Semantic Processing in Children with ASD: Differences in underlying Neural Processing in Children with High Language vs. Low Language Capabilities

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Abstract

The primary objective was to investigate semantic neural processing in children with autism spectrum disorder (ASD) as impairments in this domain have been shown in this patient population. Typically developing (TD) children and children with ASD with varying language ability did an auditory semantic categorization task. Only children with ASD with low language ability had semantic neural processing deficits, indexed by a late onset and smaller N400 component compared to TD children when the N400 component was evoked, although it was not evoked to all auditory stimuli. There were negligible neural processing differences between children with high language ability and TD children. In children with ASD, the smaller N400 component predicts lower language ability and the smaller N400 component is predicted by deficits in cortical auditory processing indexed by a smaller P1 component. Thus, language deficits in children with ASD are predicted by semantic and cortical auditory processing deficits.

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Contributions

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List of Abbreviations

ADI-R – Autism Diagnostic Interview-Revised

ADOS-2 - Autism Diagnostic Observation Schedule - Second Edition

ASD – Autism Spectrum Disorder

ASD cohort: high language – ASD cohort with high language ability

ASD cohort: low language – ASD cohort with low language ability

CAEP - Cortical Auditory Evoked Potentials

CDD - Childhood Disintegrative Disorder

CDI - MacArthur-Bates Communicative Development Inventories: Words and Sentences Vocabulary checklist

CI – Cochlear Implants

DSM-5 – Diagnostic and Statistical Manual of Mental Disorders, 5th Edition

EEG – Electroencephalography

ERP – Event-Related Potential

HA – Hearing Aids

ISIs – Interstimulus Intervals

LNC – Late Negative Component

NH – Normal Hearing

PDD - Pervasive Developmental Disorders

PDD-NOS - Pervasive Developmental Disorder Not Otherwise Specified

PLS-5 - Preschool Language Scale - Fifth Edition

PPVT-IV– Peabody Picture Vocabulary Test – Fourth Edition

RT – Reaction Time

SB-5 – Stanford-Binet Intelligence Scales - Fifth Edition

TD – Typically Developing

TW – Time Window

WPPSI-II - Weschler Preschool & Primary Scale of Intelligence – Second Edition

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Chapter One: Introduction

1.1. Overview of the Introduction

The present study utilizes electroencephalography (EEG) to examine the neural processing underlying semantic processing of spoken words in young children with autism spectrum disorder (ASD) compared to typically developing (TD) children. Section 1 of the introduction begins with a summary of the discovery of ASD and what the current diagnostic criteria is as described by the Diagnostic and Statistical Manual of Mental Disorders, 5th Edition (DSM-5) (American Psychiatric Association, 2013). The prevalence of ASD is then explained, followed by a brief explanation of how these criteria have changed from previous versions of the DSM. Finally, this section describes how ASD symptomology is assessed and quantified.

This is followed by 'Section 2: Language impairments in ASD', which discusses language impairments in this patient population. Expressive and receptive language impairments in ASD are described, with the latter focusing on deficits in semantic processing. Semantic processing is key to receptive language processing and is the theme of this study. Deficits in this domain have been shown in this patient population, but the neural processing underlying these deficits is unclear, which will be explained in section 3. This is followed by a description of how language impairment is assessed in ASD. Furthermore, the impact of language impairment in this patient population is explained.

The last section of the introduction is "Section 3: Neural Processing Underlying Semantic Language Impairment in Children with ASD", which focuses on neural processing and gives details on the event-related potentials that are recorded by EEGs and how the components of this brain response relate to semantic processing. This includes a review of cortical auditory processing components and the N400 component as well as and an explanation of the differences between adults and TD children. Furthermore, there is a literature review on ERP semantic processing studies in children with ASD and the current limitations of these studies is explained.

