although the diagnosis of autism is difficult to make before the age of 2 to 3, there are now numerous reports that describe neurodevelopmental abnormalities that are present during the early months of life. On a methodological point of view our knowledge is based on different sources: 1) parents recollection reports, 2) the design of longitudinal studies in families with a sibling diagnosed with ASD, 3) the examination of early medical and

behavioral characteristics of NICU children later diagnosed with ASD and more recently 4) prospective community-based studies.

1) Parents Recollection Reports

Retrospective videotape analyses and parental report studies provide valuable evidence that symptoms of ASDs are present during infancy. Indeed 50% of parents of children with an ASD report having concerns before 12 months of age, with many more reporting recognition of abnormalities between 12 and 24 months.

Parents recall abnormalities dating back to the first year of life, including poor eye contact, impaired either lack of response to the parents voices or attempts to play and interact (Gillberg et al., 1990, de Gicomo and Fombonne,1998; Hoshino et al.,1987). Abnormal described as extremes of temperament and behavior ranging from strang passivity tomarked irritability were also described (Gillberg 1990,). Using home videos during the first year of life several authors reported reduced social interaction, absence of social smiling, decreased orienting to faces in children later diagnosed with ASD compared to normally developing children (Mars et al., 1998; Maestro et al., 1999; Zakian et al., 2000). Reports concerning lack of spontaneous imitation, and abnormal muscle tone, posture and movements patterns were reported (Teitelbaum et al., 1998)

Although these initial retrospective studies rised important insights concerning our knowledge on early signs of autism, they were subjects to recall biases (for example exact timing of behavioral manifestations) and lacked matched appropriate control. We were also missing reports form parents from non autistic children regarding non autistic developmental disorders to evaluate the specificity of these early abnormal behavioural observations. The developmental sequence of these features was also poorly reported.

2) Longitudinal Studies in Families with a Sibling Diagnosed with an ASD

In order to avoid the recall biases from parents recollection reports, some teams designed longitudinal studies documenting development trajectories in infants diagnosed later with ASD. These studies were initiated in infants who have an older sibling diagnosed with an ASD. Recurrent risk in autism is around 9% (Szatmari et al., 1998). In the context of prospective studies, a systematicc and very early early follow up of children offers the advantage to gather enough patients in a reasonable time period to conduct statistaical and group analysis.

Some authors could recruit and and follow patients large samples infant siblings of older infants with an ASD diagnosis (Zwaigenbaum et al., 2005; Klin et al., 2008; Rogers 2009). Parrallelly they also followed low-risk infants, some of them till the age of 24 months. A series of observation grids/tools specificaly adpated for infant were used to gather information on early AD symtoms.

Evaluation Tools being Used during Longitudinal Studies

- *Infant behavioural indicators of autism:* to aid systematic data collection on early signs of autism, the *Autism Observation Scale for Infants* was developed (*AOSI*). Authors used 18 specific risk markers for autism hypothesized from retrospective studies, videotape analyses, and case reports to develop a standardized procedure for detecting each of these markers within a brief observational assessment. Infants were engaged in semi-structured play. Various target behaviors were assessed, including visual tracking and attentional disengagement, coordination of eye gaze and action, imitation, affective responses, early social-communicative behaviors, and sensory-motor development. These behaviors were rated on a scale from 0 (normal function) to 3 (highest deviation).
- *Visual orienting task:* A lot of interest has been yielded on the early visual behaviour of autisitic children. Visual attention in particular visual orientation appears to bear autistic specific components. It has been studied using diferent stet up:
- Zweigenbaum et al.elaborated a test during which infants by the age of 3 to 4 months are a engaged on a central visual fixation stimulus and submitted to a second visual stimulus presented peripherally on either the left or the right side. Latency to begin an eye movement to the peripheral stimulus is systematically measured. When performing this two options exist that seem particularly intersting to study visual attention and orientation in autistic children: either the central stimulus remains ‘on’ or is turned ‘off’ during presentation of the peripheral stimulus. This provides independent measures of the disengagement (central ‘on’) and shift (central ‘off’) operations of visual attention (Hood et al., 1993).
- *Infant temperament:* Temperament is a useful construct in understanding early differences in infants at high risk for autism. Early abnormalities in attention, behavioral reactivity, emotion regulation and activity level may compromise both the quality and quantity of early social interaction, and thus the prerequisite experiential input for developing neural systems critical to later social-communicative competencies. Temperament was measured in these studies using the Behavior Questionnaire (IBQ ; Rothbart et al., 1981) at 6 and 12 months and the Todler behavior Assessment Questionnaire at 24 months (TBAQ ; Goldsmith et al., 1996). IBQ measures different dimensions of temperament: activity level, smiling and laughing, fear, distress to limitations, soothability and duration of orienting. The inventory has been validated for use with infants aged 3–12 months, and has good test-retest reliability (Goldsmith and Rothbart, 1991). The TBAQ subscales cover activity level, expression of pleasure, social fearfulness, anger proneness, and interest/persistence.
- *Early language skills:* Some language tests scales are specifically developed for infants like the Mullen scales of early leraning or the Mac Arthur Communicative Development inventories-Words and gestures (CDI-WG) (Feldman et al., 2000). The CDI –WG provides standardized information about early use of gestures, verbal imitation and words outside of the clinic setting and was particularly adapted for describing earlycommunication skills in infants. It allowed to assess early

development trajectories, and to better understand the developmental context for the expression of early markers of autism.

What Are the Early Features of Autism Detected with Longitudinal Prospective Studies ?

From these longitudibal studies, atypical neurodevelopment features were observed in siblings that were later diagnosed with autism and that could be distinguished from other siblings and low-risk controls. They comprised:

1. Several specific behavioral markers, including atypicalities in eye contact, visual tracking, disengagement of visual attention, orienting to name, imitation, social smiling, reactivity, social interest and affect, and sensory-oriented behaviors were observed. A characteristic pattern of early temperament, with marked passivity and decreased activity level at 6 months, followed by extreme distress reactions, a tendency to fixate on particular objects in the environment, and decreased expression of positive affect by 12 months. Detailing these results, based on parent ratings on a temperament questionnaire, children with autism were observed at 6 months to be somewhat passive, with relatively few initiations and less responsiveness to efforts to engage their attention. Informal observations at home and in the research clinic also suggest that these 6-month-olds vocalize less than other infants. At 12 months of age, the AOSI found that eye contact was poor and that there were marked abnormalities in visual attention (including poor visual tracking), in social responses (reduced social smiling, social interest and expression of positive affect) and in use of play materials (lack of imitation and poor coordination of eye gaze and action). Sensory-oriented behaviors in 12-month-olds often involved the use of play materials in stereotyped, self-stimulatory ways (e.g., the child dangles a string of beads and waves them in front of his/her eyes). Data from the temperament questionnaire showed increasing irritability, intense responses to sensory input (often associated with distress) and excessive visual fixation to non-social aspects of the visual environment combined with reduced responses to social approaches from others.
2. A prolonged latency to disengage visual attention: siblings who later develop autism were also observed to have atypical development of visual attention in the first year of life. Specifically, thee are early evidence of difficulties disengaging from two competing stimuli. Interestingly, this problem may not distinguish infant siblings from controls at 6 months, nor is performance at 6 months predictive of later diagnosis. However, between 6 and 12 months, infants who developed later autism, a longer latency to disengage attention was characteristically observed compared to normal children. In contrast, typically developing infants showed clear decreases in latencies to disengage with age (Hood and Atkinson, 1993).
3. A delayed expressive and receptive language: delays in verbal and pre-verbal expressive skills and early language comprehension were also evident on standardized measures, and were corroborated by parent reports with initial impressions that infants later diagnosed with autism have relatively few vocalizations overall.

What Are the Limitations of these Early Studies?

Firstly, diagnostic assessments were limited to 24-month follow-up data in the vast majority of patients and to 36 months only for some of them, the last being the age when ASD can consistently be confirmed. A second limitation of these studies was the the lack, in the experimental design, of a comparison group of infants at risk for developmental disabilities other than ASD. Although the infant sibling group most likely included children with language delays, other developmental disorders were probably not well represented among the siblings and low-risk controls. It is important both for clinical practice and for understanding neurodevelopment to examine whether early behavioral indicators are specific to autism. Early autism evaluation scales should for example be evaluated in populations of low birth weight who have a high rate of non-autistic language, motor, and general developmental delays (Schendel et al., 2008).

Moreover, these early behavioral findings were not yet correlated with measures of early brain development. Currently there are very few MRI data in individuals with autism prior to age 2 years, which appears to be a critical period with respect to accelerated growth and premature connectivity in brain development (Courchesne et al., 2003; Volkmar et al., 2004). Very few children with autism are diagnosed prior to age 2. There are important logistic and ethical constraints to imaging infants (e.g., the need for deep sedation), as well as measurement issues that would need to be addressed prior to undertaking such studies (e.g., establishing methods to reliably differentiate white and grey matter in this age group). Moreover there are ethical limitations in proposing a systematic MRI evaluation in young infants followed in the context of seebling studies, knowing that a large proportion of these infants won't develop a clinical illness. One may consider the feasibility study of using MRI without sedation to assess brain development serially in very igh-risk infant siblings. Some eforts are currently undertaken to analyze head circumference data in sibling sample, in order to assess whether accelerated head growth as reported by Courchesne et al. (2003) is associated with particular behavioral features during the first year of life.

Another important limitation was that children with autism who have an affected sibling may not be representative of all children with the disorder, so behavioral findings from infant siblings may not fully generalize to other autistic samples. Typically, siblings have grown up in an environment already affected by ASDs which biasses the evaluation for example with the use of parents reports. Thus, numerous factors need to be considered as possible influences contributing to developmental differences, including early symptom recognition, intervention, affected parenting styles because of exposure to intervention techniques, and parental stress (Zwaigenbaum et al., 2005). These early studies emphasized the need for the set up of prospective community based studies to study the incidence and early characteristics of children suffering from ASD.

Finally, having a better knowledge of early features at play in ASD, what developing neural systems are implicated in 6- and 12-month-old infants later diagnosed with autism? Morever, having identified abnormalities in several neurodevelopmental domains, both social and non-social, how are these abnormalities interrelated? and are there primary impairments that initiate the developmental cascade towards the broader phenotype of autism?

For example, although a decrease in social orienting may be most characteristic of autism (Dawson et al., 1998), previous studies of preschoolers with autism have suggested a more general impairment in orienting to non-social stimuli as well (Dawson et al., 1998; Townsend et al., 2001). Mundy (2003) proposed that a general disturbance in visual orienting in autism

may result from impairment in a complex axis of cerebellar, parietal and frontal functions involved in the development and control of attention (Bryson et al., 1990; Carper and Courchesne, 2000; Townsend et al., 2001), and that there may be a complex interplay between the dorsal medial-frontal cortex/anterior cingulate complex, orbitofrontal and amygdala functions, and cerebellar input in the development of social and non-social attention regulation in affected individuals. Whether this model can be applied to atypical orienting patterns in early infancy is uncertain. There may be neural systems that are more operative in visual orienting during infancy than during the preschool years. For example, the observed decrease in attentional flexibility by 12 months of age in the sibling group may correlate with maturational processes of the prefrontal cortex (Johnson et al., 1991), although this remains speculative at this point. Atypical patterns of cortical activation involving the prefrontal cortex have been observed in preschool children with autism (Dawson et al., 2001; Muller et al., 2001).

3) Behavioral Characteristics of NICU Children Later Calissifed as Suffering from ASD

As already described in the autism etiological factors section, several studies identified a higher prevalence of ASD in association with obstetric and neonatal factors that result in NICU admission. When controlling for other developmental disorders a twofold increased ASD risk was identified as a result of lower birth weight and gestational age (GA; Chendel and Bhasin). None of these early studies, however, examined propectively within NICU cohorts (that are currently followed very regulrarly on a developmental point of view) very early behavioral patterns that may more likely be seen in children who later receive a diagnosis of ASD.

Karmel et al. (2010) performed a retrospective study that examined early developmental differences between infants who later received a diagnosis of ASD and matched control subjects from their sample of NICU patients. Within their cohort of NICU graduates, authors conducted serial behavioral studies from birth on a large number of NICU graduates at high medical risk as part of a prospective project to determine how brain organization interacts with autoregulatory processes over development. They made early measures involving arousal, attention, and motor regulation that predicted deficits in a number of domains. More precisely, their report included:

(1) Neurobehavioral characteristics during the neonatal period using the Rapid Neonatal Neurobehavioral Assessment (RNNA), a criterion-referenced procedure, performed at hospital discharge and 1 month postterm age (PTA) (Gardner et al., 2001). On the basis of normal versus abnormal decisions for individual items, it yields scores for categories of sensory and motor behaviors that measure visual and auditory attention and symmetry; head/neck control; trunk tone; extremity movement, tone, and symmetry; state control; feeding; and jitteriness at each age tested. It also yields a composite score that reflects number and/or severity of problems at each age. It shows a positive relation between severity of CNS injury detected in the neonatal period and the number of abnormalities identified

(2) Regulation of visual attention by states of arousal (Arousal-Modulated Attention (AModA) procedure) at hospital discharge, 1 and 4 months PTA. The AModA procedure measures an infant's ability to modulate his or her visual attention to variations in stimulation

when tested at higher and lower levels of exogenous or endogenous arousal. A visual stimulus preference is established when the infant looks longer to 1 stimulus than another when the stimuli are ordered along some systematic environmental change (eg, frequency, intensity, complexity). Specifically, infants view all possible pairs of stimuli going on and off at 1, 3, or 8 Hz for 6 trials of 15 seconds in each of 3 arousal condiiions. Healthy term and preterm neonates are excellent modulators and show greater attention to more stimulating events when less aroused (after feeding) and to less stimulating events when more aroused (before feeding or with added stimulation before each trial). Neonates with acute CNS injury are poor modulators and tend to prefer less stimulation even when less aroused. By 4 months' PTA, transitions occur such that the AModA effect no longer is apparent and more stimulation tends to be preferred (although attenuated) in all conditions irrespective of arousal. Moreover, typically, there is normalization such that AModA differences no longer are evident from CNS injury. As with RNNA, AModA has both concurrent (associated with CNS injury) and pre- dictive validity to later functioning (Gardner et al., 2003).

(3) Individual developmental trajectories from standardized assessments across age (Bayley Scales of I nfant Development, Second Edition [BSID-I I].

Using this extensive follow up tool, Karmel et al. (2010) reported that ASD neonates showed persistent neurobehavioral abnormalities and higher incidences of visual asymmetric visual tracking and arm tone deficits as measured by the rapid neonatal neurobehavioral assessment (RNNA). At 4 months, using the Arousal Modulated Attention scale (AmodA) children with ASD specifically showed a continued visual preference for higher amounts of stimulation than did control children, behaving more like newborns. Continued higher attention to more stimulating events in ASD patients at 4 months was interpreted as representing a lack of transition to more mature levels past the neonatal period, providing evidence for early atypical development, including the visual system. Similar conclusions have been reached regarding the importance of abnormally developed/organized neurally mediated visual tracking and attention systems and the importance of abnormal transitions in development of pathways that involve visual processing (Brenner et al., 2007; McCleery et al., 2007). In infants, atypical attention to stimuli and visual regard, impaired disengagement ability, and lack of typical developmental transitions in the visual system have been proposed to result in disruption of the normal bias toward social events, especially those imbedded in complex socially relevant stimuli. Unusual visual function also has been reported in older infants and children with ASD. (Mundy et al., 2009; Jones et al., 2008). Klin and associates reported that absent preferential looking to the eyes of an approaching adult in 2-year-old toddlers with ASD is related to increased level of social disability and impaired recognition of biological motion as early as 15 months (Klin et al., 2008). That group also recently reported evidence that 20month-old toddlers with ASD were still bound by physical audiovisual synchrony, whereas control subjects without ASD ignored this synchrony and attended to more socially relevant stimuli. Evidence of atypical visual system development also could be reflected in the hypersensitive visual acuity reported for high-functioning adults with ASD/Asperger syndrome (Klin et al. 2009).

(4) Prospective comunity based studies looking at early signs of ASDs.

Little research has been conducted on the prospective identification of ASD children in the context of a community based setting. For the reasons we mentioned before it is however highly warranted. They typically use a Level 1 screening tool at a single age in a community health service or general medical practice setting (Barbaro and Dissanayake, 2009).

The first studies conducted in such a context concerned the use of the Checklist for Autism in Toddlers (CHAT) at 18 months and the Early Screening of Autistic Traits Questionnaire (ESAT) at 14/15 months (Baird et al., 2000; Swinkels et al., 2006). Both type of instruments showed an excellent specificity in detecting ASD but their sensitivity was poor missing for example more than 60% of children diagnosed with an ASD at 7 years in the study of Baron Cohen. Administration at a single age is considered as the major cause for lack of satisfying sepcificity or sensitivity of these evaluation tools. A modified CHAT version (M-CHAT) and Infant-Toddler Checklist (ITC) were then developed and tested in smaller communities to improve detection sensitivity, concerning children between 9 and 24 months, but identified many children without ASDs (Kleinman et al. 2008). The ITC was unnable to distinguish children with ASD from those with general developmental or language delays. The early diagnosis is still a challenge because of the symptoms variability expressed differently at different chronological and mental ages in ASD.

Josephine Barbero et al. published a recent study (2010) in which the training of primary health care professionals in the state of Victoria (Autralia), such as maternal child health nurses was used to undertake a developmental surveillance of young children to identify those showing early signs of ASDs. Knowing that 98% of Victorian babies attended on a regular basis MCH (Maternal and Child Health) Centers soon after birth, authors chosed this institution to develop their detection program.

The aim of this prospective study using a very large community-based sample of 22,168 children monitored through 184 Maternal and Child Health Centers in Metropolitan Melbourne was to show whether routine and repeated monitoring of social and communication skills could be use to identify children with an ASD (Barbaro et al., 2010).

On a methodological poit of view, nurses from each center received a 2 and ½ hour training to practive skilled observations during their routine consultations in children at 8, 12, 18 and 24 months of age. Based on previous literature on early signs of autism, authors set up a list of key ASD signs specific for each particular age. The more relevant key items were at *8 months*, eye contact, turning to name call; at *12 months*, the use of pointing, the use of gestures; at *18 months* eye contact, pointing, gestures and pretend to play and finally at *24 months*, imitation, pointing, gestures, pretend to play and social communication. When children were detected as at "risk" they were referred from the MCH to a centralized center to follow a more specialized assessment depending again on age:

- At 12 and 18 months: the Mullen Scales of Early Learning, the Early Social and Communication scales, the CHAT-23 (18 months only), the Infant Toddlers check-list, The Early Development Interview.
- At 24 months: the Mullen Scale of Early Learning, the Autism Diagnostic Observation Schedule, the Autism Diagnostic Interview-Revised.

Authors were able to accurately identify children at risk for ASDs between 12 and 24 months with a overall positive predictive value was of 81% (90 % at 12 months, 79% at 18 months, 81 % at 24 months).

The success of this prospective study was explained by the fact that authors administrated ASD detection tool on several occasions at different ages rather than at one age as previously reported. It was also explained by the quality of the training: nurses were trained to readminister failed items on several consecutive occasions and to identify atypical behaviour.

This study showed also that primary health care professionals are able to identify and refer high risk chidren.

CONCLUSION

ASDs form a group of developmental disorders resulting in significant life-long disabilities. Early management is important. To reduce the eventual burden of disability. It is therefore crucial, to make the diagnosis early or at least to identify infants with a markedly increased risk of developing ASDs in order to offer early intervention services.

Similar efforts proved successful in another group of developmental disorders with difficult early diagnosis and life-long disability, namely cerebral palsy. Despite the high prevalence of neuroimaging abnormalities in the latter, approach to early diagnosis relies essentially on clinical features.

In this chapter we reviewed the recent advances concerning the detection of early clinical signs (before the age of one) that may be strongly associated with the developement of ASDs. Retrospective videotape analysis, parental report and longitudinal follow-up of ASD siblings taught us to recognize the presence of early semiology. The full clinical picture includes atypicalities in eye contact, visual tracking, disengagement of visual attention, orienting to name, imitation, social smiling, reactivity, social interest and affect, and sensory-oriented behaviors. A characteristic pattern of early temperament, with marked passivity and decreased activity level at 6 months, followed by extreme distress reactions, a tendency to fixate on particular objects in the environment, and decreased expression of positive affect by 12 months can be recognized. A prolonged latency to disengage visual attention from two competing stimuli was observed and a delay in expressive and receptive communication skills are also observed during the first year of life. Typically motor and behavioral stereotypies, though prevalent, are often not observed before the age of 2. Recent findings about the very particular brain structure development in children with ASD (mostly during the first months of life) probably explain some of the early typical clinical features.

The accurate and repeated observation of these early signs should help clinicians and other professionals to rise « flags ». However there remains little research into prospective identification of ASD using these early signs. Clinical experience suggests that making a diagnosis before the age of three years carries a significant risk of false positive diagnosis. There are currently no tools with sufficient specificity and sensitivity available for universal use. The CHAT at 18 months, for example, has an excellent (98 %) specificity but its sensitivity is only 38%, However, the recent report of Barbero demonstrated that well conducted detection programs organized in first line Maternal and Child Centers are able to identify children at risk for ASD with very high positive predictive values. This report however highlighted the necessity to train and educate all primary health care professionals who see children in their first 2 years of live, and to promote a general surveillance of early signs of ASD on repeated monitoring.

Currently, the setup of early ASD detection programs raises important ethical questions relating to availibility of adequate resources and validity of rehabilitation programs for ASD children younger than two years. As recently documented in the US, many early Intervention programs may not have the capability to address the expected increase in demand for ASD

services. Early intervention programs will likely need enhanced resources to provide all children with suspected ASD with appropriate evaluations and services (Wise, 2010).

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Chapter 4

DIFFERENTIAL EFFECTS OF ACUTE SEVERE HYPOXIA AND CHRONIC SUBLETHAL HYPOXIA ON THE NEONATAL BRAINSTEM

Ze Dong Jiang**[*] and Andrew R. Wilkinson
Department of Paediatrics, University of Oxford, John Radcliffe Hospital, Oxford, United Kingdom

ABSTRACT

Perinatal asphyxia and neonatal chronic lung disease (CLD) are two major problems in newborn infants, often leading to neurodevelopmental deficits or disabilities later in life. Both problems are associated with hypoxia, but the nature of the hypoxia in the two problems is different. The hypoxia after perinatal asphyxia is often acute, severe or lethal, and associated with ischaemia of the brain. In contrast, the hypoxia in neonatal CLD is chronic or prolonged and sublethal. Such differences may exert differential effects on the functional integrity and development of the neonatal brain, leading to different neuropathological changes and neurodevelopmental outcomes.

In recent years, some investigators have studied the functional integrity of the neonatal auditory brainstem in infants after perinatal asphyxia and neonatal CLD and have found differences in the effects of acute severe hypoxia and chronic sublethal hypoxia on the neonatal brainstem. In infants after perinatal asphyxia, neural conduction and synaptic function are impaired in both peripheral and central regions of the brainstem, although the impairment is slightly more severe in the more central than the more peripheral regions. In infants with neonatal CLD, however, neural conduction and synaptic function are impaired predominantly in the more central regions of the brainstem, whereas the more peripheral regions are relatively intact. These findings indicate that perinatal asphyxia affects both the central and peripheral regions of the brainstem, while neonatal CLD affects predominantly the central regions, without appreciable effect on the peripheral regions. This difference may be, at least partly, related to the different nature of hypoxia in the two clinical problems. These findings shed light on the pathophysiology underlying neurological impairment and

[*] Correspondence address: Department of Paediatrics, John Radcliffe Hospital, Headington, Oxford OX3 9DU, UK. E-mail address: zedong.jiang@paediatrics.ox.ac.uk

developmental deficits in neonatal CLD, related to chronic sublethal hypoxia, and after perinatal asphyxia, related to acute lethal hypoxia and the associated ischemia. The knowledge obtained from these studies also provides valuable information for studying and implementing neuroprotective interventions or therapies for the two neonatal problems. The interventions should target more central regions of the brain for infants with CLD, but target both peripheral and central regions of the brain for infants after perinatal asphyxia.

Recent studies have also found that in infants with perinatal asphyxia, the electrophysiological activity in the neonatal brainstem is significantly depressed, suggesting major neuronal injury and/or neuronal death after severe hypoxia-ischemia. For these infants there is a need to intervene with radical neuroprotective measures (e.g. brain cooling) as early as possible to reduce further neuronal injury and death and rescue severely injured neurons. In infants with CLD, however, there was no noticeable depression of electrophysiological activity in the neonatal brainstem, suggesting no severe neuronal injury and/or neuronal death. It appears that for infants with CLD there is no need to implement radical treatments, and well regulated supplemental oxygen may remain the most valuable therapy, along with other therapeutic adjuncts.

INTRODUCTION

In newborn infants, asphyxia and chronic lung disease (CLD) are major perinatal problems, often leading to neurodevelopmental deficits or disabilities later in life. Asphyxia occurring during the perinatal period is the most important cause of acquired brain damage in infants with subsequent life-long sequelae (Levene and Evans, 2005; Volpe, 2001). Many of the survivors have various degrees of learning difficulties, language deficits, attention deficit, hyperactivity disorders and cerebral palsy. Neonatal CLD is one of the most common long-term complications in very low birthweight or very preterm infants (Greenough and Milner, 2005; Jeng et al., 2008). Although the primary pathology of CLD is related to lungs, approximately half of severe CLD survivors have neurodevelopmental deficits, which is a major concern of CLD survivors (Böhm and Katz-Salamon, 2003; Jeng et al., 2008; Karemaker et al., 2006; Katz-Salamon et al., 2000). Therefore, neonatal CLD and perinatal asphyxia are two major problems that have attracted considerable clinical attention.