1.2. Autism Spectrum Disorder

Leo Kanner provided details of the first cases of "early infantile autism" in 1943, based on 11 clinical case studies of children between the ages of two and eleven (J. Harris, 2018; Kanner, 1944). Based on these clinical cases, Kanner recognized "autism" as a new and distinct disorder and established the unique and defining characteristics of autism, which were: 1) extreme aloneness that shuts the child off from the outside world, 2) atypical development and atypical use of language, and 3) obsessive and repetitive behaviours that are associated with an insistency on consistency. He also noted that these children, despite these characteristics, had cognitive potential and were usually, typically physically developing. Furthermore, he described them as behaving this way since birth, referring to their behaviour as innate (J. Harris, 2018; Kanner, 1944).

Concurrently, a German scientist named Hans Asperger published a paper describing four cases of children who had "autistic psychopathy" (Asperger, 1944, 1991; J. Harris, 2018; Lyons & Fitzgerald, 2007). He described these children as having typical cognitive function, but also having deficits in communication and restricted interests (Asperger, 1991; J. Harris, 2018).

Through the discoveries of both pioneers, the term "autism" was redefined as a new disorder, as it was formerly used to describe the symptom of hyper-interest to their "own world" detaching from reality in individuals with schizophrenia, before the 1940s (Bleuler, 1950; McGlashan, 2011). Prior to the new definition of autism, these children would have been deemed as "feebleminded" or "schizophrenic" (Harris, 2018; Kanner, 1943).

In both Kanner and Asperger's papers, they may be describing disorders that would now be recognized under the same diagnosis of autism spectrum disorder (ASD) (Lyons & Fitzgerald, 2007). Asperger may have encountered more mild cases of ASD with higher intellectual ability, while Kanner's patients may have been lower functioning individuals. The diagnostic criteria, as well as the name, have changed over the past seven decades since Kanner and Asperger's discovery, as knowledge surrounding this disorder increases.

The current diagnostic criteria for ASD is based on the individual's behaviour as well as developmental history and the Diagnostic and Statistical Manual of Mental Disorders, 5th Edition (DSM-5) defines ASD as possessing impairments in two domains: 1) repetitive behaviours and restricted interests, 2) social communication and interaction (American Psychiatric Association, 2013; Hyman, 2013). For repetitive behaviours and restricted interests, the deficits can manifest as: atypical bodily mannerisms, obsessive and compulsive ritualistic patterns and/or routines, and abnormal level of fixation on interests and/or atypical sensory behaviours (American Psychiatric Association, 2013; CDC, 2016; Hyman, 2013). Social and communication deficits can present as problems with social initiation and response, non-verbal communication, as well as problems developing and maintaining interpersonal relationships beyond that of a care giver (American Psychiatric Association, 2013; CDC, 2016; Hyman, 2013).

The onset of different symptoms must manifest early in life and cause clinically significant functional impairments. The phenotypic display of the core characteristics of ASD and the severity of these symptoms is highly heterogeneous. During diagnosis, the severity of both symptoms can be rated on a scale of 1-3, with 3 being the highest severity. The DSM-5 additionally allows for co-occurring specifiers to be noted during diagnosis, such as impairments in intellect and language, as well as other neurodevelopmental, mental or behavioural disorders (American Psychiatric Association, 2013).

1.2.1. Prevalence of ASD

The prevalence of ASD has increased since the 1940s when it was first defined by Kanner and Asperger, with a continually increasing trajectory as reported by the Centers for Disease Control and Prevention (CDC) (CDC, 2019). It is estimated that one in every 59 children has ASD, which is a two fold increase since 2000, when the estimation was 1 in 150 children (CDC, 2020b; Maenner, 2020). All racial, ethnic, and socio-economic groups have occurrences of ASD (CDC, 2020b). Furthermore, this neurodevelopment disorder is more prevalent in males, with a 4:1 ratio in males and females (Government of Canada, 2018).

Several factors could contribute to the increased prevalence that has been observed. Increased awareness through dissemination of information could be one factor. The public could be more aware of symptoms that are exhibited by individuals with ASD, and subsequently there would be an increase in referrals for clinical diagnoses and clarification (REAVEN et al., 2008). Other risk factors for ASD such as an increase in paternal age at birth and increased maternal obesity are now more common in society and thus could consequently contribute to the increase in prevalence in ASD (Croen et al., 2007; Halfon & Kuo, 2013; Krakowiak et al., 2012).

1.2.2. Changes to the Diagnostic and Statistical Manual of Mental Disorders, 5th Edition (DSM-5)

The advances in research and increases in prevalence of ASD have improved our understanding of this disorder, which has led to the diagnostic criteria and classification of ASD being revised several times over the past seven decades, to increase the reliability of diagnosis for ASD. The last revision happened in 2013 with the creation of the fifth edition of the DSM (DSM-5) (King et al., 2014; Catherine Lord & Jones, 2012).

One of the major modifications to the diagnostic criteria was the dissolution of the term 'Pervasive Developmental Disorders (PDD)' which included five subtypes: autistic disorder (autism), Pervasive Developmental Disorder Not Otherwise Specified (PDD-NOS), Disintegrative Disorder, Asperger's syndrome and childhood disintegrative disorder (CDD) (Kim et al., 2014; King et al., 2014). PDD and its sub-diagnoses have been replaced by an umbrella term for all of the disorders under the same diagnosis of "Autism Spectrum Disorder" (Kim et al., 2014; Lai et al., 2013). This was partly due to a lack of reliability in clinicians assigning the distinct diagnoses for the separate disorders across clinics and care centers (American Psychiatric Association, 2013), as different subtypes of PDD have overlapping symptoms with little differences between them (J. Harris, 2018; S. Sharma et al., 2012).

Another change to the diagnostic criteria for ASD was that the age of onset of symptoms no longer needed to be apparent before three years of age (Halfon & Kuo, 2013), however, the symptoms must still present during "early childhood" (American Psychiatric

Association, 2013; CDC, 2016; Kim et al., 2014). This change to the diagnostic criteria allowed for more flexibility and the inclusion of age of onset of symptoms for diagnosis (Lai et al., 2013).

Furthermore, there was a collapse of the previous DSM-IV triad of characteristics into two diagnostic criteria (Grzadzinski et al., 2013). Language and communication impairments are now combined with impairments in social interactions rather than separate criteria, as this better reflected the symptomology of ASD (Grzadzinski et al., 2013; Mandy et al., 2012).

1.2.3. Assessments for ASD

The diagnostic tools are used to assess children for ASD and these typically include some combination of parent/caregiver report of the child's development and behaviour as well as behavioural assessments conducted by a health professional which can include family physicians, pediatricians, developmental pediatricians, psychologists, psychological associates, as well as nurse practitioners in Ontario (*Autism Assessment and Diagnosis | Ontario.Ca*, n.d.; CDC, 2020a).

The most commonly used diagnostic assessments are the Autism Diagnostic Observation Schedule-2 (ADOS-2) and the Autism Diagnostic Interview-Revised (ADI-R) (*(ADI™-R) Autism Diagnostic Interview–Revised*, n.d.; *(ADOS™-2) Autism Diagnostic Observation Schedule™, Second Edition*, n.d.; Kamp-Becker et al., 2018; C. Lord et al., 1994; REAVEN et al., 2008). The ADOS-2 is an assessment which requires the examiner to administer planned social activities that will delineate if the individual has the defining characteristics of ASD (*(ADOS™-2) Autism Diagnostic Observation Schedule™, Second Edition*, n.d.; C. Lord et al., 1999). Language capabilities are not a limiting factor for using the ADOS-2, as there are different modules which can be selected based on language ability ranging from modules for children with minimal ability to use phrase speech to adolescents and adults with verbal fluency (Kamp-Becker et al., 2018; C. Lord et al., 1999). The ADI-R is a semi-structured interview that focuses on parental/primary caregiver recall on the child's full developmental history (*(ADI™-R) Autism Diagnostic Interview–Revised*, n.d.; C. Lord et al., 1994; REAVEN et al., 2008). The diagnostic tools should not be used exclusively to determine diagnosis and an additional expert clinical opinion in tandem with the results of the diagnostic tools is recommended as the "gold standard" for diagnosing ASD (CDC, 2014).