The neonatal brain, particularly the cortex, is well known to be sensitive to arterial blood oxygen tension and hypoxia. Neonatal CLD and perinatal asphyxia are both associated with hypoxia (Böhm and Katz-Salamon, 2003; Greenough and Milner, 2005; Jeng et al., 2008; Karemaker et al., 2006; Levene and Evans, 2005; Volpe, 2001). However, the nature of hypoxia in the two clinical problems is different. The hypoxia in CLD is chronic or prolonged, and sublethal. In contrast, the hypoxia in perinatal asphyxia is often acute, severe or lethal, and associated with ischaemia of the brain (Johnston et al., 2001; Levene and Evans, 2005; Volpe, 2001). Therefore, perinatal asphyxia and neonatal CLD may exert some different effects on the neonatal brain, resulting in differential neuropathological changes and neurodevelopmental outcome. Understanding the mechanisms is important for studying and implementing neuroprotective interventions or therapies for infants with the two different clinical problems (Barks, 2008; Glass and Ferriero, 2007; Tin and Wiswell, 2008).

The brainstem auditory evoked response (BAER) reflects electrophysiological activity of large numbers of neurons in the auditory brainstem in response to acoustic stimulation. As a non-invasive objective test, the BAER has been an important tool to study the functional

integrity and maturation of the neonatal, specifically auditory, brainstem and detect neural abnormalities in infants with various perinatal problems (Chiappa, 1991; Jiang, 2008,2010; Musiek et al., 2007; Wilkinson and Jiang, 2006). The BAER is sensitive to arterial blood oxygen tension and hypoxia (Inagaki et al., 1997; Jiang et al., 2005b,2006C; Sohmer et al., 1986). It has been used to assess the functional integrity of the auditory pathway and the brainstem in infants after hypoxia-ischemia (Hecox et al., 1981; Jiang, 1995,1998; Jiang and Tierney 1996; Jiang et al., 2001,2004; Karmel et al., 1988; Kileney et al., 1980). Nevertheless, there are some limitations in conventional BAER (i.e. the BAER recorded using conventional average technique) to detect neuropathology that affects the auditory brainstem. False-negative results are not uncommon.

Increase in the repetition rate of stimuli that elicit the BAER could enhance the detection of some neuropathology that affects the brainstem auditory pathway (Wilkinson and Jiang, 2006). However, in conventional evoked potential instruments (or averagers) the increase is limited by the need to prevent responses from overlapping one another. More recently, a relative new technique - the maximum length sequence (MLS) has been used to study the BAER and middle latency auditory response (Bell et al., 2001,2002,2006; Eysholdt and Schreiner, 1982; Jirsa, 2001; Jiang, 2008; Jiang et al., 2000; Lasky, 1997; Lasky et al., 1998; Lina-Granada et al., 1994; Musiek and Lee, 1997; Musiek et al., 2007; Picton et al., 1992). Unlike the uniformly spaced stimuli used in conventional BAER testing, the MLS uses patterned stimulus presentation to elicit evoked potentials. This relatively new technique permits the overlapping of responses to successive stimuli, and allows presentation of stimuli at much higher rates than is possible with conventional methods. The stimuli consist of distinct pulses of uniform polarity and amplitude occurring at pseudorandom time intervals. Each pulse sequence is actually a series of pulses. The nature of the stimuli and the newly developed processing technique make it unnecessary to wait for the response of each pulse to be completed before application of a new pulse. Thus, pulses can be delivered at maximal rates of up to 1000/sec or even higher. The higher rates provide a much stronger physiological/temporal challenge to auditory neurons, and permit a more-exhaustive sampling of physiological recovery or "fatigue" than is possible with conventional stimulation. Such a stimulus ‘stress’ provides a potential to improve the detection of some neuropathology that may not be detected by presenting less stressful stimuli (i.e. low-rate stimulation) using conventional averaging techniques.

With conventional BAER, we studied functional status of the neonatal auditory brainstem in infants with neonatal CLD (Jiang et al., 2006a,2007b), and infants after perinatal asphyxia (Jiang et al., 2001,2004,2006b). We have also studied the two clinical problems using MLS BAER (Jiang, 2008,2010; Jiang et al., 2000,2003,2009b,c; Wilkinson et al., 2007). More recently, in order to identify any differences in the functional integrity of the neonatal brainstem between the infants with neonatal CLD and those with perinatal asphyxia, we have compared MLS BAER results between the two major perinatal problems (Jiang et al., 2009a,2010). Detailed analysis was carried out, respectively, for MLS BAER wave latencies and interpeak intervals, reflecting neural conduction in the auditory brainstem, and for MLS BAER wave amplitudes, reflecting brainstem auditory electrophysiology, mainly neuronal function of the auditory brainstem. These studies have exposed some very interesting findings, suggesting that there are differential pathophysiological changes in the brainstem between neonatal CLD and perinatal asphyxia, which may have important clinical implications.

DIFFERENCES IN IMPAIRED BRAINSTEM CONDUCTION BETWEEN ACUTE SEVERE HYPOXIA AND CHRONIC SUBLETHAL HYPOXIA

In both conventional BAER and MLS BAER, the major variables that reflect the functional integrity of the auditory brainstem are wave V latency and interpeak intervals, particularly I-V interval (Wilkinson and Jiang-, 2006). The typical and major abnormality in various clinical problems is an increase in I–V interpeak interval, suggesting impaired nerve conduction and synaptic transmission in the auditory brainstem (Chiappa, 1990; Jiang, 2008; Jiang et al., 2004; Wilkinson and Jiang, 2006). Such abnormalities are often more evident at higher than at lower repetition rates of click stimuli. This is particularly obvious in MLS BAER (Jiang, 2008; Jiang et al., 2005a; Wilkinson and Jiang, 2006). The I–V interval, so-called brainstem conduction time, is the most commonly used BAER variable reflecting functional status, specifically neural conduction, of the auditory brainstem (Chiappa, 1990; Jiang, 2008; Wilkinson and Jiang, 2006). This interval is comprised of two sub-components — the early components I–III interval and the later component III–V interval. The two smaller intervals reflect functional status of the more peripheral and the more central regions, respectively, of the brainstem (Jiang, 2008,2010; Jiang et al., 2009d). Our previous BAER studies have shown that in some neuropathology the I–III and III–V intervals and, particularly, their ratio (i.e. III–V/I–III interval ratio that reflects their relative change) can uncover some abnormalities that cannot be shown by examining the I–V interval only (Jiang, 2008; Jiang et al., 2002,2009d).

In recent years, we examined wave latencies and interpeak intervals in MLS BAER in infants with neonatal CLD or after perinatal asphyxia and compared the results between the two clinical problems (Jiang et al., 2010). Particular attention was paid to the analysis of I–III and III–V intervals and III–V/I–III interval ratio to detect any differences between neonatal CLD and perinatal asphyxia in neural conduction of the more central (III–V interval) and the more peripheral regions (I–III interval) of the auditory brainstem. These analyses allowed us to gain some new insights into the effects of the two conditions on the functional integrity, related to neural conduction and synaptic transmission, of the neonatal auditory brainstem. We recruited 117 term newborn infants after perinatal asphyxia. They had clinical signs of hypoxic–ischaemic encephalopathy (hypotonia with reduced or no spontaneous movements, increased threshold for primitive reflexes, lethargy or coma, absence or very weak suck and requirement of tube feeds, or seizures), other signs of hypoxia (e.g. frequent depression and failure of breathing spontaneously at birth), and depression of Apgar score ($\leq$ 6 at 5 min) (Levene and Evans, 2005; Volpe, 2001). These infants also had meconium staining of the amniotic fluid and/or umbilical cord blood pH < 7.10. We also recruited 43 very preterm Infants with CLD, who met the following criteria of CLD: requirement for supplementary oxygen or ventilatory support beyond 36 weeks of postconceptional age to maintain PaO2 >50 mm Hg, clinical signs of chronic lung respiratory disease, and radiographic evidence of CLD (persistent strands of density in both lungs). None had any other major perinatal complications or problems that may affect the central nervous system. All were studied at term date (i.e. 38–42 weeks postconceptional age).

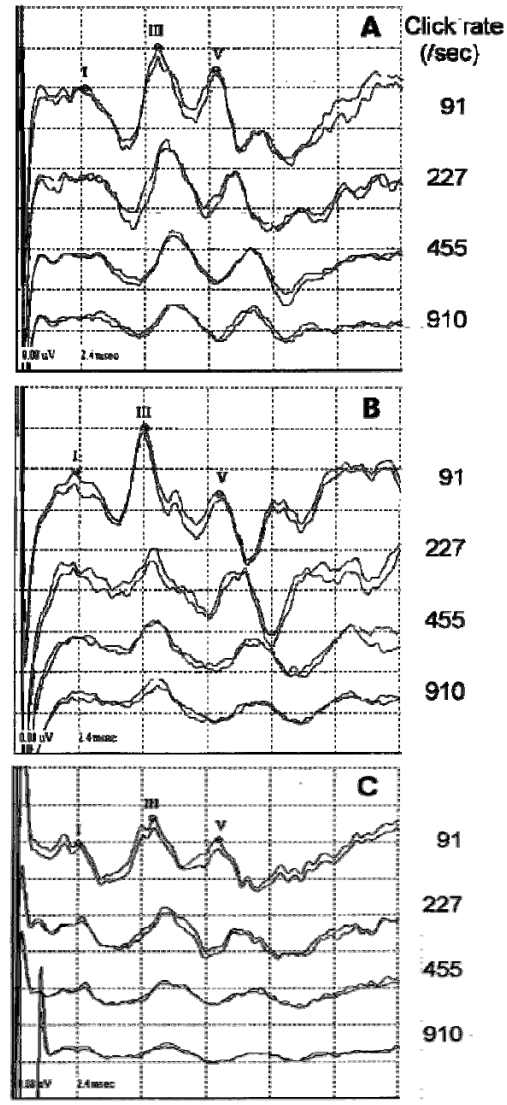

Figure 1. Sample recordings of MLS BAER, recorded with click intensity ≥ 40 dB above BAER threshold at term age, from a normal term infant (A), a very preterm infant with neonatal CLD (B), and an term infant after perinatal asphyxia (C). Compared to that in normal term infant, III–V interval is increased markedly in the infant with CLD, and moderately in the infant after asphyxia. I–III interval in the infant with CLD is similar to that in the normal infant, whereas the interval in the infant after asphyxia is moderately increased. As a result, III–V/I–III interval ratio is increased significantly in the infants with CLD. These differences are more significant at higher (e.g. 455/s) than at lower click rates (e.g. 91/s). In addition, the infant with CLD does not show any major amplitude reduction for all MLS BAER waves, but the infant after asphyxia shows a major amplitude reduction, particularly for wave V and at very high rates (455 and 910/s).

The latencies of waves I, III, and V were measured. Interpeak intervals of I–V, I–III and III–V, and III–V/I–III interval ratio were then calculated. Figure 1 shows sample recordings of MLS BAER made from each of the healthy term infants (as term controls), infants with neonatal CLD, and infant after perinatal asphyxia.

Differences in Brainstem Neural Conduction between CLD and Asphyxia

Findings in MLS BAER wave Latencies and Intervals

Compared to the normal controls, wave I latency in the infants with neonatal CLD was slightly longer at all click rates 21–910/s, but without any statistical significance. The latency in the infants after perinatal asphyxia was similar to that in the controls at all click rates. When comparison was made between the two study groups of infants, wave I latency was slightly longer in the infants with CLD than in the infants after asphyxia, though this did not reach statistical significance. Wave III latency in the infants with CLD was slightly longer than in the normal controls at all click rates between 21 and 910/s. However, the latency in the infants after asphyxia was significantly longer than in the controls at 227–910/s clicks. The latency was similar in the two study groups at lower click rates, and tended to be longer at higher rates in the infants after asphyxia than in the infants with CLD. In contrast to wave I and III latencies, the major wave latency in the BAER — wave V latency was significantly longer than in the controls for both the infants with CLD and those after asphyxia. This occurred at all click rates 21–910/s (Figure 2), though was more significant at higher than at lower click rates. The extent of prolongation in the latency was similar in the CLD infant and the infants after perinatal asphyxia at all rates, without any significant difference between the two study groups (Figure 2).

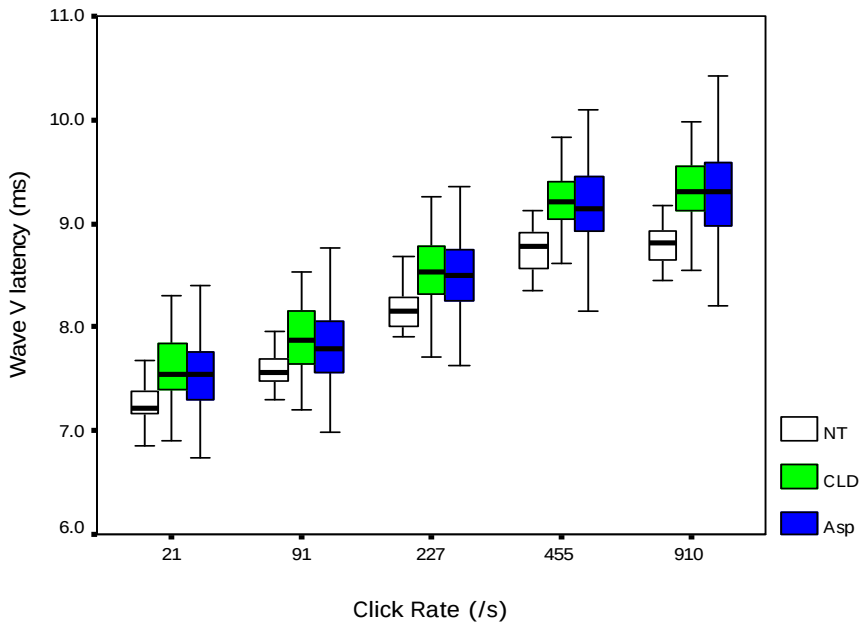

Figure 2. Boxplot of BAER wave V latency (bold line across the box, median; box, 25th and 75th centile; extensions, the largest and smallest values) at various click rates in very preterm infants with neonatal CLD, term infants after perinatal asphyxia (Asp), and normal term (NT) infants as controls. The data are recorded with click intensity ≥ 40 dB above BAER threshold at term age. These are also the case for all Figures 3-10. The p values (*$p < 0.05$) shown in the figure are for the comparison between CLD and asphyxiated infants. Wave V latency is similar in the CLD and asphyxia groups, although the latency in both groups is significantly longer than in normal infants (C).

The I–V interval in both the CLD and the asphyxiated infants was significantly longer than in the controls at all click rates 21–910/s (Figure 3). The difference for the two study groups from the controls was increased linearly with the increase in click rate. The increase in the interval was similar in the CLD and asphyxiated infants, without any significant differences between the two groups at any click rates (Figure 3). This was similar to that of the wave V latency. To detect any differences in the functional status between the peripheral and central regions of the auditory brainstem and any differences between CLD and asphyxia, we also analyzed the I-III and III-V intervals, and the III-V/I-III interval ratio. This allowed us to detect any differences between neonatal CLD and perinatal asphyxia that may not be shown by conventionally analysing the I–V interval only.

The I–III interval in the infants with CLD was similar to that in the controls at all click rates 21–910/s. However, the interval in the infants after asphyxia was significantly longer than in the controls at all rates (Figure 4). The difference was more significant at higher than at lower rates. There was a major difference in the I–III interval between the two study groups; the interval, though similar in the two groups at 21/s in conventional BAER, was significantly longer in the infants after asphyxia than in infants with CLD at all rates 91–910/s in MLS BAER. The difference was increased with the increase in click rate. In contrast, the III–V interval in infants with CLD was significantly longer than in the controls at all click rates 21–910/s (Figure 5). The difference between infants with CLD and the controls was increased linearly with increasing click rate. For the infants after asphyxia, the III–V interval was also significantly longer than in the controls, particularly at higher rates (Figure 5), but the increase was relatively less significant than that in infants with CLD. When comparison was made between the two study groups, the III–V interval tended to be longer in the infants with CLD than in the infants after asphyxia, and the difference was increased with the increase in click rate, reaching statistical significance at higher rates 277–910/s (Figure 5).

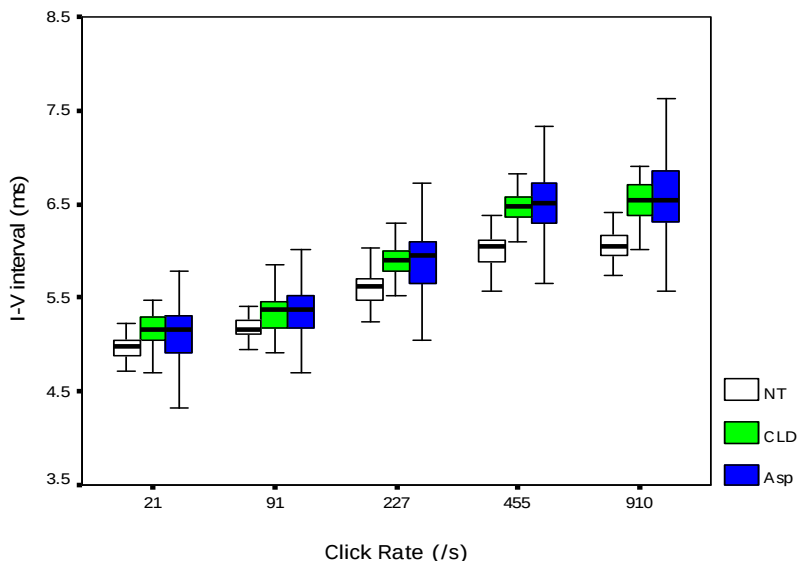

Figure 3. Boxplot of I-V interpeak interval at various click rates in very preterm infants with neonatal CLD, term infants after perinatal asphyxia (Asp), and normal term (NT) infants as controls. The interval is similar in CLD infants and asphyxiated infants, although the interval in both groups is significantly longer than in normal controls.

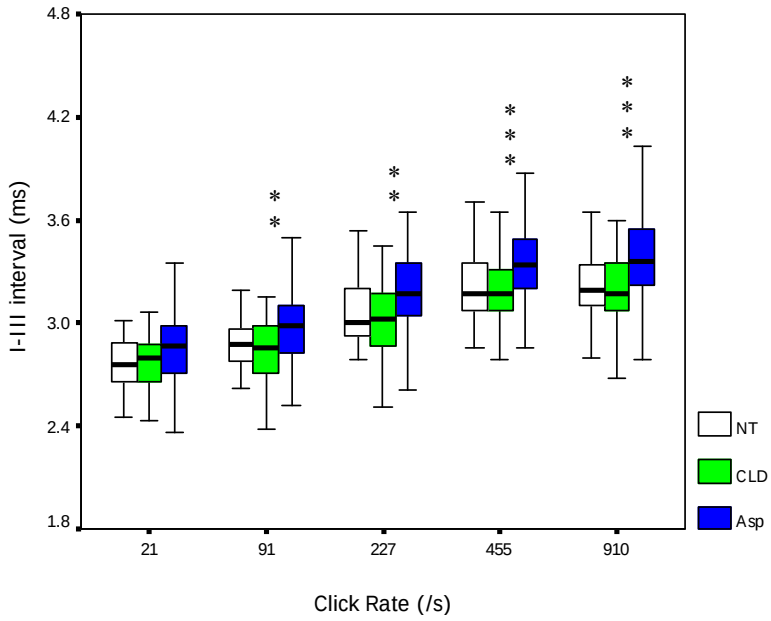

Figure 4. Boxplot of I-III interpeak interval at various click rates in very preterm infants with neonatal CLD, term infants after perinatal asphyxia (Asp), and normal term (NT) infants as controls. The p values (*p < 0.05, **p < 0.01; ***p < 0.001) are for the comparison between CLD and asphyxiated infants. The interval in asphyxiated infants is significantly longer than in CLD infants whose I–III interval is similar to that in normal controls, at all click rates 21–910/s.

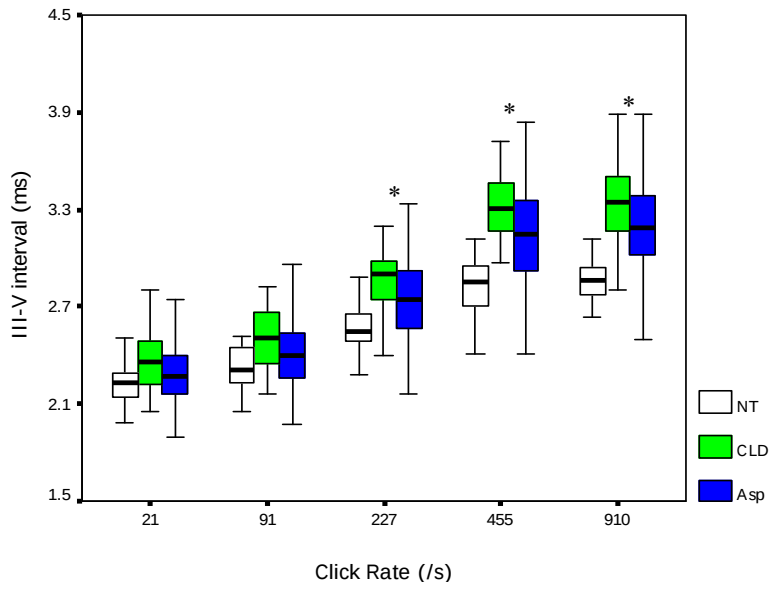

Figure 5. Boxplot of III-V interpeak interval at various click rates in very preterm infants with neonatal CLD, term infants after perinatal asphyxia (Asp), and normal term (NT) infants as controls. The p values (*p < 0.05) are for the comparison between CLD and asphyxiated infants. The interval in CLD infants is significantly longer than in asphyxiated infants at all 21–910/s, although the interval in both groups is significantly longer than in normal controls.

We further analysed the III–V/I–III interval ratio — a BAER variable that may detect some abnormality that could not be shown by analysing the I–V, I-III and III-V intervals (Jiang et al., 2009d). For the infants with CLD, the interval ratio was significantly greater than in the normal controls, and the difference between the two groups was increased linearly with increasing click rate (Figure 6). For the infants after asphyxia, III–V/I–III interval ratio was only slightly greater than in the controls, although there was a statistically significant difference at 455/s. Comparison between the two study groups revealed that the interval ratio was significantly greater in the infants with CLD than in the infants after asphyxia at all click rates.

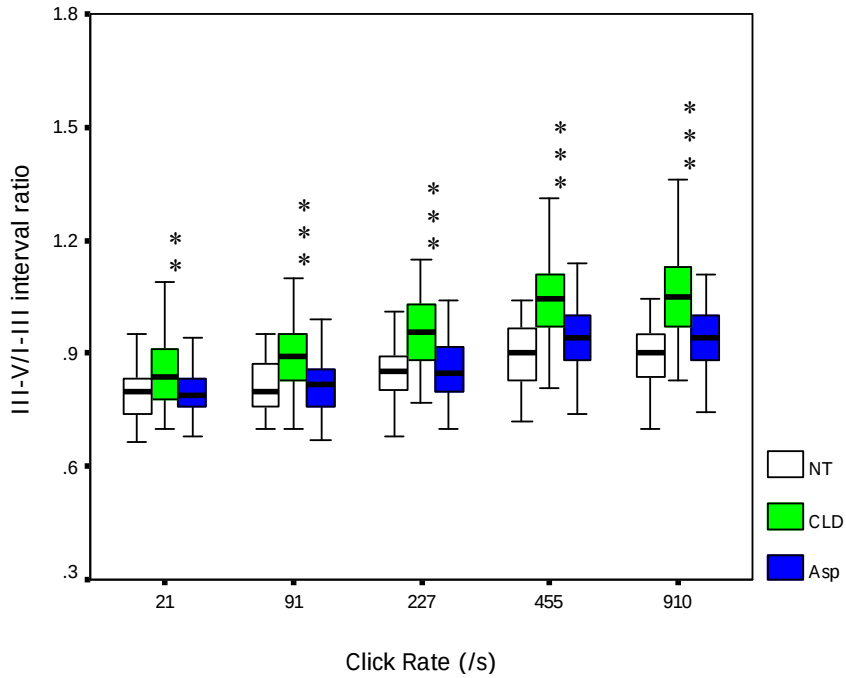

Figure 6. Boxplot of the III–V/I–III interval ratio at various click rates in very preterm infants with neonatal CLD, term infants after perinatal asphyxia (Asp), and normal term (NT) infants as controls. The p values (**p < 0.01, ***p < 0.001) are for the comparison between CLD and asphyxiated infants. The interval ratio in CLD infants, which is significantly greater than in normal controls at all click rates 21–910/s, is significantly greater than in asphyxiated infants whose interval ratio is similar to that in normal controls at 21–227/s and is greater than in controls at 455 and 910/s, at all click rates 21–910/s.

Impaired Brainstem Neural Conduction and Differences between Asphyxia and CLD

In the BAER, wave V latency and I–V intervals are the two most widely used variables that reflect neural conduction, related to myelination and synaptic function, in the brainstem or central auditory pathway (Jiang, 2008). The two variables were increased similarly in our CLD and asphyxiated infants, suggesting similar degree of impairment in neural conduction in the auditory brainstem. However, a more detailed analysis revealed that there were major differences between the two clinical problems. In the infants with CLD, no apparent abnormalities were found in wave III latency and the I–III interval (Figure 4), suggesting no appreciable abnormality in the more peripheral regions of the brainstem. In the infants after

perinatal asphyxia, however, wave III latency tended to increase and, in particular, the I–III interval was increased significantly at all click rates, suggesting impaired neural conduction in the more peripheral regions of the brainstem. On the other hand, there was a major increase in the III–V interval (Figure 5) and the III–V/I–III interval ratio in both the CLD and asphyxiated infants, but the increase was more significant in the infants with CLD than in the infants after asphyxia. Apparently, there is major impairment in neural conduction in the more central regions of the brainstem in the two clinical problems, but the impairment is more severe in neonatal CLD than in perinatal asphyxia. Such differential changes in the earlier sub-component (I–III interval) and the later sub-component (III–V interval) of the I–V interval are almost the other way around in the two clinical problems, resulting in a similar increase in the I–V interval (and wave V latency).