1.3. Language in ASD

Language is a structured system of communication used between members of a social group (Bishop et al., 2016; Chenausky et al., 2019). It can occur through spoken vocalization, written words, symbols as well as visual communication through sign language in humans (Bishop et al., 2016). Spoken and written language can be subdivided into two categories: 1) receptive language, which is the input of language, and 2) expressive language, which is the output of language (McIntyre et al., 2017). Receptive language is receiving, decoding and comprehending the language that is being expressed to the individual, while expressive language is the production and expression of thoughts and ideas through the structured language system (McIntyre et al., 2017).

Language can also subdivided into these following subcomponents; form, content and use (Cameron-Faulkner et al., 2010; Hoque, 2015; Rudd & Kelley, 2011). Form consists of phonology, morphology and syntax (McIntyre et al., 2017). Phonology focuses on the sound components and their associated meanings in language (Anderson, 2001). It also concentrates on how speech sounds behave in a syllable, word, or sentence rather than when the sound components (i.e. phonemes) are studied in isolation (Anderson, 2001). Morphology on the other hand focuses on the smallest meaningful written components (morphemes) and the rules governing their use in language (Spencer, 2001). The rules that pertain to the structured ways that sentences are formed in language are covered by syntax (Vigliocco, 2000). Semantics is the domain that covers the content (meaning) of language (Vigliocco, 2000), while pragmatics refers to the use of language in context of communication (Socher et al., 2019).

An amalgamation of both receptive and expressive abilities as well as abilities in the other subcomponents of language enables an individual to interact and communicate with others. Communication is essential for physical, identity, social and practical needs (Hewett, 2018) and deficits in either of these language domains or subcomponents would negatively affect communication capabilities, that also would consequently negatively affect the quality of life of individuals with these deficits. In individuals with ASD, impairments in these domains and subcomponents have been shown, with specific details regarding these deficits explained in the next section.

1.3.1. Language Impairments in ASD

Language impairment was one of the main characteristics of ASD initially described by Kanner, as all children in the case studies presented with atypical development of language for their age (Kanner, 1943). However, the inclusion of language impairment as a critical domain for the diagnostic criteria for ASD has been debated since and is currently removed from the core symptoms for ASD as defined by the DSM-5 (American Psychiatric Association, 2013). The general discourse surrounding language and ASD is due to the heterogenous nature of this patient population's language capabilities. Their language functionality can range from verbal language development resembling typically developing (TD) individuals to others who are minimally verbal to non-verbal (Brignell et al., 2018; Mody & Belliveau, 2013; Rapin & Dunn, 1997). However, when form, content and use of language are considered, all children with ASD have impairments in language (American Psychiatric Association, 2013; Parsons et al., 2017).

1.3.2. Types of Language Deficits in ASD: Expressive Language Impairment

Deficits in expressive language are present in children with ASD, with estimations of up to 40% of children with ASD being non-verbal (NV) as stated by the CDC (CDC, 2015). Expressive language impairment can manifest as delayed onset of speech, which is one of the first and most commonly recognized symptom noted by parents (Chawarska et al., 2007). Parents have also reported a slowing of developmental rates as well as regression (i.e., a loss of language skills) in previously acquired expressive language skills in children with ASD (Chawarska et al., 2007; Goldberg et al., 2003; Werner & Dawson, 2005). It is estimated that approximately 20% children with ASD experience language regression (Backes et al., 2013; Meilleur & Fombonne, 2009).

Impairments in expressive language are not limited to reduced vocal output, seeing as increased atypical output has been reported as well. Individuals with ASD can display stereotyped language use that would fall under restricted and repetitive patterns of behaviours (Seol et al., 2014). Echolalia is an example of a repetitive expressive language behaviour in ASD, which presents as individuals echoing a sentence or final word (Seol et al., 2014). Children with ASD also frequently have atypical verbal output in the form of jargon, which is nonsense words (Chen & Kuo, 2017). The prosody as well, which is related to the pitch, duration and intensity of speech sounds, has been shown to be abnormal in children with ASD (DePape et al., 2012; Rapin & Dunn, 1997; Shriberg et al., 2011). An example of atypical prosody was described by Zuanetti et al (2018) who reported inappropriate emphasis on syllables in the vocal output in children with ASD, revealing atypical word accentuation (Zuanetti et al., 2018). Furthermore, apraxia of speech, which is a speech disorder that is associated with deficits in the brain's ability to plan the sequence of movements required for the articulation of speech can present as a co-morbidity in ASD as well (Chenausky et al., 2019; Mody & Belliveau, 2013; Tierney et al., 2015).

1.3.3. Types of Language Deficits in ASD: Receptive Language Impairment

Receptive language capabilities have been found to be impaired in children with ASD as well (Loucas et al., 2008; Mody & Belliveau, 2013). Weismer et al. (2010) reported that children with ASD had lower receptive language ability indexed by lower age equivalent mean on the Mullen Scales of Early Learning, the Sequenced Inventory of Communication Development and the Vineland-Adaptive Behaviour Scales in comparison to age-matched children with developmental delays (Weismer et al., 2010). In a meta-analysis investigating receptive and expressive language skills in children and adolescents with ASD, Kwok et al. (2015) proclaimed that both domains of language were impaired in children and adolescents with ASD, indicated by language scores that fell 1.5 SD below the scores that were reported in the TD children and adolescents in the control group (Kwok et al., 2015). Furthermore, the language abilities of children with ASD have been reported to resemble those of children with a receptive language disorder (Howlin, Mawhood, & Rutter, 2000), emphasizing that there are language impairments in the receptive domain in a proportion of children with ASD.

Receptive language ability requires processes such as semantic processing, which is the ability to understanding the meaning of a stimuli whether it be verbal such as words or visual such as pictures, and deficits in this domain have been shown in children with ASD (Brignell et al., 2018; Mody & Belliveau, 2013; Tager-Flusberg et al., 2005; Xu et al., 2017). In a word association task, which involved participants stating as many semantically congruent words as possible after hearing a target word, the results revealed that children with ASD produced many accurate and appropriate word associations. However, they also produced more semantically unrelated responses than TD children, implicating impairments in their lexical access (Battaglia, 2012), which involves mapping words onto possible mental representations of the word that contain semantic information in the mental lexicon (Emmorey & Fromkin, 1988; Haebig et al., 2015; Taft, 2001). Additionally, deficits in integration of words have reported in ASD, with newly acquired words showing less integration overtime indexed by a lack of competition between newly acquired words and existing words with the same base (Henderson et al., 2014). Furthermore, studies have reported children and adolescents with ASD had a lack of semantic priming effects in comparison to controls (Kamio et al., 2007). There was no decrease in reaction time in response to target words that were semantically related to the preceding prime word, regardless of the degree of relatedness between the prime and target words (Kamio et al., 2007), indicating semantic processing deficits in ASD.

Overall, there are a multitude of examples of semantic processing deficits in children with ASD (Battaglia, 2012; Henderson et al., 2014; Kamio et al., 2007) that negatively affect receptive language capabilities. The focus of this study was to better understand what is contributing to the deficits in semantic processing abilities. This, in tandem, will contribute to knowledge on receptive language deficits. Furthermore, these findings are important as there is such a vital link between receptive language ability and learning, communication, and social interaction in children.

1.3.4. Assessing Language Impairment in ASD

The current approaches to assessing receptive and expressive language ability in children with ASD includes report-based assessments and standardized tests (*Assessment Tools, Techniques, and Data Sources*, n.d.; Luyster et al., 2008; Nordahl-Hansen et al., 2014).