The I–III interval was significantly longer in the infants after perinatal asphyxia than in the infants with neonatal CLD, while the III–V interval was somewhat the other way around, i.e. the interval was longer in CLD than in asphyxia. Such differential changes in the two intervals leads to a significant greater III–V/I–III interval ratio in the infants with CLD than in the infants after asphyxia.

Differences in Brainstem Synaptic Transmission between CLD and Asphyxia

Findings in MLS BAER Wave Latency- and Interval-Rate Functions

For those variables that were correlated significantly with click rate, the latency-, interval-, and amplitude-rate functions were obtained by regression analysis. The slope for each function was then calculated to assess click rate-dependent changes (i.e. changes in BAER variables with varying click rate). For each MLS BAER variable-rate function that was significantly greater than zero at the 0.05 level or better, the slope was compared between the study and control groups to detect any difference.

The slopes of latency-rate functions for waves I and III in the infants with CLD were similar to those in the normal controls. In the infants after asphyxia the slope of latency-rate function for wave I was also similar to the controls, while the slope for wave III was slightly steeper than in the controls, with no statistical significance. However, the slopes of latency-rate functions for wave V in both the CLD and the asphyxiated infants were significantly steeper than in the controls. This was also the case for the slope of the I–V interval-rate function. The slope of the I–III interval-rate function in the infants with CLD was similar to that in the controls, while the slope in the infants after asphyxia was slightly steeper than in the controls. The slopes of the III–V interval-rate function and the III–V/I–III interval ratio-rate functions in the two study groups were all steeper than in the control group, which was more significant in the infants with CLD than in the infants after asphyxia.

Comparison between the CLD and the asphyxiated infants did not show any significant differences in the slopes of wave I and V latency-rate functions and the I–V interval-rate function. The slope of wave III latency-rate function was slightly steeper in the infants after asphyxia than in the infants with CLD, with no statistical significance. The slope of the I–III interval-rate function was significantly steeper in the infants after asphyxia than in the infants with CLD ($p < 0.05$). On the contrary, the slope of the III–V interval-rate function in the infants with CLD was slightly steeper than in the infants after asphyxia. The slope of the III–

V/I–III interval ratio-rate function was significantly steeper in the infants with CLD than in the infants after asphyxia.

Abnormalities in Synaptic Efficacy and Differences between CLD and Asphyxia

As the repetition rate of clicks is increased, BAER wave latencies and interpeak intervals are usually increased progressively. Such progressive increase is attributed to cumulative decrease in the efficacy of synaptic transmission at high stimulus rates, resulting in prolonged synaptic delay along the brainstem auditory pathway. The stimulus rate-dependent changes, i.e. the changes in BAER variables with varying repetition rate of clicks, primarily reflect neural processes concerning the efficacy of synaptic transmission, as well as neural synchronisation and metabolic status of auditory neurons in the brainstem following the presentation of a temporal/physiological challenge (Jiang, 2008; Jiang et al., 2004). The efficacy of synaptic transmission in the central nervous system is related to the mechanisms for synthesis, release and uptake of neurotransmitters, and can be damaged by severe hypoxia or hypoxia–ischaemia (Jiang, 2008). The stimulus rate-dependent changes, mainly reflecting the efficacy of synaptic transmission, are presented by the slopes of BAER variable-rate functions (Jiang, 2008).

In infants with neonatal CLD, there were no appreciable abnormalities in click rate-dependent changes in conventional BAER, although I–V and III–V intervals were increased (Jiang et al., 2006a). In MLS BAER we found a significant increase in click rate-dependent changes in some variables (Jiang, 2010; Jiang et al., 2009a,2010, Wilkinson et al., 2007). In infants after perinatal asphyxia, we found a major increase in click rate-dependent changes in most MLS BAER variables and a certain degree of increase in some conventional BEAR variables (Jiang, 2008; Jiang et al., 2000,2003,2004,2009b,c).

In MLS BAER, the slopes of the wave I and V latency-rate functions and the I–V interval-rate function were increased similarly in our CLD and asphyxiated infants. However, the slope of wave III latency-rate function was steeper in the infants after asphyxia than in the infants with CLD. In particular, the slope of the I–III interval-rate function was significantly steeper in the infants after asphyxia than in the infants with CLD, while the slope of the III–V interval-rate function was slightly steeper in the infants with CLD than in the infants after asphyxia. That is, the differences in the slopes in the two interval-rate functions between CLD and asphyxia were almost the other way around. Such differential changes in the two intervals result in a steeper slope of the III–V/I–III interval ratio-rate function in the infants with CLD than in the infants after asphyxia. Therefore, there are differences in click rate-dependent changes between the two groups of infants.

The steeper slopes of these MLS BAER variable-rate functions reflect an increase in click rate-dependent changes in these MLS BAER variables, which, in turn, indicates a impaired efficacy of central synaptic transmission or a decreased ability of central neurons to recover in time to transmit the next stimulus-evoked response. The differences in click rate-dependent changes between the infants with CLD and the infants after asphyxia indicate that there are differences in the impaired efficacy of synaptic transmission along the auditory brainstem between neonatal CLD and perinatal asphyxia. The impairment in synaptic efficacy in the more central regions of the brainstem is more severe in neonatal CLD than after perinatal asphyxia. On the other hand, synaptic efficacy in the more peripheral regions of the brainstem is basically normal in neonatal CLD, but is impaired after perinatal asphyxia.

Possible Mechanisms Underlying the Differences in Impaired Brainstem Conduction and Synaptic Efficacy between CLD and Asphyxia

Hypoxemia affects the functional integrity and development of the immature brain (Jiang, 2008,2010; Johnston et al., 1995; Johnston et al., 2001). In both human infants and experimental immature animals, brainstem auditory neurons are shown to be particularly sensitive to severe hypoxemia (Inagaki et al., 1997; Jiang, 2008,2010; Jiang et al., 2005b,2006c; Sohmer et al., 1986). The differences in MLS BAER between the infants with CLD and those after perinatal asphyxia indicate that there are differences in both neural conduction and synaptic function in the brainstem between neonatal CLD and perinatal asphyxia. The infants with CLD showed a major increase in the III–V interval but no apparent change in the I–III interval, whereas the infants after asphyxia showed a significant increase in both the I–III and the III–V intervals, although the increase in the III–V interval was slightly more significant. Such results indicate that there are differences in the impaired function in different regions of the brainstem between neonatal CLD and perinatal asphyxia. These differences are most probably related to the difference in the time course and severity of hypoxia in the two clinical problems, although there are differences in some other aspects.

In perinatal asphyxia the hypoxia is often acute, severe or lethal. Furthermore, most hypoxic injuries in fetuses and infants with perinatal asphyxia are the results of combined hypoxia and ischaemia rather than hypoxia alone (Jiang, 2008; Johnston et al., 1995; Levene and Evans, 2005; Vannucci, 1990; Volpe, 2001). It is unlikely that acute hypoxemia alone severely damages the immature brain unless there is superimposed ischaemia. Such acute lethal hypoxia, associated with ischaemia, affects both the rostral/central and caudal/peripheral regions of the brainstem, although the central regions may be affected relatively more than the more peripheral regions, as shown by the slight increase in the III-V/I-III interval ratio at higher click rates (Jiang, 2008; Jiang et al., 2000,2003, 2005b, 2006c).

In neonatal CLD, the nature of hypoxia is quite different from that in perinatal asphyxia. Infants with CLD often have intermittent hypoxic episodes. During the course of CLD, the hypoxia is chronic or prolonged and sublethal. Unlike in perinatal asphyxia, the hypoxia in CLD is pulmonary in origin, and does not have any superimposed ischaemia — the major detrimental cause for brain damage after perinatal asphyxia. Experiments in animals show that chronic and sublethal hypoxia may result in severe impairments in corticogenesis in the developing brain and a significant decrease in subcortical white matter (Ment et al., 1998). Glia was significantly reduced (Schwartz et al., 2004). Magnetic resonance imaging studies in infants with CLD found impairment in cerebral cortical grey matter growth (Murphy et al., 2001). At term equivalent age infants with CLD had a reduction in cerebral volume in many regions of the brain, suggesting that chronic sublethal hypoxia may lead to severe impairments in corticogenesis in the developing brain (Thompson et al., 2007).

In our MLS BAER study, the significant increase in the III-V interval and III-V/I-III interval ratio indicate neonatal CLD has a major effect on neural conduction in the more central regions of the immature brainstem, reflecting impairment in white matter, myelination and synaptic function at the subcortical level. This effect is likely caused by or related to chronic sublethal hypoxia during the course of CLD, although other factors associated with neonatal CLD may also be involved. Our preliminary experiments in rats raised in chronic sublethal hypoxia revealed a marked reduction in staining for myelin basic protein and patchy distribution in the residual myelination in central and upper regions of the brainstem (Jiang,

2010). The results are consistent with the increase in the I–V and III–V intervals in these animals and in the infants with CLD of the present study. It appears that chronic sublethal hypoxia exerts major damage to the more central regions of the brainstem, but no major damage to the more peripheral regions.

DIFFERENCES IN BRAINSTEM ELECTROPHYSIOLOGY BETWEEN ACUTE SEVERE HYPOXIA AND CHRONIC SUBLETHAL HYPOXIA

In the BAER, in addition to wave latencies and interpeak intervals, the amplitudes of various BAER wave components are also of some value in assessing brainstem auditory function. Compared with the wave latencies and interpeak intervals, the amplitudes have a relatively large across subject variability, which limits the use of BAER wave amplitudes (Chiappa, 1990; Jiang et al., 2008a). In our previous work we have noted that with well-controlled and consistent experimental conditions, the amplitudes of BAER waves, particularly wave V, are useful variables to reflect neuronal function of the auditory brainstem (Jiang, 1998,1999, Jiang and Tierney, 1996, Jiang et al., 2001,2008a,b). For example, in children who survived perinatal or postnatal asphyxia the major abnormality is a significant reduction in wave V amplitude, even though there were no major changes in interpeak intervals (Jiang and Tierney, 1996, Jiang et al., 2008b). Similarly, in children who survived bacteria meningitis or who had cerebral palsy, there was also a reduction in wave V amplitude, which was more significant at higher than at lower repetition rates of click stimuli (Jiang, 1999; Jiang et al., 2011). These findings indicate that BAER wave amplitudes are valuable variables to assess brainstem auditory electrophysiology.

Because the nature of hypoxia is different between neonatal CLD and perinatal asphyxia, it is likely that the two neonatal problems exert different effects on the auditory brainstem, resulting in differential changes in the amplitude of BAER components. In the literature, there are some reported studies of the amplitude of BAER in infants after perinatal asphyxia (Hecox et al., 1981; Jiang et al., 2001,2006b,2008a,b,2009b), but a paucity of reports on the amplitudes of BAER wave components in infants with CLD (Jiang et al., 2009a). We carried out a detailed analysis of the amplitudes of MLS BAER components in preterm infants with neonatal CLD and compared the results with those in a large number of term infants after perinatal asphyxia for any differences.

In our study of the amplitudes of MLS BAER wave components, there were 39 very preterm infants with CLD, and 112 term infants after perinatal asphyxia (Jiang et al., 2009a). These infants were all studied at term date (i.e. 38–42 weeks postconceptional age), at the same time as our study in wave latencies and interpeak intervals (Jiang et al., 2010). The amplitude of wave I was measured from the peak of wave I to the lowest trough between waves I and III, and the amplitude of wave III from the lowest trough between waves I and III to the peak of wave III (Jiang et al., 2008a). The amplitude of wave V was measured from the positive peak to the negative trough immediately after the peak. Based on the amplitude measurements in each recording, V/I and V/III amplitude ratios were calculated. Measurements of each MLS BAER variables from two replicated recordings to each stimulus condition were averaged for data analyses.

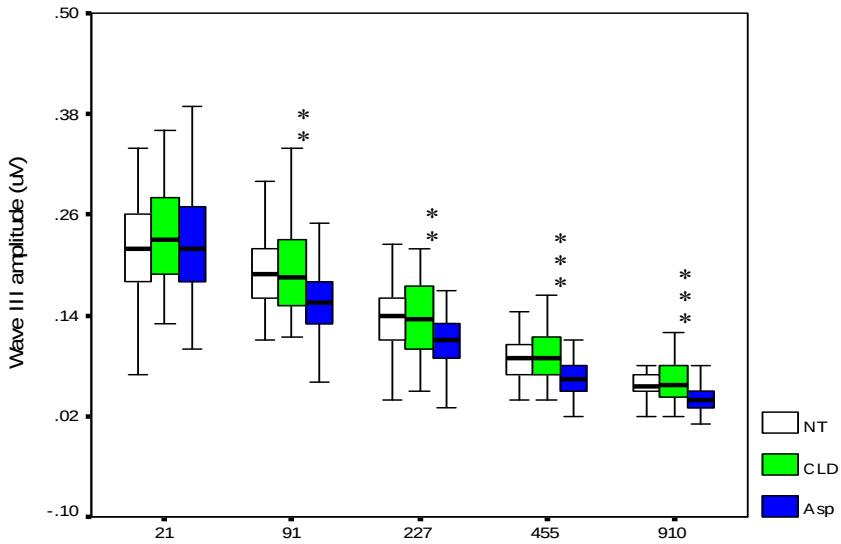

Figure 7. Boxplot of wave III amplitude at various click rates in very preterm infants with neonatal CLD, term infants after perinatal asphyxia (Asp), and normal term (NT) infants as controls. The P values (**P < 0.01, **P < 0.001) shown in the figure are for the comparison between CLD and asphyxia. Wave III amplitude is significantly smaller in infants after perinatal asphyxia than in infants with neonatal CLD at all click rates 91–910/s in the MLS BAER, although there is not difference between the two groups at 21/s in conventional BAER.

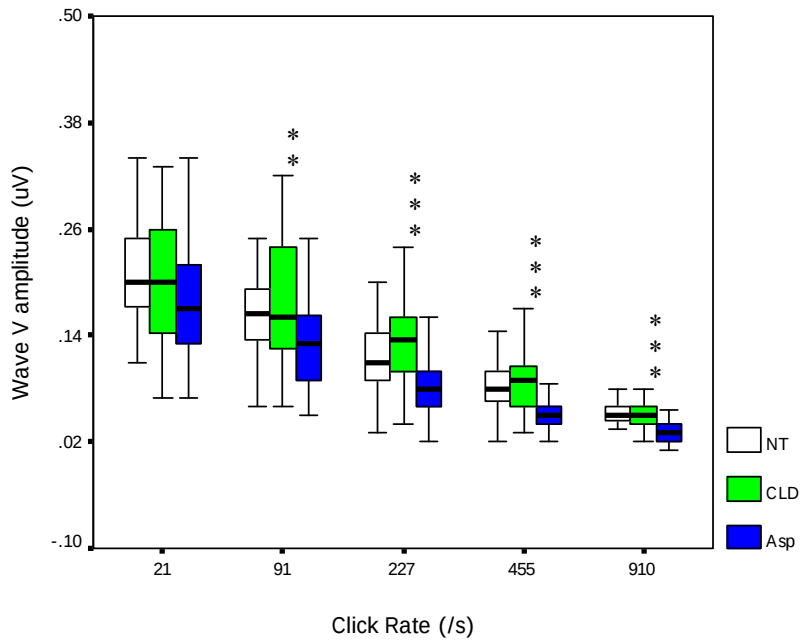

Figure 8. Boxplot of wave V amplitude at various click rates in very preterm infants with neonatal CLD, term infants after perinatal asphyxia (Asp), and normal term (NT) infants as controls. The P values (**P < 0.01, **P < 0.001) shown in the figure are for the comparison between CLD and asphyxia. Wave V amplitude is significantly smaller in infants after perinatal asphyxia than in infants with neonatal CLD at all 91–910/ses in the MLS BAER, and the differences between the two groups are more significant at higher than at lower click rates.

Differences in MLS BAER Wave Amplitudes between CLD and Asphyxia

As the click rate was increased from 21/s to 910/sec, the amplitudes of BAER wave components were reduced progressively in both the infants with neonatal CLD and those after perinatal asphyxia, which was generally similar to that in the normal controls. There were no major differences between the infants with neonatal CLD and the controls in the measurements of these wave amplitudes at all click rates. By comparison, the measurement of the amplitudes in the infants after perinatal asphyxia were apparently smaller than those in both the infants with neonatal CLD and the controls, which was more significant at higher than at lower click rates (Figure 7 and Figure 8). Similar to the controls, no clear systematic changes in the V/I and V/III amplitude ratios with varying click rates were noted in the two study groups (Figure 9 and Figure 10).

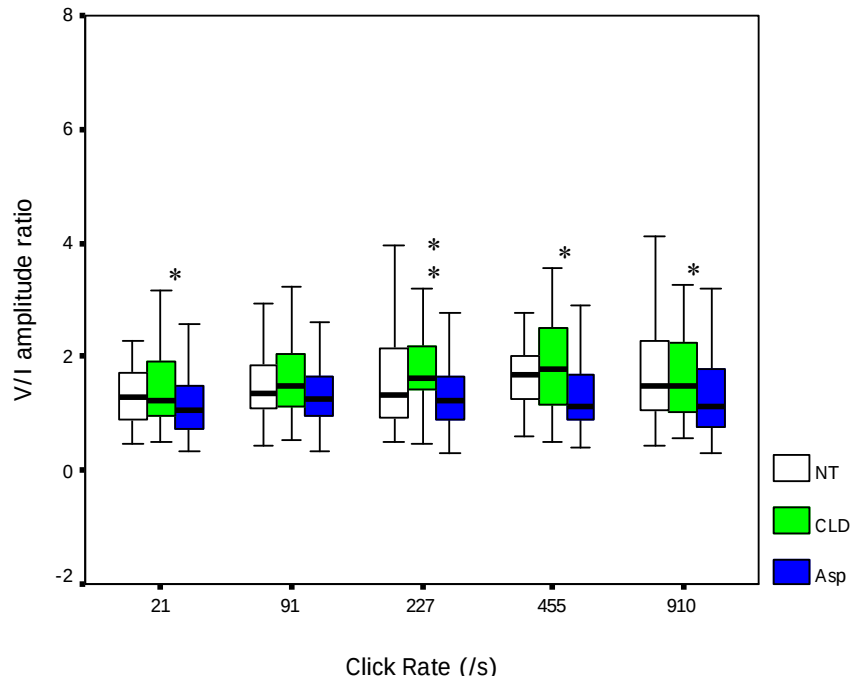

Figure 9. Boxplot of V/I amplitude ratio at various click rates in very preterm infants with neonatal CLD, term infants after perinatal asphyxia (Asp), and normal term (NT) infants as controls. The P values (*P < 0.05, **P < 0.01) shown in the figure are for the comparison between CLD and asphyxia. V/I amplitude ratio is smaller in infants after perinatal asphyxia than in infants with neonatal CLD at all click rates 21–910/s.

In the infants with CLD, the amplitudes of MLS BAER waves I, III and V were generally similar to those in the controls at all 21–910/s clicks (Figure 7 and Figure 8). No significant differences were found between the two groups in these wave amplitudes at almost all click rates. Similarly, the V/I and V/III amplitude ratios in the infants with CLD did not show any significant differences from those in the controls (Figure 9 and Figure 10). In regression analysis, the intercepts of the amplitude-rate functions for waves I and III in the infants with CLD were similar to those in the controls, while the intercept of wave V amplitude-rate function was greater than that in the controls. The slopes of these functions were all similar to

those in the controls, without any significant differences. Neither the V/I amplitude ratio nor the V/III amplitude ratio showed significant and systematic change with varying click rate.

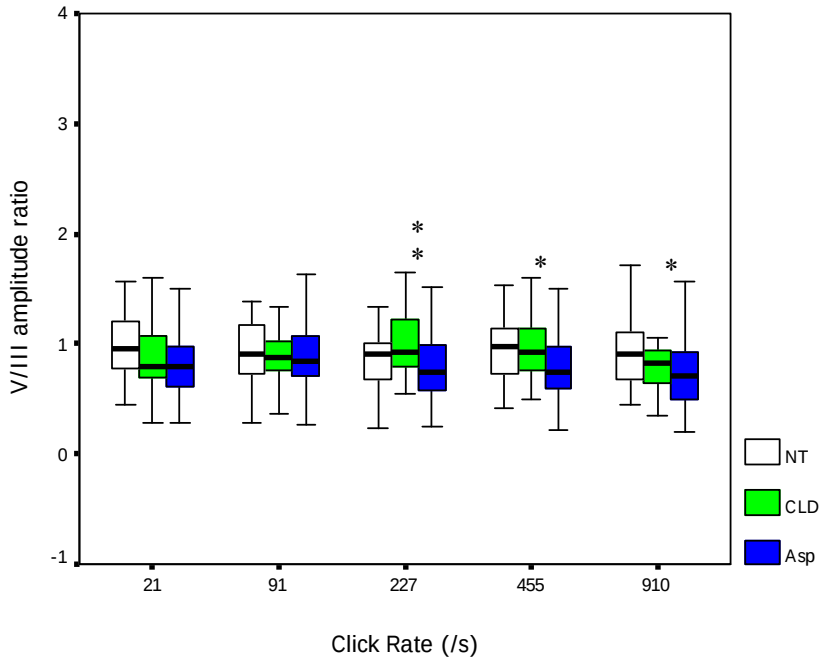

Figure 10. Boxplot of V/III amplitude ratio at various click rates in very preterm infants with neonatal CLD, term infants after perinatal asphyxia (Asp), and normal term (NT) infants as controls. The P values (*P < 0.05, **P < 0.01) shown in the figure are for the comparison between CLD and asphyxia. V/I amplitude ratio is smaller in infants after perinatal asphyxia than in infants with neonatal CLD at all click rates 21–910/s.

In the infants after asphyxia, however, all amplitudes of waves I, III and V tended to be reduced, particularly at higher rates. The amplitudes of waves I and III in these infants were similar to that in the controls at 21/s clicks in conventional BAER, but were significantly smaller at all click rates 91–910/s in MLS BAER (Figure 7). Wave V amplitude was significantly smaller than in the controls at all rates 21–910/s (Figure 8). The amplitude reduction in both waves III and V was more significant at higher than at lower rates. The V/I and V/III amplitude ratios both tended to be smaller than in the controls, and were significantly smaller at 91 and 910/s for the V/I amplitude ratio (Figure 9) and at 91, 455 and 910/s for the V/III amplitude ratio (Figure 10). In regression analysis, the intercepts of the amplitude-rate functions for waves III and V were smaller than those in the controls, but the slopes of the amplitude-rate functions for all waves I, III and V were generally similar to those in the controls.

The amplitudes of MLS BAER wave components were compared between the infants with neonatal CLD and the infants after asphyxia, and some major differences were found. At the lowest click rate 21/s, none of the amplitudes of waves I, III and V differed significantly between the two groups of infants, although wave V amplitude was slightly smaller in the infants after perinatal asphyxia than in the infants with neonatal CLD (Figure 7 and Figure 8). At higher rates, however, some clear differences were revealed between the two groups.

Wave I amplitude in the infants after perinatal asphyxia was smaller than in the infants with neonatal CLD, and differed significantly at the very high click rates 455 and 910/s. Both wave III and V amplitudes were significantly smaller than in the infants with neonatal CLD at all rates 91–910/s (Figures 7 and 8). The amplitude reduction was more significant for wave V than for wave III. These results show clearly that the differences between the two groups of infants (i.e. the amplitude reduction in the infants after asphyxia) was more significant for later BAER waves, i.e. waves with longer latencies, than for earlier waves, i.e. waves with shorter latencies (Figure 7 and Figure 8). The differences were also more significant at higher than at lower rates of clicks.

The V/I amplitude ratio in the infants after perinatal asphyxia was significantly smaller than in the infants with neonatal CLD at almost all click rates, except 91/s (Figure 9). The V/III amplitude ratio tended to be smaller than in the infants with neonatal CLD, and differed significantly at higher rates 227–910/s (Figure 10). These differences were mainly due to a more significant reduction in wave V amplitude than in wave I and III amplitudes in the infants after perinatal asphyxia.

In regression analysis of the relationship between BAER wave amplitudes and click rate, the intercepts of the amplitude-rate functions for waves III and V in the infants after perinatal asphyxia were smaller than those in the infants with neonatal CLD. By comparison, the slopes of the two functions and the slope of wave I amplitude-rate function were similar in the two groups of infants, without any significant differences. The V/I amplitude ratio was not correlated with click rate in either the infants with neonatal CLD or the infants after perinatal asphyxia. The V/III amplitude ratio did not change systematically with click rate in the infants with neonatal CLD, and was correlated weakly with the rate in the infants after perinatal asphyxia.

Mechanisms Underlying the Differences in MLS BAER Wave Amplitudes between CLD and Asphyxia

In evoked potentials of the brain, the recorded amplitude under standard conditions is proportional to the number of synchronously active elements contributing, and a reduction in amplitude reflects a smaller average contribution from a given population of cells (Chiappa, 1990; Jiang, 2008). There are several factors that can cause amplitude reduction, including (a) fewer cells contributing to the current, each cell contributing the same amount as before; (b) smaller contribution from each of the cells of the population; (c) a change in the synchrony with which the cells are activated (especially important with cell discharge); (e) a decrease in the average membrane potential of the cells making up the population; (so the driving potential for current flow is reduced); (f) a decrease in the resistivity of the bathing medium, (e.g., in children with diffuse multicystic lesions and marked atrophy of white matter).

The amplitude reduction in MLS BAER components in our infants after perinatal asphyxia reflects a depression of brainstem auditory electrophysiology. This is most likely to be attributable to neuronal impairment and/or death in the neonatal brainstem following hypoxia-ischaemia, resulting in fewer neurons contributing to BAER wave amplitudes and/or smaller contribution from each neuron, neural asynchrony, a decrease in membrane potential of neurons. We also found that the amplitude reduction after perinatal asphyxia may persist during the whole period of the first month, although the reduced amplitudes were increased

slightly in the later period of the first month (Jiang et al., 2008a). This finding indicates that there is a sustained depression of brainstem auditory electrophysiology, which may reflect sustained neuronal impairment and/or death in the auditory brainstem. It appears that neuronal damage due to hypoxia-ischaemia is unlikely to recover within a relatively short period.

After perinatal asphyxia discrete hypoxic-ischaemic lesions are very common in the auditory brainstem, including loss of neurones with gliosis or ischaemic cell changes in the cochlear nuclei, superior olive and inferior colliculus (Dambska et al., 1987; Leech and Alvord, 1977; Myers, 1977; Natsume et al., 1995; Pasternak, 1993). These findings indicate that auditory neurons in the neonatal brainstem are vulnerable to hypoxic-ischaemic insult. The inferior colliculus – the major auditory centre in rostral regions of the auditory brainstem - receives widespread auditory input from the brainstem. This nucleus has a very high metabolic rate and is particularly vulnerable to hypoxia-ischaemia. The significant reduction in wave III and especially V amplitudes in our infants after asphyxia suggests that auditory nuclei in mid and rostral regions of the brainstem are vulnerable to perinatal hypoxia-ischaemia.

The amplitude reduction in our infants after asphyxia was most significant for wave V, less significant for wave III and least significant for wave I, i.e. the later components of the BAER, reflecting more central function, were reduced more significantly than the earlier components, reflecting more peripheral function. The V/I and V/III amplitude ratios tended to be decreased, reflecting a relatively more significant reduction in wave V than the earlier waves I and III. These results suggest that taking into account the effect of amplitude reduction of wave I, which reflects peripheral auditory function, on later BAER components (i.e. waves III and V), reflecting mainly central function, the later components were still reduced significantly. Apparently, the functional status of both peripheral and particularly central auditory pathways is impaired following perinatal asphyxia. Animal experiments revealed that following hypoxia BAER wave reduction or loss began with the later waves and progressed to the earlier waves (Sohmer et al., 1986). In infants after asphyxia, we also found that the later BAER components were reduced earlier than the earlier components (Jiang et al., 2008a).

Most hypoxic injuries in fetuses and infants were found to be the results of combined hypoxia and ischaemia rather than hypoxia alone, and it is unlikely that acute hypoxemia will damage the immature brain unless there is superimposed ischaemia (Johnston et al., 1995; Vannucci, 1990). Experiments in animals found that BAER abnormalities following hypoxia are always due to ischaemia even when the initial insult is hypoxic alone (Sohmer et al., 1986). Severe, prolonged ischaemia can result in serious neuronal impairment, leading to synaptic inefficiency, or even death, fewer neurons generating the volley in the auditory brainstem. The death of neurons in the postasphyxial period may be initiated by necrosis as a direct result of the hypoxic-ischaemic insult, or occur as a result of apoptosis (Johnston et al., 1995,2001). Very severe hypoxic-ischaemic insult leads to destruction of cellular membranes and histological necrosis due to total mitochondrial failure. Less severe degrees of hypoxia-ischaemia can trigger delayed programmed cell death or apoptosis. Secondary neuronal death probably occurs as a combination of the two interrelated processes (Levene and Evans, 2005).

In neonatal CLD, however, the hypoxia is chronic and sublethal, and may be not severe enough to cause any major damage to the neural origins of the amplitudes of MLS BAER components. In infants with temporary and less severe perinatal hypoxia, there is only a mild

or subtle degree of abnormality in MLS BAER variables (Jiang et al., 2007a). Such a mild abnormality occurred only at very high-rate stimulation (455 and 910/s clicks) and during the first 3 days after birth. Apparently, temporary and less severe perinatal hypoxia does not exert any major effect on the functional integrity of the neonatal brainstem.

After perinatal hypoxia BAER abnormalities are always due to ischaemia even when the initial insult is hypoxic alone (Sohmer et al., 1986). In the case of neonatal CLD, the hypoxia does not have any superimposed ischaemia. This is probably a major reason why there is no amplitude reduction in neonatal CLD, and also a major difference in pathophysiology between neonatal CLD and perinatal asphyxia. Severe, prolonged ischaemia causes serious neuronal impairment or even death, leading to synaptic inefficiency, fewer neurons generating the volley in the neonatal brainstem. This may lead to a significant reduction in MLS BAER wave amplitudes in infant after asphyxia. In contrast, in the case of neonatal CLD the sublethal hypoxia is very unlike to cause neuronal death, as demonstrated in newborn rats with experimental chronic sublethal hypoxia (Jiang, 2010). Therefore, significant reduction in wave amplitudes in MLS BAER components is unlikely to occur in infants with neonatal CLD.

CONCLUSIONS AND SIGNIFICANCES

In summary, the above MLS BAER studies revealed that both CLD and asphyxiated infants had a significant increase in wave V latency and the I–V interval. The infants with neonatal CLD showed a significantly increased III–V interval but a normal I–III interval at all click rates. In contrast, the infants after perinatal asphyxia demonstrated a significant increase in both the III–V and I–III intervals. The I–III interval was shorter and the III–V/I–III interval ratio was greater in infants with CLD than in the infants after asphyxia. The slope of I–III interval-rate function was steeper in the infants after asphyxia than in the infants with CLD, whereas the slope of III–V/I–III interval ratio-rate function was the other way around. Thus, the infants with CLD had a major increase (i.e. abnormality) in the more central components of MLS BAER, without appreciable abnormality in the more peripheral components, whereas the infants after asphyxia had a significant increase in both central and peripheral components. These results indicate that neonatal CLD affects neural conduction in the more central regions of the brainstem, whereas perinatal asphyxia affects neural conduction in both peripheral and, though mainly, central regions.

In the study of the amplitudes of MLS BAER, all amplitude variables in the infants with CLD did not differ from those in normal term controls at all click rates 21–910/s. The slopes of amplitude-rate functions were also similar to those in the controls. In contrast, wave III and V amplitudes in the infants after asphyxia were smaller than those in both the controls and the infants with CLD, particularly at high-rate stimulation. The intercepts of amplitude-rate functions for waves III and V were smaller than in both the normal controls and the infants with CLD, although there were no significant differences in the slopes of these functions. Thus, there were no abnormalities in MLS BAER amplitudes in the infants with CLD, whereas the amplitudes were significantly reduced in the infants after asphyxia. The basically normal amplitudes in MLS BAER wave components in the infants with CLD imply that there is no noticeable depression of electrophysiological activity in the neonatal brainstem. In

contrast, the significant reduced amplitudes in infants after perinatal asphyxia suggest that there is a noticeable depression of electrophysiological activity in the neonatal brainstem. Such major differences between the two groups of infants indicate that there is no major neuronal impairment in the auditory brainstem in CLD, but there is in perinatal asphyxia.

These interesting findings contribute to our understanding of the differential effects of chronic and sublethal hypoxia and acute and severe or lethal hypoxia associated with ischaemia on the immature brain. Neonatal CLD affects the more central regions of the brainstem, without appreciable effect on the more peripheral regions, whereas perinatal asphyxia affects both central and peripheral regions. These differences between CLD and asphyxia are likely to be, at least partly, related to the difference in the time course and severity of hypoxia associated with the two problems. The knowledge obtained provide insights into the differences in the pathophysiology underlying neurological impairment and developmental deficits between neonatal CLD, related to chronic sublethal hypoxia, and perinatal asphyxia, related to acute lethal hypoxia and associated ischaemia.

Our findings have some important clinical implications, particularly in relation to early interventions or therapies for neonatal CLD and perinatal asphyxia. So far, there remain difficulties and controversies in treatment of neonatal CLD and perinatal asphyxia (Barks, 2008; Glass and Ferriero, 2007; Tin and Wiswell, 2008). We recommend that neuroprotective interventions should target more central regions of the brain for infants with CLD whereas the interventions should target both peripheral and central regions of the brain for infants after perinatal asphyxia. There is no severe neuronal injury and/or neuronal death in the brainstem in neonatal CLD. For infants with CLD there seems to be no need to implement radical treatments such as brain cooling, which is used to rescue severely injured neurons. Well regulated supplemental oxygen may remain the major therapy for infants with neonatal CLD, along with other therapeutic adjuncts. In perinatal asphyxia, however, there is major neuronal injury and/or neuronal death. For these infants there is a need to intervene with radical neuroprotective measures, e.g. brain cooling, as early as possible to reduce neuronal injury and death and rescue severely injured neurons (Barks, 2008; Glass and Ferriero, 2007). So far, hypothermia or brain cooling is the only early intervention that improves the outcome after perinatal asphyxia (Drury et al., 2010; Hoque et al., 2010). Combining hypothermia with additional treatments might achieve further improvement. Recent studies have demonstrated that Xenon, a noble anesthetic gas with an excellent safety profile, in combination additively with hypothermia offers long-term functional and histopathologic neuroprotection after perinatal asphyxia (Hobbs et al., 2008; Thorensen et al., 2009). It appears that the unique safety profile differentiates xenon as a promising combination therapy with hypothermia to improve the outcome.

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Chapter 5

CLINICAL NEUROPHYSIOLOGY IN PRETERM INFANTS: A WINDOW ON EARLY PHASES OF BRAIN DEVELOPMENT

Agnese Suppiej, Ambra Cappellari and Elisa Cainelli
Child Neurology and Clinical Neurophysiology Unit, Department of Paediatrics, University of Padova, Padova, Italy

ABSTRACT

The sensory evoked potentials in the visual auditory and somatosensory modality reflect the activity of the corresponding sensory pathways ascending to cerebral cortex and its' activation following sensory input. By contrast the event related potentials generated within specific neuropsychological paradigms reflect cognitive processing of the stimuli. It has been shown that during the first 20-45 weeks of gestation the development of the complex cortical and subcortical networks is modulated by sensory driven development of the talamo-cortical afferents and their connections with the developing cortical plate. The cortical responses recorded before the 36 gestational weeks are negative and in opposite polarity with respect to those elicited in infants born at term. It could be hypothesized that the above pattern of neurophysiological development could reflect the transient organization of immature cortex in the period of coexistence of subplate and cortical plate.

Event related potentials evoked by auditory stimulation using the oddball paradigm in the newborn is known to elicit obligate responses to sensory inputs as well as endogenous components similar to that reported in older children and adults. These responses may serve as an early index of developmental problems in the auditory cortex, in infants born pre-term.

The aim of this publication is to review the emerging evidence that evoked potential techniques may index the above maturation processes, thus providing a unique window on the brain at work during the early phases of development, in normal and pathological conditions.

1. PRETERM BIRTH

Premature birth is a risk factor for major impairments, such as cerebral palsy, deafness, blindness and mental retardation [1-3]. Over the last decades the improved obstetrical and neonatal care, as well as technical advances on neonatal intensive care, have increased survival rates of smaller premature infants. However, neonates born prematurely who survive without major neurological handicap are at high risk of cognitive, motor, psychiatric and behavioral disorders [4-6]. These long-term sequelae are the result of neonatal brain injury or interruption of the normal process of brain maturation that occurs during the last trimester of pregnancy [7,8].

In the third trimester of gestation crucial events occur in the formation of the nervous system and knowledge of these mechanisms is important to understand the origin of the neurodevelopmental abnormalities in dose surviving preterm birth, without major neurological sequelae. Data from the above experimental studies may help the interpretation of development in preterm infants.

2. ANATOMY OF BRAIN DEVELOPMENT

The third trimester of gestation is the most important developmental period for the formation of cerebral pathways: path-finding, target selection and growing into the cortical plate. Essential features of this period are the transient organization of neuronal circuitry and of fetal brain lamination [9,10]. The main structural feature that distinguish early preterm from term neonatal cortex [11,12,13,14]. This transient organization is supported by the presence of the subplate, the most prominent lamina on fetal brain histology known to disappear at the end of the first year of postnatal life [7]. Post mortem studies have also shown that the development of transient embryonic and fetal zones closely correlates with other cellular neurogenetic events [12,13,15,16].

The third trimester comprise the period between the 20 and 45 weeks' gestation; on the basis of major characteristic of the transients pattern of organization it can be divided into four broadly defined phases: fetal (below 24 PCW), early preterm (24 - 32 PCW), late preterm (33-35 PCW) and neonatal phases (36 -45 PCW).

Fetal Phase (< 24 PCW)

This period is characterised by a great production of neurons by the germinative matrix and by the migration of neurons to the cortical subplate (SP). This transient structure is the thickest lamina of this period and its development is driven by thalamocortical afferents, callosal and basal forebrain fibers. Thalamocortical fibers approach, in the late fetal phase, their subplate target in different cortical regions (thalamic afferents are documented in the frontal [7], auditory [8], visual [17] and somatosensory cortex [18], where they stay "waiting" with others afferent fibers for a prolonged period prior to their ingrowth into the cortical plate [19].

Early Preterm Phase (24 – 32 PCW)

During this phase the thalamocortical fibers grow into the cortical plate of the frontal, somatosensory, visual and auditory cortices [7,8,17]. The SP has a crucial role in this process. The formation of synapses in the deep cortical plate and the continuation of synaptogenesis in the subplate parallels the coexistence of transient endogenous circuitry and thalamo-cortical permanent circuitry during the formation of gyri and sulci [9,10]. At this time is possible to record a clear cortical signal in response to any sensorial stimuli [20,21].

These events were thoroughly described in the developing visual cortex of the cat [22] where thalamocortical afferents were found to terminate on subplate neurons and their activation was monitored upon the stimulation of the lateral geniculate body [22]. Other thalamic terminals activate cells in the cortical plate and build the first framework for sensory-driven circuitry [22]. These interactions between endogenous circuitry of the subplate zone and thalamic, sensory-driven circuitry [9,10] coincides with appearance of electrophysiological transients [20,21,23,24] and behavioral phenomena [25,26].

The majority of synapses in the cortical plate are located on the dendrites, which suggests that they are probably excitatory in nature. Furthermore studies by acetylcholinesterase (AChE) histochemistry in the human brain shows a transient modular distribution and intracortical elaboration of thalamocortical fiber branches, which coincides with incipient areal differentiation [7,8,12]. Gradually the elaboration of synapses and growing of dendrites will changes the vertical organization of the cortical plate into a radial organization. Ventricular and subventricular zones will became thinner due to the decreased proliferative activity.

Late Preterm Phase (33 – 35 PCW)

At this time the subplate zone start to decline in parallel with the ingrowth of the callosal and long cortico-cortical pathways [19]. Major afferent subcortico-cortical and efferent cortico-subcortical pathways have completed their growth, selected their targets and established synapses. The afferent fibers build up the corona radiata and adapt their course to the process of gyration. The intracortical circuitry develop with the first evidence of intracortical differentiation [9]: the major histogenetic event of this period is the appearance of six layers in neocortex [27]. The cortical layers remain immature, as result of an incomplete development of dendritic trees. The substrate for interaction between cortical areas and the two hemispheres is incomplete primarily because cortico-cortical pathways are still growing. However, the maturation in the organization of the cortical pathways brings to a better connection between the periphery and the cortex [19]. The influence of the sensorial stimulation in this phase is not completely understood; animal studies show, however, the stimulation do not affect the number of synapse since after birth. This suggests an endogenous, programmed control of this process before birth [28]. Although, changes in the laminar distribution of synapses indicate a structural synaptic plasticity [28].

Also the organization of the white matter undergoes changes: in late preterm infants, the tangential fetal fiber-architectonic stratification transforms completely into a corona radiata system, the centrum semiovale is formed and the gyral white matter becomes visible [29].

This period is characterized also by an intensive differentiation of dendrites of pyramidal neurons [30,31]. In comparison to postnatal ages, there are very few spines on dendrites of pyramidal neurons in premature infants [30,31,32]. As dendritic spines in adults represent the most common postsynaptic element with a presumably excitatory function, their paucity in late preterm suggests a functional immaturity of cortical neurons.

The Neonatal Period (36- 44 PCW)

In this period axonal arborization, dendrites, spines and synapses within the cortical plate layers develop rapidly [26,32]. No further growth of long afferents and cortico-cortical pathways and corpus callosum's fibers along the interhemispheric pathways trajectory occur but, the retraction of exuberant axons begins. In parallel to cessation of thalamo-cortical growth and early long cortico-cortical callosal fibers, short cortico-cortical fibers develop during several postnatal weeks. The maturation of thalamo-cortical circuitry and interaction with sensory input shapes fine connectivity within the cortical columns [9], underlining the great importance of the sensory-driven cortical activity in neonates during critical periods of development.

3. NEUROPHYSIOLOGY OF BRAIN DEVELOPMENT

The evaluation of early human brain development should ideally combine both structural and functional information. In fact, the development of cerebral connections cannot be studied directly with neuroimaging techniques but, clinical neurophysiology can give important information about normal and abnormal development of brain function.

Neurophysiological techniques comprise the electroencephalogram (EEG) and evoked potentials (EPs). These techniques have the advantage over structural magnetic resonance imaging of being "functional" and over the more spatially precise functional MRI of providing more accurate temporal information and of being performed noninvasively at bedside [33,34,35]. Knowledge of neurophysiological developmental patterns may provide inside into brain mechanisms underlying brain maturation and are, at present, regarded as possible markers of brain damage during the early phases of development [35].

3.1. The Electroencephalogram

The electroencephalogram (EEG) represents the synchronous activity of neurons arranged perpendicular to the surface of the cerebral cortex, recorded from the scalp. The EEG of the premature infant reflects the immaturity of fetal brain. The immature EEG background electrical activity is characterized by discontinuity, labiality and fragmentation. The greater the prematurity, the more marked are these EEG aspects [36,37]. During development the discontinuity gradually decreases providing the most visually striking aspect of EEG maturation. Although normative EEG data for early prematurity are only emerging, normal EEG characteristics and their maturational patterns in premature infants are well

established [36]. The correlation of research on early phases of structural brain development with ontogenesis of EEG is a crucial step in the knowledge of functional brain development.

3.1.1. Maturation of the EEG

Fetal Phase (< 24 PCW)

For infants born below 24 week's gestation survival rate is very low and thus the possibility to record the EEG. Engel, 1964, has recorded the EEG of a 19 weeks old fetus, detecting initially activity in the 9 to 10/sec range, flattened gradually during the recording. In this period the thalamo-cortical fibers are still waiting in the subplate, thus explaining the lack of EEG activity [38].

Early Preterm Phase (24 -32 PCW)

In the early preterm period the EEG activity is characterized by a discontinuous pattern consisting in high amplitude synchronized burst of physiological rhythms alternating with periods of low voltage activity (amplitude < 10 μV) of duration corresponding to gestational age [36]. The EEG inactivity reflects the immaturity of the cortex and its connections, whereas the bursts activity, endogenous self-organizing figures, probably reflects thalamic subcortical impulses [39], with a probably crucial role in the maturation of the thalamo-cortical connections [9,40-42]. The increasing activity of the cortical circuitry parallels the decrease of discontinuity. In this phase transient EEG phenomena can be related to the coexistence of transient endogenous circuitry of the subplate and permanent sensory-driven circuitry. With maturation these EEG transients such as centro-occipital delta brushes and temporal sowtooth show a focal distribution over sensory areas [43]. With development of the cortical circuitry higher frequencies gradually replace of slow activity [44]. Finally, in this period waking and sleeping states cannot be recognize in EEG, although a cyclic organization of the EEG activity is emerging [45]. Using the direct current EEG (DC-EEG), methodology that allows to avoid the high-pass filter of the conventional EEG, it has been demonstrated that the most prominent spontaneous EEG activity of premature infants consist of very slow, large amplitude transients(SAT), possible electrical correlates of cortical developement [44].

Late Preterm Phase (31 – 40 PCW)

In the late preterm phase brain maturation provide the neurophysiological basis for a more continuous EEG pattern. At 34 PCW the EEG activity reaches a continuity of about 80%, and by 35 PCW the differentiation between quiet and active sleep becomes more evident, and discontinuity is observed only during quiet sleep (in the co called "tracé alternant" pattern) [46]. The continuous background activity depend on maturation of thalamo-cortical loops, that can arise only after the thalamo-cortical fibers have established their final connections at specific cortical layers (near term age) [40]. The maturation of EEG transients characterizes this period; the temporal sawtooth disappears in active sleep at 32 PCW and in quiet sleep at 33-34 PCW; by contrast delta brush activity decreases in amplitude and frequency and has its full expression between 32 and 34 PCW. Frontal transients appear at 34 PCW and become mature at 36 PCW in quiet sleep [47]. From 34 PCW the EEG activity become synchronous between hemisphere, thanks to development of the corpus callosum. At About 36-38 weeks, the premature babies show EEG features similar to those of

full-term neonates. Delta brush activity begins to decline and then disappear [48]. Wakefulness, active and quiet sleep show three distinctive EEG pattern. From full-term into infancy the maturation of intracortical interneuronal circuits and of inhibitory neurotransmission allow the cortical networks to become able to generate robust higher frequency activity (β and γ), believed to be essential for many cognitive functions [49,50,51].

3.2. Sensory Evoked Potentials

Sensory Evoked Potentials (EPs) are electrical potential changes of sensory receptors, neural pathways and the brain generated in response to external stimuli [13]. They are usually elicited by visual, auditory or somatosensory stimulation and reflect the activity of the sensory pathways ascending to cerebral cortex and early obligatory cortical responses to specific sensory stimuli. EPs are recorded from electrodes located on the surface of the scalp and become recognizable from other electrical signals such as the EEG thanks the averaging technique, a summation of a sufficient number of stimulus-locked responses. The resulting waveform can be viewed as a sequence of waves; an oversimplified analysis of these waves is based on measurement of latency (the time of occurrence following the stimulus) reflecting mainly myelin function, and measurement of amplitude witch express the energy of the signal and is related to the number of activated neurons.

As with the EEG evoked potentials can be recorded at bedside in neonatal intensive care units, even in the incubator, in a short time with minimal handling and can be regarded as a non invasive technique even in infants born prematurely [52,53].

Sensory EPs allow the evaluation of the functional integrity of the sensory systems, from the periphery to cerebral cortex. In premature infants sensory receptors, peripheral nerves, the retina and visual pathways as well as the cochlea and auditory pathways are immature. Thus, in order to elicit and study the somatosensory, auditory and visual cortices maturation it is important that methodology is tailored to this immaturity.

The study of maturational changes of sensory evoked potentials in infants born preterm may offer a window on the development of thalamo-cortical connections and their ingrowths from the subplate into the cortical plate, during the early phases of brain development.

3.2.1. Visual Evoked Potentials and their Maturation

Visual Evoked Potentials (VEPs) can be evoked by brief changes either in the luminance (Flash FVEPs) or in the contrast (Pattern PVEPs). VEPs are recorded by three electrodes located on the occipital scalp, they reflect the activation of primary and secondary areas in the visual cortex. The PVEP waveform consist in adults of three main waves called N 70, P 100 and N 145 based on their negative or positive polarity and their time of occurrence after stimulation. The FVEPs adult waveform consist of six peaks named I to VI according to Ciganek (1961) [54]; most reliably recorded peaks are III e IV corresponding in latency and polarity to N70 and, P100 of PVEP. In infants and children the FVEP components III and IV are reliably recognized at the usual adult latency from 6 months of age and in childhood literature they are named N1 (N70) and P2 (P100) [55]. However before 6 months main changes occur in immature FVEP [56]. Responses can be recorded from 28 PCW; the FVEP up to 32 PCW is characterized by a prominent negative wave at about 300 ms (N300) inconstantly preceded by a small occipital positive wave with a latency of about 200 ms

(P200). Between 35 and 40 PCW the positive wave increases in amplitude and is preceded by a small negative wave. This sequence of morphological changes of VEPs' development is the most reliable indicator of occipital cortex maturation; these changes have long been recognized to parallel of the anatomical maturation of the human visual cortex [57]. Further morphological changes occur soon after birth: the P200 becomes bifid with an earlier positivity usually seen just before 4 weeks of age while by 6 months the typical N1 P2 complex has it's mature morphology [37].

3.2.2. Auditory Evoked Potentials and their Maturation

Auditory Evoked Potentials (AEPs) reflect sequential activation of the structures of the auditory system from the cochlea to the primary and secondary projection areas in the auditory cortex. According to their time of appearance after the stimulus AEPs have been classified as short, middle and long latency responses. The cortical AEP (CAEP), comprising middle and long latency responses, is one of the ontogenically earliest response. Measurements of CAEPs can be made prenatally, however, the uterine environment makes these of measurements difficult. CAEPs can be recorded as early as 24 weeks gestation in infants born prematurely, it is present at a time when cortical development is still very immature and afferent fibers are only beginning to be seen in the cortical plate [8]. It has even been suggested that the CAEP recorded at this time represents cortical activity due to activation of non-lemniscal pathways arising from the reticular formation. In fact at this time myelination of cochlear nerve and auditory brainstem structures is immature thus, the synchrony of neural response needed to obtain an auditory brainstem potential cannot be seen until 28–29 PCW [37]. Kurtzberg et al. [58], describe five maturational stages of CAEP maturation, evolving from preterm to term age. Predominantly negative responses are seen both in medial and lateral scalp sites in less mature infants with gradually more positive responses in more mature infants. Maturation start at the central scalp sites, followed by the lateral sites where predominantly positive responses are seen only at term age. This pattern of maturation is thought to be the result of earlier maturation in primary auditory areas followed by maturation in secondary areas [58].