Report-based assessments are evaluations made by parents/caregivers of the child's language skill in a natural setting (Nordahl-Hansen et al., 2014). These assessments are easier to administer as they are not subject to the motivation levels or cooperation level of the child (Nordahl-Hansen et al., 2014). However, parents/caregivers tend to lack the expertise required to evaluate language skills and may not be able to accurately report the child's language capabilities (De Houwer et al., 2005; Law & Roy, 2008).

Standardized tests assessing language are conducted by trained professionals with expertise in assessing language skills (Condouris et al., 2003). These assessments are standardized; therefore, participants are tested in a similar fashion (Condouris et al., 2003; Nordahl-Hansen et al., 2014), which reduces the confounding factors of differences in the environment, as well as differences between caregivers for report-based assessments. However, results from standardized tests reflect the abilities in a predefined set of language skills but do not capture real-time language performance in a natural, real-world setting (Condouris et al., 2003). Additionally, standardized tests require participants to have a certain level of cognitive and language skills as well as attentive and cooperative capabilities (Koegel et al., 1997; Luyster et al., 2008), that can be problematic when assessing children with ASD, who are known to have deficits in those domains (Charman, 2004; Luyster et al., 2008; Mody & Belliveau, 2013). Nonetheless, standardized assessments allow for the comparison of performance relative to a large, standardized sample of age-matched individuals that is essential for determining level of impairment (*Assessment Tools, Techniques, and Data Sources*, n.d.).

The advantages and drawbacks of both approaches supports the notion of assessing language ability through a mixed method approach that includes both report assessments and standardized tests in the future.

1.3.5. Impact of Receptive Language Impairment in ASD

Receptive language impairments can negatively impact the quality of life for children with ASD by affecting social capabilities and success in the traditional academic environment.

Social relationships with friends and family have been shown as a predictor of happiness in children and have been attributed to increasing academic success and reduction in dropping out of school (Amati et al., 2018; Brendgen et al., 2005; Farmer et al., 2008; Holder & Coleman, 2009; Rotheram-Fuller et al., 2010). These relationships require pragmatic skills, which refers to social communications skills that involves both receptive and expressive language skills, (Philofsky et al., 2007). Children with ASD have shown lack of social reciprocity and social initiation for conversational interactions as well as increased socially inappropriate comments and difficulties understanding gestures and emotional cues, emphasizing their deficits in pragmatics (Baltaxe, 1977; Loveland et al., 1997; Philofsky et al., 2007). This may decrease the quality of life of children with ASD because there is evidence showing an inverse relationship between pragmatic skills and quality of friendships as well as feelings of loneliness in children and adolescents with ASD (Bauminger & Kasari, 2000; Locke et al., 2010; Parsons et al., 2017).

Furthermore, the traditional teaching style that is predominately used in the academic environment focuses on auditory learning, which can be affected by language impairments. Educators are typically situated in front of the classroom, verbally delivering information to the classroom-(N. Harris & Bacon, 2019). Impairments in receptive language, such as those seen in children with ASD, would affect an individual's ability to learn in this environment. Furthermore, there is movement towards active learning, which emphasizes involvement and engagement of students in their own learning (*Active Learning in Secondary and College Science Classrooms : A Working Model for Helping the Learner to Learn / Joel A. Michael, Harold I. Modell - Trove*, n.d.; Michael, 2006). In this model, discourse among students and teachers is encouraged, which requires both expressive and receptive language skills (Michael, 2006). Deficits in either language skill would severely hinder an individual's capabilities to succeed in active learning, and this is especially pertinent in children with ASD who are known to have deficits in these domains (Chenausky et al., 2019; Howlin et al., 2000; Mody & Belliveau, 2013; Seol et al., 2014).

1.4. Neural Processing Underlying Semantic Language Impairment in Children with ASD

Knowledge surrounding the underlying neural processing associated with semantic processing deficits in children with ASD remains limited, despite significant evidence showing deficits in this domain. To delineate these underpinnings of semantic processing deficits, non-invasive neurophysiological techniques such as electroencephalography (EEG) allow for investigation of known neural signatures of cortical auditory and semantic processing (Bell & Cuevas, 2012). The temporal resolution of EEG is high, therefore this technique is able to record how language processing unfolds in real time in the brain as it allows for capture of rapid neural changes associated with language processes (Bell & Cuevas, 2012). Additionally, EEG is non-invasive (Light et al., 2010), which may lower attrition rates in studies with young children and children with ASD.