3.2.3. Somatosensory Evoked Potentials and their Maturation

The somatosensory EPs (SEPs) are characterized by a sequence of waves recorded at different locations, with different latency, polarity and presumed generators, responses are both difficult to obtain and highly variable in premature infants [23,34,59]. At present only short latency SEPs elicited by stimulation of the upper limb (median nerve at wrist) have been clinically validated in neonate. The waves are identified with an abbreviation characterized by N for the negative peak and P for the positive peak; every peak has a number that identify the latency. Upper limb SEPs offer the opportunity to evaluate the somatosensory pathway from peripheral nerve to somatosensory cortex: the N9, is a negative short latency component corresponding to the peripheral-ascending volley; the P11 is generated in the cervical cord dorsal column; the P14 originates in the lower brainstem close to the cervico-medullary junction; finally, the early cortical components N20-P27 and P22-N30 are recorded respectively in the parietal regions controlateral to stimulation, and in the central and frontal regions. Cortical responses following electrical stimulation of the median nerve may be recorded from 25 PCW and are present in all normal infants from 27 weeks, they are both difficult to obtain and highly variable [23,34,59]. The first negative component N1, thought to

correspond to the adult N20, show a latency decrease from 120 msec (at 27 PCW) to 24-26 msec (at 40 PCW) [37]. Maturation shapes the evolving topography, latency and morphology of short latency SEPs, in the period of the ingrowth of the thalamo-cortical fibers in the cortical plate, of the process of gyration of the cortex and of short cortico-cortical circuitry maturation [34]. Thereafter SEP development is characterized by other two coexisting phenomena with opposite effects: myelinogenesis, causing a progressive increase in conduction velocities and synchronization of potentials, decreases the latency, while body growth has the opposite effect. The interaction of these two phenomena will explain the complex pattern of SEP development in childhood [37]. The maturational patterns described for cortical AEPs and VEPs is not found in short latency SEPs used in routine clinical practice. Topography studies showed that SEP fronto-occipital voltage gradient lasted significantly longer than the sharp N1 response that is typically recorded using only few electrodes over the central areas [60,34]. Analyses of these later components demonstrated a maturational pattern similar to CAEPs and VEPs.

3.2.4. The Anatomical Substrate of Sensory EPs Ontogenesis

The patterns of EPs maturation in preterm infants, summarized in the previous chapters, appears to parallel basic science studies of histological and neurochemical processes and neuroimaging studies, it opens a unique window on the brain at work during the early phases of development [52].

Sensory input traveling in thalamo-cortical connections and subsequent cortical activation can be explored by sensory evoked potentials in vivo at bedside.

Anatomic studies in the early seventies already pointed out that the gradual shift in the synaptic and dendritic formation from predominantly deep to superficial cortical structures, was underlying the immaturity of evoked potentials in preterm infants; by contrast brain maturation was explaining the consequent change of cortical electrical dipole [11].

Thanks to the advancements in knowledge of brain maturation we now know that the ingrowths of these connections from the subplate into the cortical plate occurs at the time when very preterm babies are viable to life; during this time so crucial for brain development cortical responses start to emerge following visual, auditory and somatosensory stimulation. The very long depolarization responses with negative polarity shown by all three sensory modalities occur at the time when studies demonstrate the thalamo-cortical fibers waiting in the subplate, while when the thalamo-cortical fibers growth into the cortical plate, the responses become more well-defined peaks, with positive polarity. In the period of coexistence of both the subplate and cortical plate intermediate waveforms may be recorded. Thus in the early stages cortical responses appear to depend from the activation of transient structures and their endogenously generated intermittent activity; sensory input will only gradually begin to take part in shaping the developing circuitry [24].

3.3. Event-Related Potential (ERPs)

Unlike EPs, ERPs are long-latency potentials elicited in response to stimuli requiring some type of cognitive operation, thus providing a unique opportunity to evaluate higher-order cortical processes [61,62]. Whereas evoked potentials reflect the activation of well-defined sensory pathways, ERPs are generated in parietal, temporal and frontal association

areas, rending the identification of their neural generators very difficult. This make ERPs more variable and less reliable, for clinical use in neonatal neurology. Research however showed that specific neuropsychological paradigms can elicit different ERP components, that, although variable, show characteristics according to the supposed underlying cognitive operation stimulated by the paradigm. Interesting examples of ERP components elicited within a specific paradigm are: - the MMN, an automatic change detection response; - the N170, a negative wave elicited in response to facial stimuli; - the P300 wave, a late positive wave recognizable when a subject discriminate a stimulus target from series of standards, etc… [33]

As EPs, ERPs can be elicited using in any kind of sensory modality but auditory stimulation is most frequently used with neonates, because it can be recorded also in sleep [63]. Research in the last decade mainly focused on several versions the auditory change-detection paradigm, the so called "odd-ball". The typical experimental procedure consists of occasionally replacing repetitive "standard" stimuli by a physically "deviant" stimulus, with tones and speech sounds as the more frequently used stimuli [33]. Responses elicited by the standard stimuli allow evaluation of sound perception but, meanwhile a memory trace is automatically formed holding a representation of the standard stimulus [64]; when the deviant occurs, it is automatically compared to the memory trace and, if a difference is detected, the mismatch negativity (MMN), a small negative wave, is elicited [64,65,67,68,69](Figure 1). Thus, the MMN is an automatic and pre-attentive cortical response that reflects auditory discrimination, sensory memory and automatic attention [65,66].

The generators, in adults, are thought to be in auditory cortices, specifically in the supratemporal plane, and in the right frontal area [70,71].

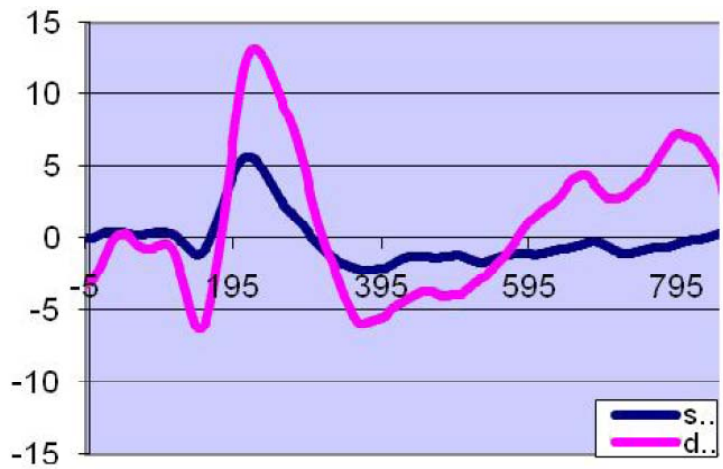

Figure 1. Responses to "standard" (pink trace) and "deviant" (blue trace) stimuli in a odd-ball paradigm of a 38 PCW infant born at 34 PCW.

MMN has been said to develop rather early in comparison to other event-related potential waves [72]. It has even been suggested to be the ontogenetically earliest discriminative response of the human brain [73]. Indeed, an adult-like MMN response has been reported in preterm and full-term newborns and in early infancy [64,67,68,72,74,75,76,77]. However, MMN in newborn infants remains controversial. Although the original studies showed a

frontal-negative difference wave [67,73], its scalp topography differed from adults [78]; other studies have recorded a positive difference wave [79,80]; finally, some authors failed to find a MMN response in normal term infants [67,74,81,82]. Possible explanations of the discrepant findings in the literature are probably differences in methodology and inter individual variability [83,84,85,86]. By comparing the change-detection ability in premature infants reaching term or near term age with that in full-term, atypical pattern of maturation has been described in prematures. Discriminative responses to deviant stimuli are more spread and delayed [67], smaller and more negative [87], atypical or even absent [61], or present only in active sleep [88]. Notably, the immature morphology persists even when the age at measurement is corrected to term [64]. Leppanen et al. [89] reported ERPs maturational changes in the MMN paradigms consisting in positive responses in more mature infants and concluded that maturational patterns could affect ERPs measured in the MMN paradigm; these effects could depend on changes in the infant brain during the pre- and perinatal stages of development [72,]but the developmental timetable of the auditory cortex and the main neural generators of the neonatal MMN, remain at present largely unknown [90].

We can hypothesize that the observed changes in amplitude and polarity of MMN could reflect the rapid development of thalamo-cortical connections, cortical lamination, and synaptic activity in early development [90].

CONCLUSIONS

Correlation between structural and functional brain maturation seem possible using data on EEG, EPs and ERPs maturation. These techniques could be used in the future to differentiate normal from abnormal preterm neonates. Following extremely preterm birth, developmental disability has been described in a high proportion of school age children even in the absence of neurological sequelae [91]. It has been said that the mystery of preterm birth prompts a search for better models [92]. Clinical conditions leading to premature birth may affect the maturation of cortical circuitry consisting in establishment of the thalamo-cortical connections between subplate and cortical plate, thus affecting development of cognitive function. This could explain why premature infants reaching term age may show a delayed maturation or atypical ERPs. Changes may reflect abnormal elaboration of intra cortical auditory circuitry or of long cortico-cortical pathways between auditory and other cortical areas [70,93,88,94.]ERP differences between preterm and full-term infants during the first year of life have been described; the correlation between ERPs and developmental indices at 2 years of age confirms the possibility that ERP could be used as an early indicator of impaired cognitive development [61,95,96,97]. Although few data correlate ERPs in preterm neonates with long-term outcome [96,97], correlations of ERP components with later development have been found in other clinical populations [4,61,97,98].

Identification of early markers of brain dysfunction following preterm birth is needed for prevention of long-term cognitive sequelae.

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Chapter 6

AUDITORY EVOKED POTENTIALS IN RETT SYNDROME: PERIPHERAL AND CENTRAL AUDITORY FUNCTION

Joseph P. Pillion** and Sakkubai Naidu
Kennedy Krieger Institute, Baltimore, USA

ABSTRACT

Rett Syndrome (RTT) is a disorder caused by mutations in the methyl-CpG-binding protein 2 gene (MECP2) located at Xq28 that predominantly affects females. MECP2 mutations account for as many as 96% of cases with the classical features of RTT. RTT is characterized by a progressive loss of cognitive and motor skills, communication disorder, and deceleration of head growth. RTT syndrome is characterized by a period of apparently normal prenatal, perinatal and psychomotor development for the first 6 to 18 months, followed by a period of loss of previously developed language skills and purposeful hand use. Seizures, intermittent hyperventilation, ataxia and stereotypical hand movements develop over time.

The marked impairment in expressive and receptive language in patients with RTT has led to a number of studies investigating the peripheral and central auditory status in patients with RTT. These studies have included measures of the peripheral and central auditory status of patients with RTT. Procedures utilized have included tympanometry, otoacoustic emissions, the auditory brainstem response, middle latency response, long latency response, frequency following response, and long latency auditory evoked potentials. In the literature on RTT, there are conflicting reports as to the presence of abnormalities in the interpeak latency intervals of the ABR. The majority of studies have reported no abnormalities in the ABR interpeak latency intervals in RTT. It has also been reported that the interpeak latency intervals do not change over time in RTT, suggesting that RTT is not characterized by degenerative changes over time. However, several studies have reported prolongation in the I-V or the III-V interpeak latency intervals in RTT and an increased rate of ABR abnormalities has been found in patients with RTT syndrome with seizures requiring use of anticonvulsants. The use of sedation may also

* Address correspondence to: Joseph P. Pillion, Ph.D. Department of Audiology, 801North Broadway, Kennedy Krieger Institute, Baltimore, MD 21205USA.

impact on the ABR in RTT. Evoked potential and other studies have shown that while the majority of patients with RTT have normal peripheral auditory function, hearing loss is present in some patients with RTT. Abnormal or absent middle latency responses and in the late vertex response have been reported and suggest the possibility of central auditory processing disorder in RTT. Atypical developmental patterns in auditory evoked potential responses mediated at brainstem levels (FFR) as well as at cortical levels using a passive oddball task have been reported. These studies will be systematically reviewed in the present chapter. The objective will be to consider the implications of auditory dysfunction as reflected in audiological and evoked potential studies, for the overall speech and language disorder characteristic of individuals with RTT.

INTRODUCTION

Rett Syndrome (RTT) is a neurological disorder caused by mutations in the methyl-CpG-binding protein 2(MeCP2) gene located at Xq28 that predominantly affects females [1]. MECP2 mutations account for as many as 96% of cases with the classical features of RTT. The disorder is characterized by a progressive loss of cognitive and motor skills, communication disorder, and deceleration of head growth [2]. RTT is characterized by a period of apparently normal prenatal, perinatal and psychomotor development for the first 6 to 18 months, followed by a period of loss of previously developed language skills and purposeful hand use [3]. Seizures, intermittent hyperventilation, ataxia and stereotypical hand movements develop over time. RTT occurs with a prevalence of 0.72 per 10,000 female live births [4].

Disordered speech and language is a fundamental component of RTT [5] much as it is the autism spectrum disorder. Girls with RTT typically function at a pre-verbal level in terms of language development [6]. Typically individuals with RTT do not exhibit behaviors indicative of an intention to speak [7, 8] to a greater degree than individuals not diagnosed with RTT and who are functioning at comparable levels of cognition [8]**.** In additions, girls with RTT have been found to exhibit fewer communicative behaviors as compared to similarly delayed children not diagnosed with RTT [8]. More than 50% of patients with RTT produce words before 20 months of age but stop production of words by 40 months of age [9].The majority of cases produce only single words and function at approximately a one year level of development in terms of expressive language [9].The severity of communication disorder appears to be related to the loci of mutation in genes of methyl-CpG-binding protein 2 (MECP2)[9]. These findings suggest that speech and language disorder is a significant component of RTT much the same as it is for ASD.

The marked loss of acquired language skills in girls with RS discussed above has led investigators to question the auditory status of patients with RS. These studies have included measures of peripheral auditory status utilizing measurements of otoacoustic emissions, tympanometry, and the auditory brainstem response. The central auditory processing abilities in RTT have also been examined to a very limited degree utilizing developmentally appropriate behavioral test techniques but primarily with auditory evoked potential measurements.

PERIPHERAL AUDITORY FUNCTION

The assessment of the peripheral auditory status of children and adults with RTT presents challenges as the patients have significant developmental delays and are unable to participate for many of the available conventional audiometric procedures. Consequently, investigators have often utilized objective test procedures that require no behavioral response from the subjects. Measures such as tympanometry to assess the status of the middle ear have been utilized in patients with RTT in several studies [10-14]. It has been suggested that there is a high incidence of otitis media in RTT [10]. Otitis media and associated conductive hearing loss is a frequent problem in school age children [15] and can have a significant impact on language learning and social development in the early years (Hasenstab, 1987). Children and adults with developmental delays have a significant incidence of otitis media with associated conductive hearing loss [16]; [17, 18]. Several genetic syndromes are particularly susceptible to high incidence of otitis media with associated conductive hearing loss. These include Down syndrome [19], Hurler-Hunter syndrome and Turner's syndrome [20]. Measures of acoustic immittance have proved to be especially useful in assessment of the middle ear status of children and adults with developmental delays who cannot effectively cooperate for audiologic assessment of their monaural sensitivity for air and bone conduction. Pelson et al. reported that 6/17 (35%) of their sample of subjects with RTT had evidence of middle ear disease.The subjects ranged in age from 5.7 to 17.4 years. Stach et al. [11] reported that 12/36 (33%) of their subjects with RTT who ranged in age from 2 to 28 years had evidence of middle ear dysfunction. Pillion et al. [12] reported a prevalence of middle ear dysfunction of 27% (18/66 ears) in 34 children with RTT ranging in age from 2 to 15 years. In another study [13], on the basis of abnormal tympanograms and ABR findings, a prevalence of conductive or mixed hearing loss in 17 of 162 ears (10.5%) of 81 subjects with RTT ranging in age from 1 to 39 years of age. In contrast, a low prevalence of abnormal tympanograms was reported by Nicholas et al. who found only one abnormal tympanogram in 10 subjects with RTT who ranged in age from 10 to 28 years [14]. As part of a large study of RTT, measurements of acoustic immittance were undertaken on 62 children and adults with RTT at the Kennedy Krieger Institute. The subjects ranged in age from 2 to 26 with a mean age of 9.58 (SD= 6.85). Measurements of acoustic immittance were obtained with a Virtual model 310 acoustic immittance measurement unit and an Apple Macintosh computer. Measures of static admittance (Peak Ytm in mmho), ear canal volume (Ya in mmho), peak pressure (Pme in daPa) and gradient (in daPa) were obtained. Findingswere compared to normative data [21]. For 27 of the subjects, measurements of acoustic immittance were undertaken after a 90-120 minute period of sedated sleep. Subjects were administered 75-100 mg./kg. of chloral hydrate.

Results for the four tympanometric variable are shown in Table 1. The prevalence of middle ear dysfunction was only 9.67% utilizing normative data [21]. By comparison, a point prevalence of 15.2% has been reported in some developmentally delayed patients (Pillion et al, 1986).Because measurements of acoustic immittance were undertaken after a period of conscious sedation for 27 of the subjects, comparisons were made for the four tympanometric variable for sedated and unsedated subjects. Results indicated for the right ear a significant difference between patients with RTT who were sedated for pressure ($t=-2.57$, $df=57$, $p<.05$), but not for compliance ($t= -033$, $df= 60$, $p>.10$), equivalent volume ($t=-1.49$, $df= 60$, $p> .10$), or gradient ($t=-1.77, 1.77$, $df= 54$, $p>.05$). For the left ear, the difference between sedated and

unsedated patients was significant for pressure (t=-2.40, df=54, p<.05), but not for compliance (t= 1.93, df= 58), p>.05), equivalent volume (t= 0.60, df= 59, p>.10), or for gradient (t= -171, df=51, p>.05). Findings indicate the patients with RTT are susceptible to middle ear dysfunction to a degree roughly comparable with other patients with developmental delays but not to the extent that some patients with syndromes with much higher prevalence of middle ear dysfunction (e.g., Down syndrome).

Table 1. Mean and standard deviations for Compliance, Equivalent Volume, Pressure and Gradient for subjects with RTT

Compliance	Equivalent Volume	Pressure	Gradient
0.73 (0.64)	0.59 (0.23)	-20.80 (89.27)	97.05 (52.40)

Measurements of otoacoustic emissions (OAEs) have allowed investigators to study cochlear function and have also been utilized with patients with RTT. In the absence of middle ear dysfunction or conductive involvement, measurements of OAEs provide evidence indicative of the presence of cochlear dysfunction; specifically, outer hair cell dysfunction. Pelson and Budden[10] reported that 2/17 (12%) of their subjects with RTT had sensorineural hearing loss. Wu et al [22]reported an elevated ABR threshold in one patient with RTT and 3 patients with a delayed Wave I which is suggestive of peripheral hearing loss out of nine individuals. However, Stach et al. [11]found no evidence of peripheral auditory dysfunction in any of the subjects that they were able to complete ABR testing despite their finding that 1/3 of their sample of 36 subjects had middle ear dysfunction in at least one ear. In other study, ABR Wave I abnormalities were found in the presence of normal tympanograms in 6/34 patients with RTT, suggesting the presence of cochlear hearing loss [12]. Pillion et el. reported that bilateral hearing loss was found in 16/81 (19%) patients and unilateral hearing loss in 13/81 (16%) patients [13]. The majority of the hearing loss was in the slight to mild range of hearing impairment. The prevalence of sensorineural hearing loss was estimated to be 17.3% (28 of 162 ears). In addition, the prevalence of hearing loss was increased in the older patients and in those with seizures requiring use of anticonvulsants[13]. The discrepancy between the findings of Stach et al and Pillion et al. may be explained by methodological issues in that Pillion et al. utilized sedation which may have permitted milder forms of hearing loss to be identified. One study utilizing measurements of otoacoustic emissions has shown evidence of outer hair cell dysfunction in patients with RTT [14] who reported that 7/10 individuals with RTT had reduced or absent OAEs, suggesting the possibility of a relatively high proportion of peripheral hearing loss, presumably sensorineural, as compared to that found in their control group consisting of age and sex matched subjects. The prevalence of peripheral hearing loss in individuals with developmental delays has been estimated to be significantly greater, in the range of 10-15%, as compared to the general population [23].

CENTRAL AUDITORY FUNCTION

There is some very limited evidence that deficits in central auditory processing may be present in RTT [24]. Lenn et al [24] examined auditory processing in a 5 year old child with RTT using visual reinforcement infant speech audiometry procedures (VRISD). The child was conditioned to turn in response to a change stimulus presented in the background of a standard stimulus. Normal detection thresholds as well as normal frequency discrimination ability was demonstrated for sinusoidal stimuli. However, discrimination for tones of changing frequency was at chance levels. This study has not been replicated or undertaken in larger numbers of patients with RTT. Other investigators have suggested on the basis of auditory evoked potential studies that there is a "systematic decline" in auditory function in RTT from peripheral to central levels, suggesting the presence of a central auditory disorder [11]. Studies relating to central auditory function in RTT are summarized below.

ABR STUDIES

The early ABR studies in patients with RTT were undertaken when RTT was only beginning to emerge as a diagnostic entity. The numbers of subjects studied in several of the early ABR studies were small including only 5-6 patients [22, 25-27] or nine patients [28, 29]. Studies [10-12, 30] with larger numbers of cases enrolled subjects on the basis of clinical diagnosis of RTT as the importance of mutations in the MECP2 gene was not recognized until 1999 [1]. Information regarding the MECP2 mutation status of the patients was included in only two studies [13, 31]and in only one study [31]were patients suspected of RTT on the basis of their clinical profile excluded on the basis of negative findings upon MECP2 mutation analysis. Many of the studies report sufficient details as to the procedures utilized but several provide only the barest details [22, 25]. Use of sedation was reported in some studies [10, 12]but is not always noted [22, 25-28].

As noted above in the section in the Section on Peripheral Auditory Function, from 0% to 36% per centof patients with RTT has some degree of peripheral hearing loss. This has significant implications for interpretation of the neural integrity of the central auditory pathways on the basis of ABR interpeak latency intervals. For example, in patients with Down Syndrome peripheral auditory dysfunction may be a factor contributing to the shortening of the interpeak latency intervals under some conditions that has been reported [32]. It has been shown that the presence of a peripheral hearing loss can significantly impact on estimates of interpeak latency intervals [33-35]. Given these concerns, it is appropriate to utilize a battery of audiological tests including tympanometry to assess the central auditory status when measurements of the auditory brainstem response are undertaken in patients with DD who are susceptible to hearing loss or middle ear dysfunction [32].

At the Kennedy Krieger Institute a study was implemented to compare the ABR of a group of 71 children and adults with RTT. Auditory evoked potentials were recorded utilizing shielded electrodes affixed at Cz, C3, C4, Fz and on each mastoid. When ABR measurements were conducted, one or two channel concurrent differential recording was employed with the Cz electrode serving as the active electrode and the Fz electrode serving as the ground. Each mastoid served as reference for one of the two recording channels. The auditory stimuli were

delivered monaurally via shielded earphones. ABR measurements were conducted using rarefaction clicks presented at the rate of 8.4/sec. Responses were averaged following filtering (100-3000 Hz), amplification ($2x10^5$ or 10^5) and rejection of artifacts. Results of ABR testing were evaluated with respect to normative data collected at the Kennedy Institute [36], consisting of a group of multiply handicapped, neurologically impaired children with normal peripheral auditory sensitivity. Estimates of peripheral auditory sensitivity were made through consideration of the latency-intensity function and the lowest level at which a response could be identified. Assessment of the integrity of the central auditory pathways included comparison of Wave I, III and V interpeak latency intervals and amplitude ratios with laboratory norms. Measurements of tympanometry were also included.

Table 2. ABR findings for three patient groups (RTT, DD, and Neurodegenerative Disease)

	RTT	DD	Neurodegenerative
Normal ABR	61.2	47.7	51
Peripheral Impairment	12.9	35	6.1
Abnormal Morphology	20	15.9	34.7
Could not test	5.9	1.4	8.2

Subjects were classified into one of four groups: Normal; Peripheral Involvement; Neurological Involvement; and CNT. Subjects with evidence of peripheral hearing loss of mild or greater degree were placed in the Peripheral Involvement group. Subjects were placed into the Neurological Involvement Group if examination of interpeak latency intervals and amplitude ratios exceeded laboratory normal (>2 SDs). Results are summarized in Table 2. Results suggest that ABR abnormalities are found in RTT in that 28% (20/71) of the patients were found to have abnormalities in interpeak latency interval or amplitude relationships which significantly deviated from our clinic norms for one or both ears. Evidence of peripheral involvement of mild or greater degree was found in one or both ears in 14% of patients. 53% of the patients ABRs indicated the presence of normal or near normal peripheral auditory sensitivity, normal interwave intervals and waveform morphology bilaterally. Three subjects could not be assessed due to failure to sedate satisfactorily for testing.

In order to place the above findings in perspective with findings obtained in other groups with developmental disabilities, comparison was undertaken to examine differences among three groups: a) the 71 patients with RTT described above, b) a group of 110 consecutive DD patients seen at the KKI for delineation of their auditory status [37], and c) a group of 49 patients with diagnoses of neurodegenerative disease. The purpose of the analysis was to assess the degree to which ABR findings in patients with RTT differ from patients representing our clinical caseload consisting of predominantly the pediatric DD population and a group of patients with diagnosed neurodegenerative disease. Findings indicate that the RTT group had the highest percentage of normal ABRs in the three groups. The DD group, who were referred for ABR testing primarily due to concerns regarding their auditory status, had the highest percentage of peripheral involvement. The neurodegenerative disease group had the highest percentage of neurological dysfunction as evidenced in the ABR. In another investigation, it has been shown that there were no significant differences in the ABR

interpeak latency intervals (i.e., I-III, III-V, and I-V) consistent with the presence of progressive white matter degeneration in 27 patients with RTT tested at intervals from one to 9 years apart [30].

FFR

The frequency following response is an auditory evoked potential that reflects the waveform properties of the eliciting stimulus. It is evoked by periodic stimuli that are relatively low in frequency (100-500 Hz). It can be elicited by tonal or speech stimuli. Because it occurs in the first 5-6 msec. following the eliciting stimulus, the generators are believed to be at brainstem levels. Unlike the ABR which reflects a synchronous response to transients, the FFR is produced by neurons which are unresponsive to transients but which are phase-locked to the eliciting stimulus [38]. Consequently, while the FFR is generated at brainstem levels, it reflects different neural encoding that that manifested in the ABR. The FFR has been shown to be sensitive to temporal auditory processing deficits in children with specific language impairment (SLI) [39] and has been extensively studied as a measure of auditory processing [40-42]. The FFR has been utilized to assess auditory processing in nine patients with RTT who ranged in age from 26-55 years [43]. The FFR obtained in the patients with RTT were smaller in amplitude and with less consistent waveform synchrony than those obtained in the control group of subjects similar in age. The authors suggested that the responses from the patients with RTT were more similar in morphology to those obtained in infants [43]. The authors related their findings to studies which have posited that RTT is characterized by central nervous system immaturity [44] on the basis of cerebral blood flow studies which have shown reduced flow in patients with RTT. No other studies of the FFR have been undertaken in patients with RTT.

MLR

The middle latency response occurs within the first 100 msec. following stimulus onset. The MLR consists of a series of peaks which are designated Po, Na, Pa, Nb, and Pb. The MLR appears to have multiple generators with major contribution from the thalamocortical pathway with additional contributions from the inferior colliculus and the reticular formation [45]. The auditory reception area in the temporal lobes also makes a contribution to generation of component Pa of the MLR [45]. An absent MLR is considered a good indication of higher auditory dysfunction in patients 10 years of age or older [46] as there are maturational effects that need to be taken into account in younger children [47]. Abnormalities in the MLR have been reported in patients with cortical lesions [48] Component Pa of the MLR is influenced by lesions affecting the auditory cortex and thalamic projections in the temporal lobe[48]. The MLR has been shown to be sensitive to dysfunction in central auditory processing in children [49].

Measurements of the middle latency response have been undertaken in RTT [11, 29]. Bader et al studied the MLR in 7 patients with RTT and found that peak Pa was always present but was delayed in 4 of the patients; the group mean was significantly delayed in

comparison to that found in a control group [29]. On the other hand, Stach et al. were able to measure the MLR in only 52% of ears of 28 subjects; the MLR was present for stimulation of both ears in only 50% of their subjects. It should be noted that in the Stach et al study, measurements of the MLR were undertaken on 36 subjects but data for 8 subjects were not considered valid due to obvious movement artifact. Their subjects were tested while awake and ranged in age from 2 to 28 years with a mean age of nine years [11]. The subjects in the Bader et al study were apparently older and ranged in age from 10 to 22 years [29].

At our facility measurements of the MLR have been undertaken under sedated conditions. All subjects were sedated with chloral hydrate (75 mg./kg.). When measurements of the middle latency response (MLR) were undertaken, the auditory signals were 2000 Hz tone pips with a duration of 10 msec. andrise/decay times of 4 msec. The signals were presented at a rate of 2/sec. Electrodes were affixed at Cz, C3, C4, Fz and on each mastoid. When measurements of the MLR were undertaken, the C3 or C4 electrode location was used for the active electrode and the Fz electrode as ground. Each mastoid electrode served as a reference for one of the recording channels. Responses were averaged following filtering (10-3000 Hz), amplification ($2x10^4$) and rejection of artifacts. The MLR was identified in both ears in 20 of 27 (74%) of subjects and in only one ear in 6 (22.2%) of subjects. In two of the latter patients, the patient awoke from sedation before testing could be completed. Thus, the MLR was present in 46 of 52 ears completed (80.5%). In only one case was the MLR absent or poorly resolved in both ears. The trend appears to suggest that the MLR is consistently present and measureable in patients with RTT who are sedated for testing with only a few exceptions. In contrast, other investigators [11], who tested their patients while awake, have concluded that abnormalities in the MLR and late latency response suggested the presence of a central auditory disorder. Results discussed above do not support that conclusion. It is concluded that movement artifact may make it difficult to record the MLR in awake patients with RTT.

LLR

The components of the auditory evoked response that occur in the 50-650 msec range are variously termed the long latency or the auditory cortical response (ACR). The early components (e.g., component P1) of the LLR reflect stimulus encoding. Later components (e.g., P3) reflect stimulus related information processing at higher neural levels. Bader et al. [29] measured the ACR in 7 patients with RTT. Component N1 of the ACR was identifiable in all subjects although it was at a delayed latency in 2/7 subjects. Component P2 was present in 7/7 of the subjects with delayed or near delays in latency in 3/7 subjects. An oddball paradigm was also utilized in a passive listening procedure with tone burst of 1000 Hz (standard stimulus) and 2000 Hz (rare stimulus) as stimuli. Component P3 was recordable in all of the subjects but delayed in latency overall in the group data; the response to the rare stimulus was enhanced in 2/7 of the subjects. Overall, the authors interpreted their findings for N1 and N2 as indicative that higher level sensory functions are intact in RTT. In addition, Bader et al. [29] suggested that some discrimination function is intact for the tonal stimuli utilized in the investigation.

Stach et al. [11] examined components N1 and P2 of the ACR in response to a 500 Hz tone burst stimulus in 36 patients with RTT. Components N1 and P2 had to be both present and occurring within a specified temporal window for the ACR to be considered normal based upon local normative data. Recording conditions were considered acceptable in only 25/36 (69%) of the patients during recording of the ACR. A measureable ACR was evident in only 10/25 (40%) of the right ears and 8/25 (32%) of the left ears in the patients with RTT. Thus, a significant majority of the patients with RTT had un-recordable or absent ACRs.

McCulloch et al [50] also studied long latency ERPs but in the visual modality. They used ERPs to study basic cognitive visual functions such as discrimination and face recognition. Eleven subjects with RTT who ranged in age from 5-24 years, had repeatable VEPs to flash and pattern visual stimuli, suggesting normal function of the central retina and visual afferent pathways [50]. McCulloch et al [50] also attempted to measure component P3 of the visual event related potential in 8 subjects with RTT who ranged in age from 4-38 years. Discrimination and face recognition paradigms were utilized. Sufficient artifact free trials could be obtained on only 3/8 subjects for the discrimination experiment and 4/8 for the recognition component of the experiment. For both the discrimination and facial recognition protocols, not only was P3 immeasurable but early activity reflecting sensory processing of the stimuli at the posterior electrodes sites could not be measured, suggesting the data were of poor quality. All ERP data were un-interpretable in the subjects with RTT.

Stauder et al [51] examined the development of auditory and visual processing using passive oddball methodology in a group of 17 patients with RTT who ranged in age from 2 to 60 years. A control group of 18 subjects who ranged in age from 4 to 56 years was also included. The subjects were distributed into one of three age groups. Patients with RTT were excluded if they had seizures or severe irregular breathing that could potentially impact on the recordings. Two investigators were present in the room during testing to monitor the subjects and to pause the recordings if the subjects failed to maintain attention to the screen during the visual task or if the patients made sudden movements or closed their eyes.Despite these efforts, data from one subject with RTT was excluded for both visual and auditory tasks, one for the auditory only tasks and one for only the visual tasks. The auditory stimuli were pure tones at 440 Hz (frequent stimulus) and 880 Hz (rare stimulus).The visual stimuli were circles or squares and red or green in color on a black background. The rare visual stimuli were small colored blocks on a white background. For the auditory conditions, component P3 was evident in only the oldest control group. Components P1 and P3 were not present in any of the groups with RTT. The morphology of the averaged responses across subjects was markedly different between controls and patients with RTT across all age groups. For the visual stimuli, component P1 was only present in the control groups. The youngest RTT group did show a reduced response to the novel stimuli. However, the two older groups with RTT did not demonstrate any activation to the novel stimulus. Overall, the authors concluded that the patients with RTT exhibited reduced, slower and less synchronized processing in both visual and auditory modalities. In addition, the patients with RTT did not exhibit the clear developmental changes in ERP components over age groups as seen in the control subjects for any component with the exception of the auditory N2 component.

SUMMARY AND CONCLUSIONS

Speech and language disorder is a significant component of RTT much the same as it is for ASD. Girls with RTT have been found to exhibit fewer communicative behaviors as compared to similarly delayed children not diagnosed with RTT and the severity of communication disorder in RTT appears to be related to the loci of mutation in genes of methyl-CpG-binding protein 2 (MECP2). The marked loss of acquired language skills in girls with RS discussed above has led investigators to question the auditory status of patients with RS. These studies have included measures of the peripheral auditory status utilizing measurements of otoacoustic emissions, tympanometry, and the auditory brainstem response. The central auditory processing abilities in RTT have also been examined to a very limited degree utilizing developmentally appropriate behavioral test techniques but primarily with auditory evoked potential measurements. The prevalence of peripheral hearing loss in patients with RTT has been estimated to range from 0% to 36% in the studies reviewed above. Overall, it appears that the prevalence of peripheral hearing loss in individuals with RTT is significantly greater than what is found in the general population.Patients with RTT are susceptible to middle ear dysfunction to a degree roughly comparable with other patients with developmental delays but not to the extent that some patients with syndromes with much higher prevalence of middle ear dysfunction (e.g., Down syndrome). ABR findings indicate that there is no evidence consistent with the presence of progressive white matter degeneration in RTT. The MLR is consistently present and measureable in patients with RTT who are sedated for testing with only a few exceptions. Movement artifact may make it difficult to measure the MLR in RTT. The difficulty in assessment of patients with RTT in the awake state may be a contributing factor in the variability in findings reported in other EPs in RTT as well. Overall, findings do not support the view that peripheral or central auditory dysfunction is the predominant factor contributing to the marked language disturbance that is one characteristic of RTT.

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Chapter 7

PEDIATRIC EPILEPSY

Batool Kirmani
Scott and White Healthcare;
The Texas A&M Health Science Center College of Medicine, Texas, USA

ABSTRACT

Even in this age of modern medicine, managing pediatric epilepsy still poses a challenge. The seizures seen in childhood are grouped in different types of epilepsy syndromes. The epilepsy syndromes are classified on the basis of age of onset, family history, and type of epilepsy, progressive nature of the disease, EEG abnormalities, precipitating factors, family history, neuropsychological features, underlying genetic abnormalities and prognosis. Once the diagnosis is established, the next step is management. Pharmacological management of pediatric epilepsy is still the main cornerstone. The pharmacological treatment differs in terms of acute versus chronic seizure management. Despite the availability of new anticonvulsants, there is still a portion of pediatric population which remains intractable. These patients may be evaluated for epilepsy surgery or brain neurostimulation at a specialized epilepsy center. Data have shown that effective treatment of epilepsy has improved quality of life and cognitive outcomes in children. This chapter will provide a in-depth review of various aspects of pediatric epilepsy and recent advances in diagnosis and management of this condition.

INTRODUCTION

Management of pediatric epilepsy requires an in depth understanding of the various pediatric epilepsy syndromes and how to approach the epilepsy patient. Pediatric epilepsy syndromes are a group of evident clinical features in epilepsy patients which labels them as part of a specific syndrome. The classification of epilepsy syndromes is based on the Committee on Classification and Terminology of the International League Against Epilepsy (1989) [1] . The diagnosis is established based on the clinical features and diagnostic testing which includes electroencephalography, long term intensive seizure monitoring, magnetic

resonance imaging, positron emission tomography, and functional MRI . In cases where a surgical option is pursued, neuropsychological testing, WADA testing , SPECT and interictal SPECT testing are utilized. Medical management is still the cornerstone with both old and newer anticonvulsants available. Acute seizure management is done as an inpatient. For children with obvious lesion on MRI brain, which includes focal cortical dysplasias , prenatal stroke, tumors or mesial temporal sclerosis, surgery is the better option. Temporal lobectomies, lesionectomies and hemispherectomies, subpial transections resections are the surgical options for intractable epilepsy patients.

APPROACH TO THE PEDIATRIC EPILEPSY PATIENT

A. History

The history can provide clues as to whether the patients have generalized or localized epilepsy or syndrome. Generalized epilepsy syndrome exhibits generalized tonic clonic seizures, and focal seizures are seen with localization related epilepsy. A careful history can further help the physician to further characterize the syndrome. The following areas should be emphasized while taking the epilepsy history from patients and caregivers.

1. Age of Onset

Generalized Epilepsies

The age of onset varies in different types of generalized epilepsies. *Childhood absence epilepsy* is seen in children from age 2 to 13 years of age with the peak incidence between 6 and 7 years old. *Benign neonatal familial convulsions* are seen in the first week of life and benign myoclonic epilepsy is seen between 1 and 2 years of age. Benign neonatal convulsions are also known as fifth day fits . *Lennox Gastaut syndrome* begins between the ages of 1 and 6 years of age and persists during adulthood. Progressive myoclonic epilepsies begin in childhood but some forms do occur during adolescence. *Juvenile absence epilepsy* and *Juvenile myoclonic epilepsy* as the names suggest begin during adolescence between 12 and 18 years of age. Juvenile absence epilepsy can be seen in children as early as 10 years of age. *Epilepsy with generalized seizures on awakening* is seen in children between 8 and 15 years of age [2].

Localization Related Epilepsies

Mesial temporal epilepsy with hippocampus sclerosis, familial temporal lobe epilepsy and autosomal dominant frontal lobe epilepsy are seen in childhood or early adolescence. *Idiopathic childhood occipital epilepsy* is seen in children from 3 to 16 years of age. *Panayiotopoulas syndrome* is seen between 1 and 14 years of age. The Panayiotopoulos syndrome occurs in children around 1 and 14 years of age [2].

2. Childhood Medical History

Birth history is important in localization related epilepsies. On careful interview of the parents or the caregivers, there is usually a history of febrile seizures, meningitis, stroke, head

trauma, encephalitis, developmental delay or family history of seizures. This provides important information in case a surgical option is pursued later.

3. Seizure Semiology

Generalized Epilepsies

Careful history is important regarding the seizure semiology. In *Juvenile myoclonic epilepsy,* the adolescent gives a typical history of being "clumsy" in the morning. Further history shows the presence of myoclonic jerks. Generalized tonic clonic seizures are triggered by stress, alcohol intake, photosensitivity, sleep deprivation and fatigue [3]. Generalized tonic clonic seizures are also seen within 30 minutes of awakening with *epilepsy with generalized tonic clonic seizures on awakening*. These children have a different type of generalized seizures including absence, tonic, atonic, myoclonic and generalized tonic clonic in generalized seizures with febrile seizure plus. Complex partial seizures are also seen such as *Lennox Gastaut syndrome*, *progressive myoclonic epilepsies, and west syndrome i*n addition to the seizure types described above with generalized seizures with febrile seizure plus. Abrupt behavior arrests with occasional automatisms are seen in children with brief staring spells in *childhood absence epilepsy* as well as generalized tonic clonic seizures. Similar presentations are seen in Juvenile absence epilepsy but absence seizures are seen less in childhood absence epilepsy [3,4].

Localization Rrelated Epilepsies

Focal motor seizures localized to the face and upper limbs with preserved consciousness are seen with *Benign childhood epilepsy with centrotemporal spikes*. The seizures seen with this syndrome are usually nocturnal in nature and duration is 2 minutes. Autonomic seizures are seen with *Panayiotopoulos syndrome*. These seizures begin with nausea, vomiting, flushing, hypersalivation, and urinary incontinence without alteration of awareness. The initial phase is followed by unresponsiveness and prolonged focal seizure which may last for 30 minutes (autonomic status epilepticus). Visual seizures are seen with *idiopathic childhood occipital epilepsy* with and without secondary generalization [4]. *Temporal lobe epilepsy with hippocampal sclerosis* results in focal seizures. The seizures begin as simple partial seizures (aura) without alteration of awareness. The simple partial seizures are brief and are characterized by déjà vu sensation, rising epigastric sensation, intense fear, gustatory or olfactory sensations. These are followed by complex partial seizures with alteration of consciousness, oroalimentary automatisms, dystonic posturing of the limb and limb automatisms which include fumbling of the fingers and picking hand movements. Some patients try to move aimlessly and become combative if attempts are made to restraint them. These seizures may also secondarily generalize. The duration of these seizures is 1 to 3 minutes followed by postictal confusion and amnesia which may range from 5-10 minutes to half an hour [5-8]. Lateral temporal seizures are seen with familial temporal lobe epilepsy. The auditory hallucinations (simple partial seizures) characterized by tinnitus or buzzing are typical for lateral temporal seizures followed by alteration of awareness (complex partial seizures) [5]. Frontal lobe epilepsy is characterized by brief nocturnal seizures with bizarre body movements. Frontal lobe seizures rapidly generalize and there is no postictal confusion. These seizures can easily be confused with pseudoseizures which are a form of conversion

disorder [9]. *Supplementary motor area seizures* show asymmetric posturing of the limbs, brief in onset and consciousness is preserved [10].

Parietal lobe seizures begin with a simple partial seizure with somatosensory sensations which includes tingling, numbness, pain or shock like sensation contralateral to the seizure focus [11].

4. Seizure Frequency

The parents and caregivers are advised to maintain a seizure diary. Appropriate medical management is possible only if the frequency of seizures is known.

5. Failed Anticonvulsants

A careful history is required regarding failed anticonvulsants before making any adjustments in the medication regimen. Patients failing more than two anticonvulsants and are still having seizures fulfills the criteria for intractability. These patients may prove to be good surgical candidates.

B. Physical Examination

Careful physical examination is extremely important in the determination of a particular epilepsy syndrome based on the associated neurological findings.

Focal neurological deficits are seen in localization related syndromes due to childhood strokes or tumors.

Mental retardation is seen with *Lennox –Gastaut syndrome, West syndrome,* and *Progressive myoclonic epilepsies* which include *Unverricht-Lundborg disease, Lafora's disease, Neuronal Ceroid Lipofuscinosis and myoclonic epilepsy with ragged red fibres.*

Progressive myoclonic epilepsies are devastating group of epilepsy syndromes. The neurological manifestations seen in *Unverricht-Lundborg disease* includes ataxia, intention tremor, dysarthria, and progressive decline in mental status. The myoclonus can be so severe that the patient develops difficulty with gait, speech and swallowing. *Lafora's disease* is characterized by visual loss, apraxia, dementia and ataxia. Visual loss , ataxia, dysarthria, severe myoclonus and extrapyramidal symptoms are seen in different types of *neuronal ceroid lipofuscinoses.* Dementia, ataxia, myopathy, lactic acidosis, dystonia neuropathy, optic atrophy and lower motor neuron abnormalities are seen in *Myoclonic epilepsy with ragged red fibres* [12].

Café au lait spots are seen with *neurofibromatosis*. *Tuberous sclerosis* can be diagnosed with careful physical examination based on the presence of adenoma sebaceum and hypomelanotic spots.

C. Diagnostic Tests

a. EEG

The EEG is an important diagnostic tool in epilepsy. The EEG test gives us a clue that the syndrome is either localized or generalized.

Localization Related Epilepsies

Benign childhood epilepsy with centrotemporal spikes (BECTS) is named so because of the characteristic electroencephalographic findings. Spikes are seen in the central and temporal regions which becomes more prominent during drowsiness and sleep. These spikes have characteristic morphology and are identified by the presence of a polyphasic dipole tangential to the rolandic region [13]. In *Panayiotopolous syndrome* , spikes are similar in morphology to BECTS but occipital spikes predominate in this syndrome. High amplitude occipital spikes are seen *idiopathic childhood epilepsy* [4] *Mesial temporal epilepsy with hippocampal sclerosis* and *familial temporal lobe epilepsy* are characterized by focal slowing and spikes in the temporal regions. Ictal EEG shows rhythmic 4-5 Hz theta activity in the anterior mid temporal regions [5]. The EEG is normal in frontal lobe epilepsies.

Generalized Epilepsies

Generalized discharges are seen in generalized epilepsies but the patterns differ depending on the type of syndrome. *Childhood absence seizures* are characterized by 2-4 Hz generalized frontally dominant spikes and wave discharges. In *Juvenile myoclonic epilepsy,* 4-6 Hz frontally dominant spikes and polyspikes and wave discharges are seen. No evidence of generalized epilepsy is seen Benign neonatal convulsions [3,4,14]. Generalized polyspike discharges are seen with myoclonic jerks on EEG in *Benign myoclonic epilepsy of infancy* [15]. *Lafora body disease* is characterized by generalized epilepsy with diffuse slowing in the earlier stage followed by extremely disorganized background with continuous multifocal epileptiform discharges. EEG shows generalized epileptiform discharges in *neuronal ceroid lipofuscinoses* [14,16].

Lennox –Gastaut syndrome has a typical EEG pattern characterized by the presence of generalized frontally dominant slow wave discharges in the range of 1.5 -2.5 Hz [17].

b. Ambulatory EEG

Ambulatory EEG is important to rule out spells which are suspicious for seizures by the presence or absence of epileptiform discharges. Non-epileptic spells are diagnosed by the absence of epileptiform abnormalities [18]. Patients with diagnosed epilepsy can also benefit from this test as this may ascertain the frequency of seizures if the parents or caregivers are not sure. This will help in the appropriate adjustment of anticonvulsant medications. The pitfall of the outpatient ambulatory EEG is the quality of the study since it is difficult to keep the leads in place in children. Another pitfall is that the patients cannot be withdrawn from their medications which results in masking the epileptiform discharges. So, this test cannot be used for accurate presurgical localization.

c. Long Term Video EEG Monitoring in the Epilepsy Monitoring Unit

Video- EEG Monitoring is essential for localization and characterization of seizures for the epilepsy surgery workup. The advantage of this test over ambulatory EEG is that the patients can be withdrawn from their anticonvulsants so that adequate and reliable data are available to accurately diagnose the type of epilepsy and to rule out non-epileptic spells. Video- EEG Monitoring in a fully equipped epilepsy monitoring unit is an essential part of surgical work up of patients if the surgical work is pursued based on the intractable nature of

the epilepsy. The definition of intractability is uncontrolled epilepsy despite adequate trials of two or three anticonvulsants [19].

The children evaluated for surgery are accompanied by parents or caregivers. The medications are withdrawn completely or decreased depending on the intractable nature of the disease. The goal is to capture at least 4 to 5 seizures to determine the seizure onset. The EEG data under medication withdrawal determines the extent of interictal epileptiform abnormalities and ictal localization of the seizure focus.

The semiology of seizures is determined by the video EEG monitoring. The localization of seizures determine the specific semiology. Temporal lobe seizures of mesiotemporal onset are characterized by an aura of rising epigatric sensation, déjà vu feeling, olfactory aura, followed by alteration of awareness and oroalimentary automatisms. Limb automatisms are seen ipsilateral to the seizure focus and dystonic posturing contralateral to the seizure focus. Lateral temporal seizures are different since they are characterized by auditory aura. EEG shows rhythmic theta activity in the temporal leads. Interictal EEG shows temporal spikes [5-8]. Frontal lobe seizures are short, nocturnal with bizarre body movements. They have little or no postictal confusion. Scalp EEG is usually normal so they may be confused with pseudoseizures [9]. Occipital lobe seizures are characterized by visual hallucinations or loss of vision with forced blinking. Interictal EEG may show occipital spikes and ictal EEG may show focal rhythmic activity in the occipital regions [20].

Parietal lobe seizures have somatosensary aura. Language disturbance is seen in the dominant hemispheric involvement and neglect in case of non-dominant hemisphere. Epileptiform discharges are poorly localized [11]. In generalized epilepsies, staring spells are seen in absence epilepsies and myoclonic jerks are evident in juvenile myoclonic epilepsy. Typical tonic, atonic and clonic movements are captured on the video EEG recording. Ictal EEG consists of generalized rhythmic spike and wave activity, the frequency of which differs depending on the type of syndrome (3).

d. Intracranial EEG

Intracranial EEG is required in case of non localization of seizure onset on scalp recordings. Subdural grids , subdural strip and depth electrodes are used for this purpose for precise localization of the seizure focus. Intracranial EEG is also important in extratemporal and dominant hemisphere epilepsy cases to map the language, memory and frontal cortices to minimize deficits post surgery. The contacts on the subdural grid electrodes are stimulated for functional brain mapping. Minor complications occur in 1 to 2 % of the patients undergoing intracranial EEG monitoring [21].

e. Imaging Tests

Magnetic Resonance Imaging (MRI)

MRI Brain is the most important tool to detect the seizure focus and to rule out structural abnormalities. Special seizure protocols are used in the epilepsy centers to detect these lesions. T1 sequences with thin cuts are used to detect the lesions like cortical dysplasias, hamartomas, polymigrogyria, schizencephaly, arteriovenous malformations, cavernous hemangiomas and low grade tumors seen in younder children. The availability of 3-tesla magnets greatly improved the quality of scans in the recent years [22]. Mesial temporal

sclerosis can also be detected by special techniques and is more common in adolescents and adults. These patients are excellent candidates for epilepsy surgery [23]. Localization of lesions is extremely important in order to pursue surgical work up for intractable epilepsy.

SPECT

The SPECT scan measures the distribution of radioactive isotopes in the brain. There are two types of SPECT scans used for the epilepsy surgery work up. The interictal SPECT which measures the distribution of radioisotopes in the resting state as compared to ictal SPECT where the injection should be administered within 30 seconds of seizure onset. The ictal SPECT has much higher yield than interictal SPECT [24].Regions of hypometabolism are seen with ictal SPECT and hypometabolism with interictal SPECT (25).

Positron Emission Tomography (PET)

A PET scan detects changes in brain glucose metabolism. It has been shown that epileptic foci have decreased glucose metabolism as compared to the non-epileptogenic regions of the brain. PET scan can detect focal dysplasias which are common causes of pediatric epilepsy. These dysplasias can be missed on magnetic resonance imaging. The most commonly employed tracer is 18 –flourodeoxyglucose [25].

Functional Magnetic Resonance Imaging

Functional MRI shows that metabolically active areas have high ratios of oxygenated to deoxygenated hemoglobin secondary to increased neuronal activity in the brain. The functional MRI is employed in the epilepsy centers to localize language as part of pre-surgical work up. It is used to supplement WADA testing [26].

f. Neuropsychological Testing

Neuropsychological testing in children is important in order to assess neurocognitive deficits due to chronic epilepsy. It includes a series of neuropsychometric testing. Neuropsychological testing is extremely important in the pre-surgical work up of epilepsy since it localizes the deficits in the areas of memory and language which are extremely important to predict post surgery outcome [27].

Intracarotid Amobarbital Test (WADA Test)

The WADA test localizes memory and language and determines cerebral dominance. A routine angiogram is done by an interventional neuroradiologist. During the procedure, sodium amobarbital is injected in the carotid artery. This leads to the development of hemiparesis. Language dominance is determined by the development of aphasia, and memory is tested during hemiparesis. The procedure is again repeated with the other carotid artery. WADA testing is difficult in younger children because of behavioral problems and anxiety issues. Simplified protocols are used in those instances [28-30]. It also has been recommended to obtain the help of a pediatric psychologist to reduce anxiety in children [31,32].

D. Management of Pediatric Epilepsy

a. Medical Management

Acute Management

Children with epilepsy are frequently admitted to the hospital because of status epilepticus or acute exacerbation of seizures. Status epilepticus is defined as continuous seizure activity or recureent seizures without recovery of consciousness for 30 minutes [33]. The new definition is single seizure or recurrent seizure lasting for 5 minutes. The definition of acute repetitive seizures is seizures that recur over a set period of time, typically hours in children [34].

The acute management starts with general measures which includes establishing the diagnosis of status epilepticus or acute repetitive seizures, assess the patient's airway and oxygenation and circulation and obtain intravenous access. The next step is to obtain IV access and assess circulation. Once the above procedures are accomplished, etiology should be assessed by laboratory testing including blood count with differential, serum electrolytes, blood urea nitrogen, creatinine, serum toxicology screen, anticonvulsant levels and blood alcohol levels. Thiamine should be given to prevent Wernicke's hypoglycemia.

Anticonvulsant therapy should be initiated as soon as possible and continuous EEG should be arranged to rule out non-convulsive status epilepticus. The first line agents include benzodiazepines. These include intravenous lorazepam and diazepam. Lorazepam can be administered at a rate of 1-2 mg/min (0.1 mg/kg). The maximum dose is 8 mg. Rectal diazepam can also be used 0.5 mg/kg but is more effective in acute repetitive seizures. Consider loading with intravenous phenytoin or fosphenytoin 20 mg/kg. The above measures should be completed within 30 minutes. The other choices are midazolam and Phenobarbital. Midazolam can be used in a intravenous dose of 0.1-0.3 mg/kg within 5-10 minutes. It has been shown to abort seizures within one minute. If the seizures still continue, the choices are pharmacologically induced coma by pentobarbital, propofol or thiopentane. The last choice is neuromuscular blockade by or halothane administration by an anesthesiologist [35]. Newer antiepileptic medicines which are not approved by FDA are still used as choices based on the literature data. These agents include valproate and levetiracetam. Valproic acid was found to be effective in controlling refractory status epilepticus as an alternative to diazepam in an open label , randomized, controlled study conducted by Mehta and colleagues in children. No hepatotoxicity is seen with valproic acid [36]. Intravenous levetiracetam has been found to be effective in the treatment of status epilepticus and acute repetitive seizures. Data have already been published in different age groups regarding the safety and efficacy of levtiracetam in children. It has been shown in several retrospective studies that intravenous levetiracetam has been effective in treating status epilepticus and acute exacerbation of seizures in children and neonates [37-39]. However, randomized, blind comparison trials for newer anticonvulsants are needed in order to change the protocol of status epilepticus in the future.

Chronic Management

Chronic management with anticonvulsants is needed for adequate control of seizures in patients with a well diagnosed history of epilepsy.

These include first generation anticonvulsants which includes phenytoin, carbamazepine, Phenobarbital, primidone, valproic acid, ethosuximide and benzodiazepines. The second generation newer anticonvulsants include felbamate, lamotrigine, gabapentin, topiramate, levetiracetam, oxcarbazepine, tigabine, pregabalin and newly approved anticonvulsant lacosamide.

First Generation Anticonvulsants -Phenytoin is a sodium channel blocker and is used in the management of tonic clonic seizures and partial seizures [40]. Gingival hyperplasia, acne and hirsutism are the common side-effects of phenytoin therapy. Carbamazepine is also a sodium channel blocker and used to treat simple partial, complex partial seizures with and without secondary generalization. It is also used to treat generalized tonic clonic seizures but should not used in patients with myoclonic and absence seizures. It has been shown in the literature that carbamazepine causes worsening of myoclonic and absence seizures [40]. Common side-effects include drowsiness, diplopia, blurred vision, and vertigo. Immusuppression is also reported with carbamazepine. Phenobarbital is the oldest anticonvulsant and has been used since 1912. Phenobarbital is also a sodium channel blocker like phenytoin and carbamazepine and is used to treat tonic clonic and complex partial seizures. Phenobarbital is effective in neonatal seizures and status epilepticus [41]. The common side-effects include increase sedation in adults and hyperactivity and irritability in children. Primidone is similar to Phenobarbital in treating epilepsy. Valproic acid acts on the GABA receptors and causes blockage of the sodium channel. It is the first line of treatment in primary generalized epilepsies. These include generalized epilepsy syndromes like juvenile myoclonic epilepsy, juvenile absence epilepsy and other syndromes associated with absence seizures. Valproic acid has teratogenic effects and spina bifida is seen in children exposed to valproate. The common side effects include gastrointestinal disturbances, drowsiness, hair loss and tremor [42].

Ethosuximide is a T-type calcium blocker and is effective in childhood absence seizures [41].

Benzodiazepines inhibit GABA receptors and should not be used as first line treatment of epilepsy. Clonazepam is used as adjunctive therapy for generalized epilepsies but not as effective for partial seizures [43]. Clorazepate can be used for both generalized and absence seizures.

Second generation anticonvulsants - Felbamate is used for partial epilepsy and in Lennox gastaut syndrome. Felbamate has serious side-effects including fatal aplastic anemia (1 per 3000) and hepatic failure (1 per 10,000) which make it a less popular drug [44].Lamotrigine is a broad spectrum second generation anticonvulsant used in the treatment of primary generalized and partial epilepsy. It has also been shown to be effective in seizures associated with Lennox Gastaut syndrome. Risk of Stevens Johnson syndrome is more common in children younger than 16 years with concomitant valproate therapy. Lamictal causes sodium channel blockade. Gabapentin has been approved by FDA for adjunctive therapy in children aged 3 to 12 years for the treatment of partial epilepsy. Common side-effects include dizziness, fatigue and sometimes weight gain which is dose related. Gabapentin increases release and synthesis of GABA. Topiramate has been approved as adjunctive therapy in children aged 2 to 6 years for both partial and primary generalized epilepsies and seizures associated with Lennox Gastaut syndrome. The common side-effects include weight loss, paraesthesias, cognitive slowing and dizziness. In children, renal stones, open angle glaucoma and hypohidrosis is seen. In rare cases, non anion gap metabolic acidosis has been reported in

children. Oxcarbazepine is also a sodium channel blocker used in the treatment of partial epilepsy. Tigabine is also effective in the treatment of partial epilepsy.

Levetiracetam was first approved as adjunctive therapy for partial seizures but later received approval for primary generalized and juvemile mypclonic epilepsies. The common side-effects include irritability, somnolence and dizziness. Zonisamide and pregabalin are also used for partial seizures [45,46]. The choice of the anticonvulsant depends on the patient's history, type of epilepsy, other medical comorbidities or cost constraints.

b. Surgical Management

This includes epilepsy surgery in intractable cases. The type of epilepsy surgery depends on the type of lesions. Focal lesions such as low grade tumors and focal cortical dysplasias are treated by lesionectomies. Total resection of the lesion results in seizure freedom in 80% of the patients [47]. Functional mapping is required prior to resction. Hemispherectomy is done in patients with Sturge-Weber syndrome, prenatal stroke or Rasmussen encephalitis. Anterior temporal lobectomy and amydalo-hippocampectomy are the common surgical procedures in patients with mesial temporal sclerosis which is seen in adolescents and adults. Callostomy which is the division of anterior two thirds of the corpus callosum and posterior one thirds if needed to prevent secondary generalized seizures, Lennox Gastaut syndromes and drop attacks. Subpial transections are palliative procedures where respective surgery is not possible. In subpial transections, the lateral cortical connections are disconnected to prevent the spread of epileptiform discharges. This procedure is used with Landau-kleffner syndrome and is found to be beneficial [48].

Cerebral Stimulation

This includes chronic stimulation of the anterior nucleus of the thalamus and the subthalamus. Deep brain stimulation is still awaiting approval from the FDA for use in epilepsy patients. Deep brain stimulation causes disruption of the cortical reticular network which results in seizures. It has been shown to be effective in both generalized and partial epilepsy [49].

It has been reported that a 30-50% decrease in refractory partial and secondary generalized seizures is seen with vagal nerve stimulation. Vagal nerve stimulation is used for complex partial seizures with and without secondary generalized seizures. There are not enough data to conclude if it is beneficial in generalized epilepsy. Stimulation parameters can be varied to achieve better seizure control [50].

CONCLUSION

Despite the treatments available, more basic research and clinical trials are needed to uncover hidden aspects of pediatric epilepsy in order to achieve better control.

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Chapter 8

CEREBROSPINAL FLUID LEVELS OF CYTOKINES AND CHEMOKINES IN PATIENTS WITH WEST SYNDROME

Gaku Yamanaka*, Hisashi Kawashima, Tasuku Miyajima, Shingo Oana, Yu Ishida, Yasuyo Kashiwagi and Akinori Hoshika
Department of Pediatrics, Tokyo Medical University,
6-7-1 Nishi-Shinjuku, Shinjuku, Tokyo, Japan

ABSTRACT

We assessed the cytokine and chemokine profile of cerebrospinal fluid (CSF) in patients with West syndrome (WS) to elucidate whether an immunological processes are concerned with the pathophysiology of WS. We analyzed CSF levels of twelve patients with WS, influenza-associated encephalopathy (IE), as a representative disease with high cytokines storms and twelve controls (cont). All samples of CSF were obtained the first 24 hours after the tonic spasms with informed consent. Seventeen items were measured using the Bio-Plex Multiplex Cytokine Assay, Interleukin (IL)-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, IL-13, IL-17, G-CSF, GM-CSF, INF-γ, MCP-1, MIP-1β and TNF-α.

Results: All of the cytokine and chemokine levels in the WS groups were significantly higher than those in the cont group. The CSF levels of IL-2, IL-6, IL-8, IL-10, G-CSF and IFN-g in the WS group were significantly lower than those in the IE group. In a comparison of symptomatic and cryptogenic patients, the IL-12 levels of patients with symptomatic were significantly higher than those of cryptogenic patients. In a comparison of AED-response and ACTH-response group, the IL-12 levels of the ACTH-response group were significantly higher than those of AED-response group. Our results demonstrated that the increased CSF levels of cytokine and chemokine profile in patients with West syndrome. The CSF IL-12 levels may be a valid predictive marker of the etiology, and became the basis for determining the direction of treatment in patients

* Correspondence: Gaku Yamanaka, MD, Department of Pediatrics, Tokyo Medical University, 6-7-1 Nishi-Shinjuku, Shinjuku, Tokyo 160-0023, Japan. Phone: +81 3 3342 6111. Fax: +81 3 3344 0643. E-mail: gaku@tokyo-med.ac.jp.

with WS. A larger study should be conducted to clarify which cytokines are mainly concerned with the pathophysiology of WS.

Keywords: cytokine, epilepsy, West syndrome, infantile spasm, ACTH, IL-12.

INTRODUCTION

Many studies with experimental models and humans have shown that immunological processes and oxidative stress are involved in the pathogenesis of epilepsy [1-3]. In rodents, epileptic seizures induced rapid expression of cytokine mRNA in glial cells, and intrahippocampal injections of interleukin (IL) -1β enhanced seizure duration [4]. In adult patients with epilepsy, an increased IL-6 level in the cerebrospinal fluid (CSF) and serum has been observed several hours after partial or generalized tonic-clonic seizures [5-7].

West syndrome (WS) is an age-dependent epileptic encephalopathy characterized by *tonic spasms* and a particular pattern of hypsarrhythmia on electroencephalography (EEG) and psychomotor delay or arrest. Recently, immunological analyses have been conducted in patients with WS [8-11]. The evaluation of the serum cytokine levels revealed higher levels of serum IL-2, tumor necrosis factor (TNF)-α, and interferon (IFN)-α [8], IL-1 receptor antagonist (RA), IL-5, IL-6, and IL-15 in patients with WS than in the control subjects [10]. A recent our clinical and EEG assessment of WS patients showed elevated serum IL-1RA levels [12]. Haginoya et al evaluated the CSF cytokine levels in patients with WS and reported lower IL-1RA levels in the WS group than in the control group [13]. It has been shown that the clinical symptoms of WS are transiently alleviated by viral infection [14,15] and intravenous gamma-globulin treatment successfully cures WS in some patients [8, 13, 16]. Although some studies have implicated the involvement of immunological processes in the pathophysiology of WS, its pathogenesis is thus far unclear. Therefore, in this study, we aimed at assessing whether immunological processes are associated with the pathophysiology of WS, and hence, we examined the cytokine and chemokine levels in the CSF of patients with WS.

MATERIALS AND METHODS

Twelve Japanese patients with WS (5 cryptogenic, 7 symptomatic), eight influenza-associated encephalopathy (IE), as a representative disease with high cytokines storms and twelve controls (cont) were enrolled in this study (Table 1). The diagnosis of WS was made based on observations of typical tonic spasms and hypsarrhythmia. The mean age of patients was 9.7±0.1 months (range 5—15 months). All of the CSF sampling was carried out before improvement of both of tonic spasms and hypsarrhythmia by medical treatment within 24 hours after the tonic spasms. Although some anticonvulsants (AEDs) such as vitamin B6, sodium valproate, and zonisamide were used parenterally, these samples were obtained before adrenocorticotropic hormone (ACTH) administration. We defined the group showing improvements in clinical features and electroencephalographic (EEG) findings in response to AEDs as the AED-response group and the group showing improvements in response to ACTH, as the ACTH-response group.

IE was defined clinically and totally by the following features: seizures, impaired consciousness lasting 24 hours and cerebral edema. Influenza virus infection was proved in all children by using an influenza rapid detection kit and/or usual viral isolation from the throat. CSF sampling was conducted within three days after the onset of seizures or unconsciousness, respectively. The control samples were obtained from patients on whom lumbar puncture was performed to exclude neurological and central infectious disease. At the time of admission CSF samples were obtained after receiving informed consent from the patients and stored below −20 °C until use, all had normal cell counts and glucose levels.

Seventeen items were measured using the Bio-Plex Multiplex Cytokine Assay, Interleukin (IL)-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, IL-13, IL-17, G-CSF, GM-CSF, INF-γ, MCP-1, MIP-1β and TNF-α. Statistical differences between three independent groups were tested using the nonparametrick Kruskall Wallis test followed by paired t testing the Mann-Whitney U test, p-value of less than 0.05 was considered significant. The results were expressed as mean (SE).

Table 1. Clinical characteristics of patients with West syndrome

Case	Age (months)	Gender (M/F)	Etiology	An Effective Treatment
1	7	M	Cryptogenic	AED
2	11	F	Cryptogenic	AED
3	14	M	Bacterial meningitis	AED
4	7	F	LBW	AED
5	14	M	Asphyxia	AED
6	9	F	Cryptogenic	ACTH
7	9	M	Cryptogenic	ACTH
8	6	M	Cryptogenic	ACTH
9	7	F	CMV infection	ACTH
10	5	F	PEHO syndrome	ACTH
11	15	M	ELBW, VD	ACTH
12	12	F	PVL	ACTH

ELBW, Extremely low birth weight baby; LBW, low birth weight baby; VD,Ventricular dilatation; PVL, Periventricular leukomalacia; CMV, Cytomegalovirus; AEDs, anticonvulsants; ACTH, adrenocorticotropic hormone; PEHO syndrome;Progressive Encephalopathy with (O) Edema, Hypsarrhythmia and Optic atrophy.

RESULTS

All of the cytokine and chemokine levels in the WS and IE groups were significantly higher than those in the cont group. The CSF levels of IL-2, IL-6, IL-8, IL-10, G-CSF and IFN-g in the WS group were significantly lower than those in the IE group (Table 2). In a comparison of symptomatic and cryptogenic patients, the IL-12 levels of patients with symptomatic (average 37.6 ±19.7, range= 1.59-154) were significantly higher than those of cryptogenic patients (average 9.9 ±3.9, range= 0.43-17.5 pg/ml). In a comparison of AED-response and the ACTH-response group, the IL-12 levels of the ACTH-response group (average 29.1±21.0, range=0.43-154 pg/ml) were significantly higher than those of AED-response group (average 21.7±2.6, range=16-30 pg/ml) (Figure 1).

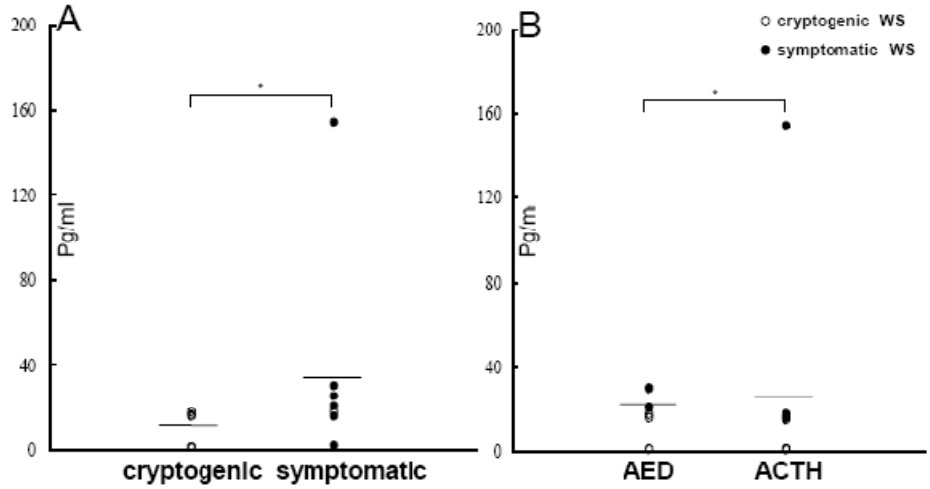

Figure 1. The IL-12 levels were compared between symptomatic and cryptogenic patients with WS: the IL-12 levels were significantly higher in the former than in the latter. Similarly, the IL-12 levels were significantly higher in the ACTH-response group than in the AED-response group. The CSF IL-12 levels in the patients with the cryptogenic (n = 5) and symptomatic groups (n = 7) (A), of the ACTH-response (n = 7) and AED-response group (n = 5) (B). Median values presented as arrows.

Table 2. The CSF levels of all the assessed cytokines and chemokines were significantly higher in the WS group than in the cont group (*). The CSF levels of IL-2, IL-6, IL-8, IL-10, G-CSF, and IFN-□ in the WS group were significantly lower than those in the IE group (**)

	WS	IE	cont
IL-1β*	171.0±72.5	251.6±121.3	0.36±0.1
IL-2**	20.0±6.3	53.2±16.4	0.35±0.1
IL-4*	82.7±26.1	36.9±10.1	0.2±0.1
IL-5*	11.3±2.8	14.2±3.3	0.1±0.
IL-6**	269.7±142.8	3604.1±1913.0	8.2±1.24
IL-7*	45.6±28.4	58.2±20.6	1.9±0.5
IL-8**	1833.3±1003.1	10291.8±3935.0	13.4±1.7
IL-10**	140.8±48.9	202.5±54.3	1.2±0.1
IL-12*	26.0±11.9	46.11±13.5	1.0±0.2
IL-13*	21.2±9.6	71.4±58.4	0.3±0.2
IL-17*	86.8±25.3	130.6±31.9	3.4±0.3
G-CSF**	50.9±25.7	868.0±537.8	20.0±5.3
GM-CSF*	119.0±24.8	132.9±31.9	3.9±0.5
IFN-γ**	50.9±25.7	148.6±50.9	14.5±3.6
MCP-1*	9012.4±2256.3	17000.8±4065.2	911.4±338.2
MIP-1β*	529.7±220.9	418.9±111.1	7.5±0.6
TNF-α*	70.7±44.0	184.3±67.0	14.7±3.3

No significant differences were noted between the WS and IE groups with respect to the other cytokine levels.

DISCUSSION

In the present study, we analyzed the CSF cytokine levels and found higher levels of various cytokines and chemokines in patients with WS exhibiting tonic spasms than in the control subjects. Although an immunological alteration in the serum level has been suggested in patients with WS [8, 10-12], only a few studies have reported the analysis of CSF cytokine levels in patients with WS. However, these previous studies have not reported a significant difference was in the CSF levels of IL-lβ, IL-6, and TNF-α in patients with WS except for a significant decrease in the IL-1RA levels of WS patients [13, 16]. These results are in disagreement with our findings. The time course and extent of the CSF cytokine changes after epileptic seizures, and the factors that influence these changes are not well defined, which makes the assessment of these changes complicated. Recently, it was found that in patients with localization-related epilepsy, the IL-1RA levels in the CSF increased significantly with a decrease in the IL-1β levels, as observed in the samples obtained within 24 h after the seizure [7, 17]. These results support the fact that the time of sampling is important to accurately determine the cytokine levels. In our study, CSF sampling was carried out within 24 h after tonic spasms; the timing of sampling might be responsible for the difference in the cytokine levels between the studies.

In comparison with patients with IE, whose pathophysiology includes intense inflammatory reactions, our patients with WS showed lower levels of some cytokines such as IL-2, IL-10, G-CSF, and IFN-γ as well as the representative pro-inflammatory cytokines IL-6 and IL-8; no significant difference in the levels of other cytokines was detected between the WS and IE groups. Recently, another type of encephalopathy was categorized during the IE except for the group characterized by a systemic cytokine storm [18]. Although a factor of the small number of cases may affect the outcome of the statistical analysis, the various pathophysiologies of IE may ultimately reflect no significant differences in the CSF cytokine levels, except IL-1RA, between the IE and WS groups.

Previous studies that have assessed the serum cytokine and free-radical levels and have reported higher cytokine levels and oxidative stress in symptomatic epilepsy patients than in cryptogenic epilepsy patients [3, 8], whereas another study reported no significant difference in serum cytokine levels between the 2 groups [10]. In the present study, the symptomatic WS patients showed higher CSF levels of IL-12 than the cryptogenic patients. IL-12 is a cytokine that can exert regulatory effects on T and NK cells and promote Th1 responses and is mainly secreted by microglia in the central nervous system (CNS) [19,20]. Certain reports have suggested that IL-12 plays a crucial role in the induction of the critical autoimmune responses involved in the initiation of experimental autoimmune encephalomyelitis [21-23]. Some other studies have reported increased IL-12 levels in the CSF and plasma during active disease and local expression of IL-12 within the CNS in patients with multiple sclerosis [24-26]. We must agree that the number of patients enrolled in our study was not sufficient to allow the etiological evaluation of the pathophysiology of symptomatic WS. Although it may not be practical to assess the extent to which IL-2 elevation contributes to the pathophysiology of symptomatic WS, the CSF IL-12 level may be a valid predictive marker of the etiology of WS. A study comparing the AED- and ACTH-response groups showed that the ACTH-response group had higher IL-2 levels than the AED-response group. Thus, the

CSF IL-12 level may be a potential marker to help decide between the use of AED or ACTH therapy for patients with WS.

In the present study, elevated levels of CSF cytokines were detected in patients with WS, indicating that immunological alterations are partly associated with the pathophysiology of WS. Our conclusions are confounded by a number of factors. Therefore, future studies with a larger patient population are required to assess whether or not immunological processes are associated with the pathophysiology of WS.

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Chapter 9

FOCAL EPILEPSIES AND MULTIPLE INDEPENDENT SPIKE FOCI

Tomoyuki Takano*
Department of Pediatrics, Shiga University of Medical Science, Seta-Tsukinowa, Otsu 520-2192, Japan

ABSTRACT

In order to investigate the mechanism of seizure spread in patients with focal epilepsy, the interictal and ictal epileptiform activities were analyzed with special reference to multiple independent spike foci, and examined individual seizure semiology by video-EEG monitoring. The ictal EEG was recorded in 18 (9 in focal epilepsies, and 9 in generalized epilepsies and epilepsies undetermined whether focal or generalize) of the 77 epilepsy patients. Nine patients with focal epilepsies include two cases with temporal lobe epilepsy (TLE), 5 with frontal lobe epilepsy (FLE), and one each with parietal lobe epilepsy (PLE) and occipital lobe epilepsy (OLE). Four patients had underlying disorders, including gray matter heterotopia, periventricular leukomalacia (PVL), viral encephalitis and West syndrome (WS). Interictal EEG recordings verified multiple independent spikes in three patients, and their ictal EEGs resulted in generalized epileptiform discharges after onset. Two cases had past histories of profound brain insult such as PVL and viral encephalitis before the appearance of multiple independent spike foci. We suggest that these etiological backgrounds are closely associated with the multiple cortical excitability producing multiple independent spike foci, resulting in generalized epileptiform discharges.

Keywords: Ictal EEG, Multiple independent spike foci, Video-EEG monitoring.

* Communication should be addressed to: Tomoyuki Takano, MD.Department of Pediatrics, Shiga University of Medical Science, Seta-Tsukinowa, Otsu 520-2192, Japan; E-mail: tmyktkn@belle.shiga-med.ac.jp; Telephone: +81 77 548 2228; Fax: +81 77 548 2230.

INTRODUCTION

An epileptic seizure is an acute event that can be a natural, although pathological, and a reaction of a brain to a noxious insult (Engel et al., 2008). The interictal state represents a period of relative quiescence, during which the enduring pathophysiological disturbances that predispose patients to the spontaneous recurrence of epileptic seizures are minimally active, or held in abeyance by some active seizure-suppressing mechanism. On the other hand, the ictal onset represents a functional transition from the interictal state. It can reflect a variety of processes, depending on the underlying pathophysiology and the type of ictal manifestation (Engel, 1990). In focal epilepsies, the interictal electroencephalogram (EEG) spikes are generated in a specific area of the cortex called the irritative zone. This is clearly related to, but frequently not identical to the ictal onset zone, which is the area of cortex that initiates seizures. The irritative zone can include multiple spike sites, some of which are located at a distance from the ictal onset zone, even in the opposite hemisphere (Engel and Pedley, 2008).

Video-EEG monitoring helps to classify the type of seizure, to define whether it is partial or generalized, and to pinpoint where seizures arise in the brain. Moreover, it can provide some information about the mechanisms of ictal precipitation, ictal propagation, ictal termination, and postictal phenomena. In this study, in order to investigate the mechanism of seizure spread in patients with focal epilepsy, the interictal and ictal epileptiform activities were analyzed with special reference to multiple independent spike foci, and examined individual seizure semiology by video-EEG monitoring.

Table 1. Clinical characteristics of the epilepsy patients

Case No.	Age/ Sex	Epilepsy syndrome	Etiology or underlying disorders (age at onset)	Age of first seizure	Psychomotor development	Antiepileptic drugs	Seizure frequency
1	3y 11m/ M	TLE	None	3y8m	Normal	CBZ	1-2/w
2	16y 5m/ M	TLE	Gray matter heterotopia	12y	Normal	TPM	1-2/m
3	7y 7m/ M	FLE	Periventricular leukomalacia (at birth)	4y	Mild delay	CBZ, ZNS	1-2/d
4	9y 1m/ M	FLE	None	5y	Mild delay	TPM, LEV, PB	5-8/d
5	10y 8m/ M	FLE	Viral encephalitis (6y)	6y	Severe delay	VPA, TPM, LTG	3-5/w
6	17y 6m/ F	FLE	WS (5m)	5m	Severe delay	TPM, LTG	3-5/d
7	23y 4m/ F	FLE	None	10y	Normal	PHT, TPM	1-2/m
8	15y 7m/ M	PLE	None	15y	Normal	ZNS	5-10/d
9	3y 6m/ M	OLE	None	8m	Normal	ZNS, TPM	3-5/d

Abbreviations: No. = Number, d = Day, w = Week, m = Month, y = Year, M = Male, F = Female, TLE = Temporal lobe epilepsy, FLE = Frontal lobe epilepsy, PLE = Parietal lobe epilepsy,OLE = Occipital lobe epilepsy, BMEI = Benign myoclonic epilepsy in infancy, CAE = Childhood absence epilepsy, WS = West syndrome, SGE = Symptomatic generalized epilepsy, NS = Neonatal seizures, CSWS = Continuous spike and waves during slow wave sleep, OS = Ohtahara syndrome, CBZ = Carbamazepine, TPM = Topiramate, ZNS = Zonisamide, LEV = Levetiracetam, PB = Phenobarbital, VPA = Valproate, LTG = Lamotrigine, PHT = Phenytoin, ESM = Ethosuximide.

METHODS

Patients

Patients with seizure disorders referred to the video-EEG monitoring unit of the Department of Pediatrics, Shiga University of Medical Science between January 2009 and December 2010 were evaluated. All patients underwent a detailed seizure history and neurological examination. The examination assessed the reason for referral and the diagnosis after video-EEG monitoring, and selected the patients with focal epilepsy. The age, sex, epilepsy syndrome, etiology or underlying disorders, age of first seizure, psychomotor development, antiepileptic drugs, and seizure frequency of the selected patients was recorded.

Video-EEG Monitoring

This study used one monitoring bed unit with a digital 21-channel video-EEG system (Neurofax EEG-1714, Nihonkoden Co. Ltd). Surface electrodes were placed according to the international 10-20 system. Video-EEG monitoring was recorded for 20 hours (2 PM to 10 AM in the next morning) per session. One to three sessions were performed in each patient. These monitoring records were used to select the patients with focal epilepsy in whom the ictal video-EEG was recorded. The interictal epileptiform discharges and their dominant focuses, seizure semiology, ictal epileptiform discharges at onset and after onset, and the onset zones were analyzed in these patients.

RESULTS

Clinical Characteristics of the Patients (Table 1)

A total number of 248 sessions of video-EEG monitoring was recorded from a total of 106 patients with seizure disorders, including 77 cases of epilepsy and 29 cases of non-epilepsy patients. The ictal EEG was recorded in 18 (9 in focal epilepsies, and 9 in generalized epilepsies and epilepsies undetermined whether focal or generalize) of the 77 epilepsy patients. The clinical characteristics of these 9 patients with focal epilepsies are summarized in Table 1. These patients include two cases with temporal lobe epilepsy (TLE), 5 with frontal lobe epilepsy (FLE), and one each with parietal lobe epilepsy (PLE) and occipital lobe epilepsy (OLE). Four patients had underlying disorders, including gray matter heterotopia (Case 2), periventricular leukomalacia (PVL) (Case 3), viral encephalitis (Case 5) and West syndrome (WS) (Case 6). The age at the time of the first seizure was 5 months to 15 years old. Four cases (44%) showed mild to severe delay of psychomotor development. Six cases (66%) required polytherapy with more than 2 antiepileptic drugs, and five cases (55%) showed refractory seizures every day.

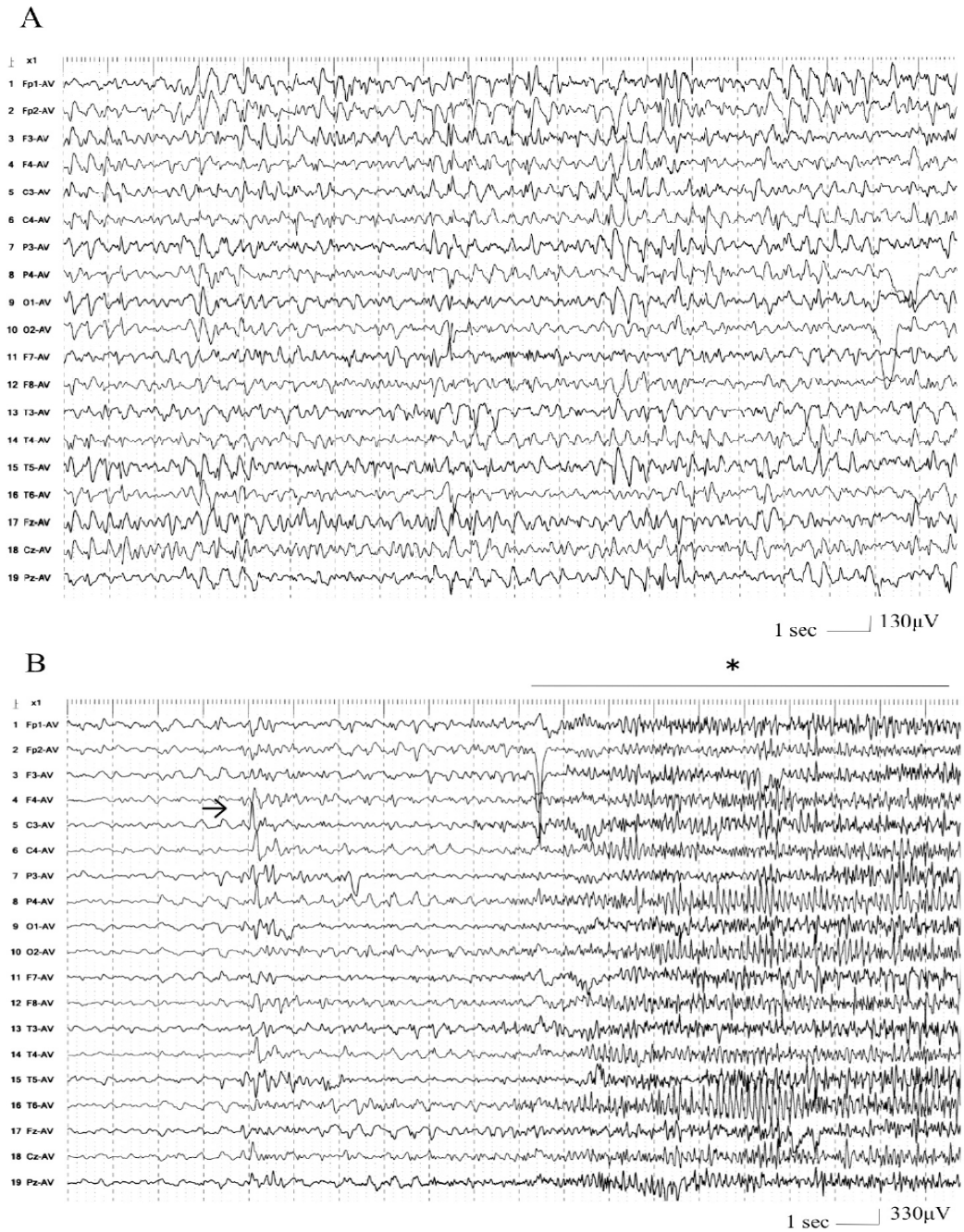


Figure 1. A: Interictal EEG of FLE (Case 3). Multiple independent spike foci appeared frequently on Fp1, Fp2, F3, P3, O1, O2, and T5. B: Ictal EEG of the same patient. Epileptiform discharge started with a high voltage focal spike on C3 (arrow) with the sudden onset of tonic extension of the right arm. This was followed by a generalized high voltage fast activity, at 8 to 10 Hz, during a generalized tonic seizure (*).

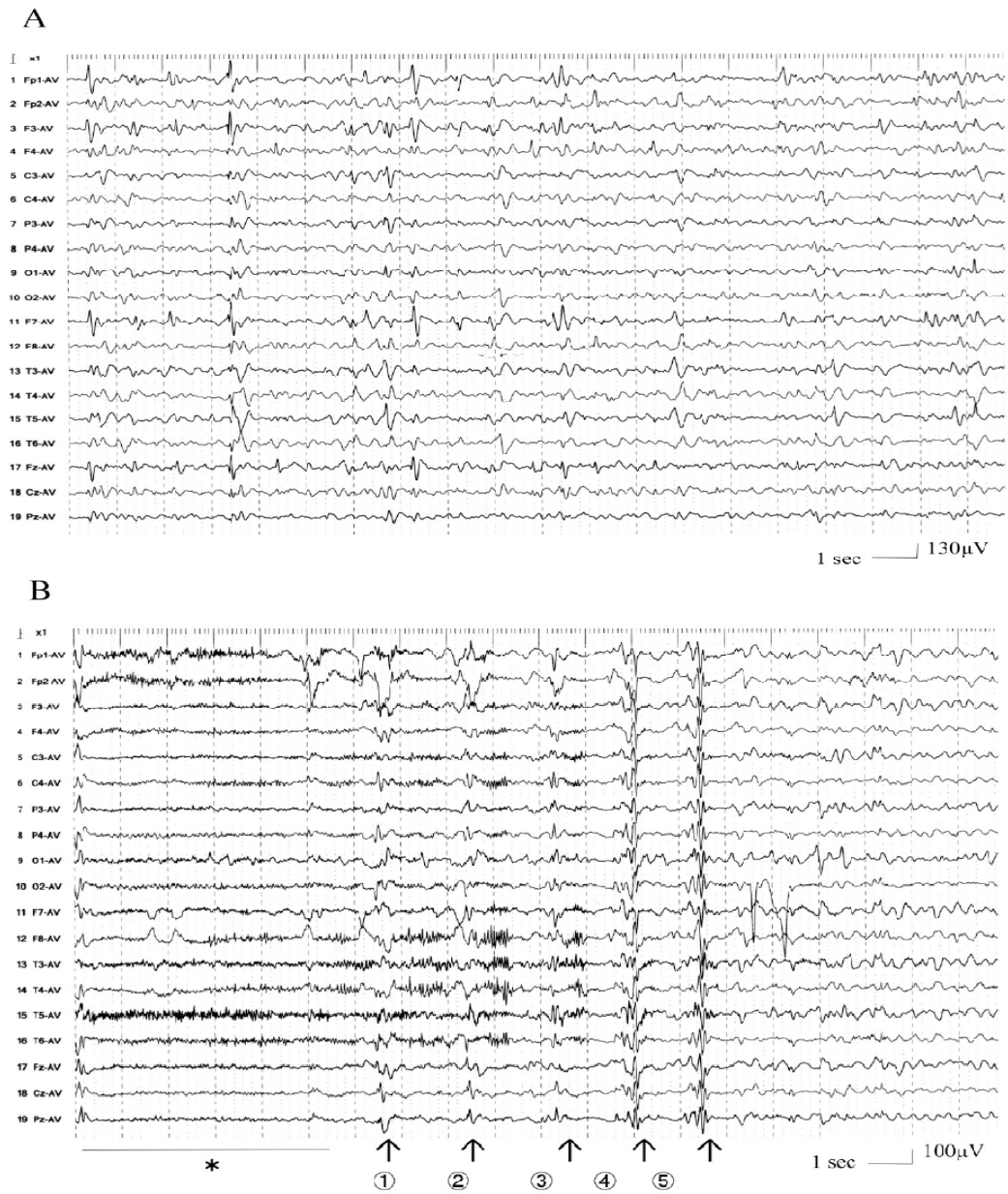


Figure 2. A: Interictal EEG of FLE (Case 5). Multiple independent spikes appeared frequently on Fp1, Fp2, F3, F4, C3, O1, F7, and T5. B: Ictal EEG of the same patient during sleep. Fast rhythms started on Fp1, Fp2 and T5 with the onset of tonic seizures of right and left arms (*). Ictal discharges were followed by the three rhythmic spikes (□, □, □), and two generalized polyspikes (□, □) , which corresponded to three clonic seizures of the upper limbs and two generalized clonic seizures, respectively.

Table 2. Characteristics of seizures and EEG findings

Case No.	Interictal		Ictal events			
	EEG	Dominant focus*	Seizure semiology	EEG at onset	Onset zone**	EEG after onset
1	Multiple independent spikes	C4, P4, F3, F4	Autonomic seizure showing prolonged epigastric sensation	Rhythmic slow wave activity	T3, F3, F4	Generalized rhythmic slow wave activity
2	Focal spike	T4, F8, T6	Tonic seizure of left arm	Rhythmic slow wave activity	T4, T6, F8	Propagation of rhythmic slow wave activity to Fp2 and F4
3	Multiple independent spikes	Fp1, Fp2, F3, P3, O1, O2, T5	Tonic seizure of right arm, and secondary generalized tonic-clonic seizure	Focal spike	C3	Generalized rhythmic slow wave activity, followed by rhythmic spike discharge
4	No epileptiform discharge	None	Tonic- clonic seizure of left arm and leg	Progress build-up of fast activity	Fp1, Fp2, F3, F4	Localized rhythmic sharp wave activty on bilateral Fp and F
5	Multiple independent spikes	Fp1, Fp2, F3, F4, C3, O1, F7, T5	Tonic- clonic seizures of trunk	Fast activity	Fp1, F8, T4, T6	Generalized rhythmic poly spike activity
6	No epileptiform discharge	None	Complex partial seizures with automatism	Focal spike	Fp1, Fp2	Fast activity, propagating to right hemisphere
7	No epileptiform discharge	None	Tonic- clonic seizure of right arm and leg	Fast activity	C3, C4	Rhythmic spike activity, propagating to left hemisphere
8	No epileptiform discharge	None	Somatosensory seizure showing right abdominal pain	Low voltage rhythmic alpha wave activity	O1, O2	Same pattern
9	Focal spike	T3, F7	Visual seizure	Rhythmic slow wave activity	P3, P4, O3, O4	Same pattern

Abbreviations: no. = Number, ECD = ethyl cysteinate dimmer, IMZ = iomazenil,
*, **: Electrode nomenclature is according to the International Federation of Clinical Neurophysiology 10-20 system.

Characteristics of EEG and Seizure Semiology (Table 2)

Interictal EEG recordings verified that four patients (Cases 4, 6, 7 and 8) presented no epileptiform discharges, two patients (Cases 2 and 9) showed focal spike, and three patients (Cases 1, 3 and 5) had multiple independent spikes. The dominant foci for interictal epileptiform discharges varied. Semiological observation during ictal events revealed that five patients with focal motor seizures showed tonic (Case 2) or tonic-clonic seizures (Cases 4, 5 and 7) and with secondary generalized seizures (Case 3), and other each four patients had autonomic seizure (Case 1), complex partial seizure (Case 6), somatosensory seizure (Case

8), and visual seizure (Case 9). Ictal epileptiform discharges at onset appeared as rhythmic slow wave activity in 3 cases (Cases 1, 2 and 9), alpha wave activity in one case (Case 8), fast activity in 3 cases (Cases 4, 5 and 7), and focal spike in 2 cases (Cases 3 and 6). Ictal onset zones were closely associated with each of the symptomatogenic zones recognized by each seizure semiology, that is, tonic or clonic seizures were evolved from the frontal or central cortex (Cases 2, 3, 4, 5 and 7), autonomic seizure from the temporal cortex (Case 1), and visual seizure from the occipital cortex (Case 9). A complex partial seizure with automatism (Case 6) was thought to have originated from the cingulum, and a somatosensory seizure (Case 8) from the parietal operculum. The ictal onset zones in those seizures were the frontal (Fp1, Fp2) and occipital (O1, O2) cortices, respectively.

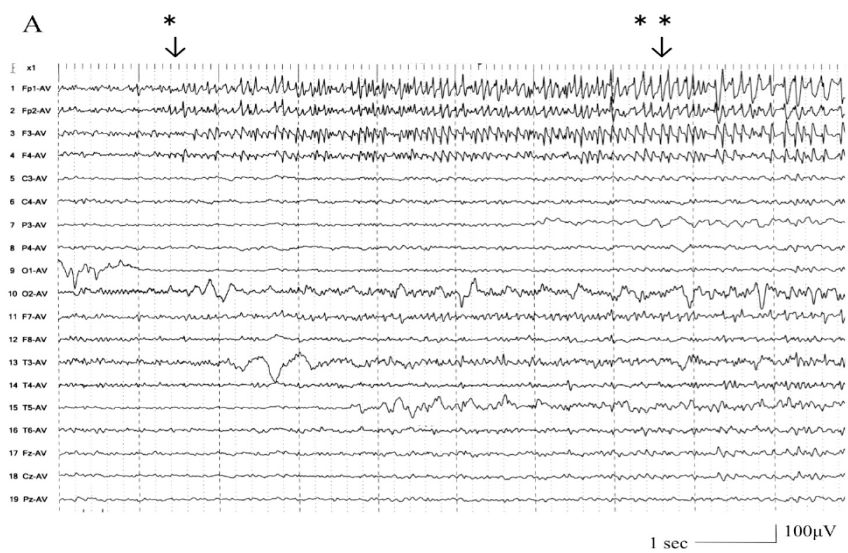
The ictal epileptiform discharges after onset presented three patterns: generalization, propagation and the same pattern. Three cases with multiple independent spike foci showed generalized ictal activity after onset: generalized rhythmic slow wave activity (Case 1), generalized rhythmic slow wave activity and subsequent rhythmic spike discharge (Case 3), and generalized rhythmic poly spike activity (Case 5). Case 3 was a male patient of FLE with multiple independent spikes on Fp1, Fp2, F3, P3, O1, O2, and T5 during interictal state (Figure 1A). His ictal epileptiform discharge started with a high voltage focal spike on C3 by the sudden onset of the tonic extension of the right arm at the same time, followed by a generalized high voltage fast activity during a generalized tonic seizure (Figure 1B). Case 5 was a male patient with FLE after viral encephalitis. His interictal EEG presented frequent multiple independent spikes on Fp1, Fp2, F3, F4, C3, O1, F7, and T5 (Figure 2A). Ictal EEG revealed that fast rhythms started on Fp1, Fp2 and T5 with the onset of tonic seizures of right and left arms, and were followed by the three focal rhythmic spikes and two generalized polyspikes, which corresponded to each of three focal clonic seizures and two generalized clonic seizures, respectively (Figure 2B). Three cases revealed focal or hemispheric propagation of ictal epileptiform discharges after the focal onset (Cases 2, 6 and 7). The other three cases showed continued restricted focal epileptiform discharges (Case 4) or the same patterns of ictal discharges (Cases 8 and 9). Case 4 was a male patient of FLE without epileptiform discharges during the interictal state. His ictal discharges started from the rapid rhythms on the bilateral Fp and F at the sudden onset of tonic seizure of the left arm, followed by focal rhythmic sharp wave discharges during the clonic phase of the left arm and leg (Figure 3A). No generalization was found until the end of the seizure. Case 8 was a male patient with PLE without interictal epileptiform discharges. His ictal discharge was low voltage rhythmic alpha wave activity that was continuously observed during the somatosensory seizure manifesting severe abdominal pain, in which ictal activity did not show any generalization or propagation (Figure 3B).

DISCUSSION

The EEG pattern of multiple independent spike foci is characterized by epileptiform discharges that arise from at least three noncontiguous electrode positions with at least one focus in each hemisphere (Blume, 1978). More than 70% of the patients with multiple independent spike foci have identifiable causative factors, such as hypoxic ischemic encephalopathy, hydrocephalus, tuberous sclerosis, central nervous system infection, and

neuronal migration disorders (Blume, 1978; Mizukawa, 1992; Noriega-Sanchez and Markand, 1976), thus suggesting variable and widespread occurrence of neuropathological findings. Most patients have significant cognitive and motor deficits: motor disturbances in 40 to 45% and mental retardation in 68 to 82%. Noriega- Sanchez and Markand (1976) and Blume (1978) found most patients with multiple independent spike foci to have multiple seizure types, including various types of generalized seizures, which occur with high frequency and prove refractory to medication. Multiple independent spikes appear as paroxysms of brief multiple spike and waves, irregular spike and waves, or sharp waves during interictal state. However, ictal EEG findings in patients with multiple independent spike foci, which have been reported in association with certain seizure types, have not been entirely clarified. Mizukawa (1992) reported ictal EEGs in 17 patients with tonic spasms, that is, desynchronization in 9, recruiting rhythm in 1, and rapid synchronization in 7. Diffuse polyspike and wave or irregular spike and wave are also reported in myoclonic seizures. Yamatogi and Ohtahara (1981) suggested that these ictal EEG patterns are similar to those of generalized motor seizures seen in patients with Lennox- Gastaut syndrome.

Some researchers have proposed that this electroclinical syndrome with multiple independent spike foci with severe epilepsy should be called Markand-Blume-Ohtahara syndrome, because of extensive studies on multiple independent spike foci by Markand and Blume for defining its clinical correlates, and by Ohtahara for integrating clinical characteristics with other epileptic encephalopathies (Yamatogi and Ohtahara, 2003, 2006). Interictal multiple independent spikes were observed in 3 patients with focal epilepsies in the current series (temporal lobe epilepsy in 1, and frontal lobe epilepsy in 2). Two cases had past histories of profound brain insult before the appearance of multiple independent spike foci such as PVL and viral encephalitis with widespread neuropathological findings. It is suggested that these etiological backgrounds are closely associated with the multiple cortical excitability producing multiple independent spike foci, resulting in generalized epileptiform discharges.



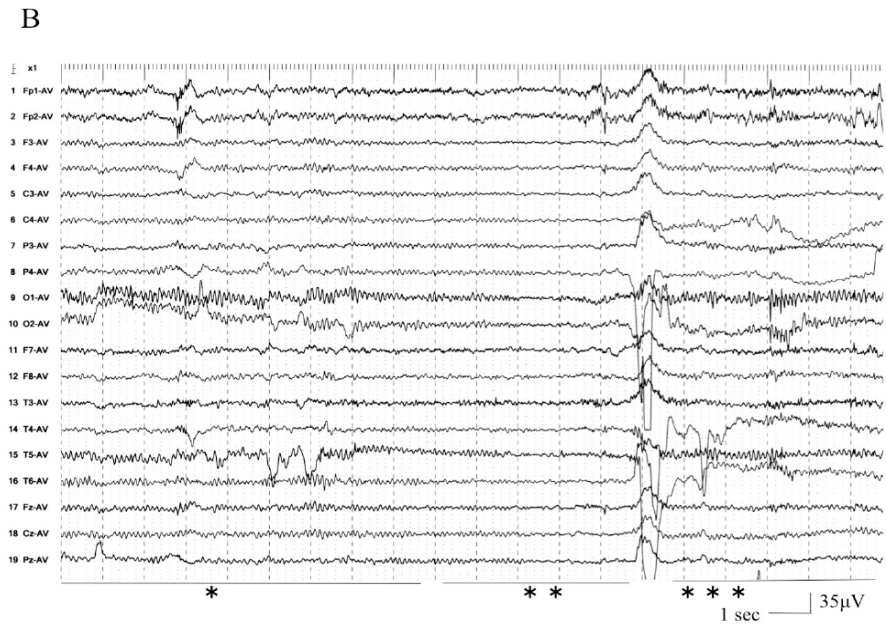


Figure 3. A: Ictal EEG of TLE (Case 4). Epileptic discharges started from the rapid rhythms on the bilateral Fp and F at the sudden onset of tonic seizure of the left arm (*), followed by rhythmic sharp wave discharges of the left arm and leg during the clonic phase, 7 seconds later (**). B: Ictal EEG of PLE (Case 8). Low voltage rhythmic alpha wave activity was continuously observed during the somatosensory seizure accompanied by severe abdominal pain (*). Ictal discharges attenuated (**) or activated (***) in proportion to the decrease or increase of the abdominal pain.

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