1.4.1. Event-Related Potentials (ERPs)

EEGs record event-related potentials (ERPs), which are brain responses evoked by a presentation of an external stimulus (Blackwood & Muir, 1990; M. Kutas & Hillyard, 1980; Picton et al., 2000). ERPs reflect the summation of simultaneous post-synaptic potentials, which are voltages that occur when a large number of cortical pyramidal neurons fire in synchrony as a function of stimulus processing (Beres, 2017; Government of Canada, 2018; Peterson et al., 1995; Sur & Sinha, 2009).

ERPs are depicted as a waveform that contains peaks and troughs, which are called components (Beres, 2017; Marta Kutas & Federmeier, 2011). These components have well-established cognitive correlates, some of which are specifically associated with language processing, allowing the ability to visualize language processing as it unfolds over time (Sur & Sinha, 2009). The components can be characterized according to their latency of occurrence, polarity (positive or negative), amplitude, and distribution on the scalp in response to a stimulus (Sur & Sinha, 2009; Woodman, 2010). The nomenclature begins with their polarity (Negative/Positive) designated by a letter (N/P), followed by their latency designated by the time in milliseconds or ordinal position in the waveform for components (Beres, 2017; Woodman, 2010).

1.4.2. Components of ERPs related to Semantic Processing

For receptive language processing, obligatory cortical auditory evoked potentials (CAEP) are thought to reflect cortical auditory processing of the auditory stimuli generated by the auditory cortex (Munro et al., 2020; Ponton et al., 2000). The occurrence of the CAEPs is thought to be automatic and pre-attentive, involving no cognitive processes occurring between 50 to 300 ms after onset of auditory stimuli (Beres, 2017; Ceponiene et al., 2002; Ibrahim et al., 2018; Martin et al., 2007). The latency and amplitude of the CAEPs reflect the speed of cortical auditory processing as well as the sensitivity to the physical characteristics of the auditory stimuli (Davies et al., 2010; Hyde, 1997).

The obligatory CAEPs are followed by late components, which are associated with higher order processing such as semantic processing (Woodman, 2010). The CAEPs which index cortical auditory processing of auditory stimuli, needs to occur before semantic processing and their integrity can affect these later stages of processing.

1.4.3. Cortical Auditory Evoked Potentials

Adults

In adults, the obligatory CAEPs consist of the P1-N1-P2 component complex seen at the central electrodes and is associated with detection of sound in the auditory cortex (Čeponien≐ et al., 2002; Picton et al., 1999). This complex of components is shown in **Fig. 1a**. The components can be evoked by both brief auditory stimuli such as clicks and tones as well as complex sounds such as phonemes and words (Ceponiene et al., 2002; Martin et al., 2007). Physical features of auditory sound such as frequency, intensity and presentation rate of auditory stimuli are factors that affect the amplitude and latency of the P1-N1-P2 complex (Pratt et al., 2008; Wagner et al., 2017). Additionally, this complex is sensitive to duration, and delivery of stimuli to ears (binaural versus monoaural) as well as subject state (Martin et al., 2007). The main neural generator of the P1-N1-P2 complex has been reported to be located bilaterally in auditory cortex in the temporal lobes (Ceponiene et al., 2002; Picton et al., 1999).

The first component, the P1 component, also referred to as the P50 component, peaks at approximately 50 ms in adults as a positive deflection in the ERP (Davis, 1939), shown in **Fig 1a**. The neural source has been shown to arise from Heschl's gyrus that contains the primary auditory cortex in the temporal lobes (Howard et al., 2000; Liégeois-Chauvel et al., 1994; Lightfoot, 2016).

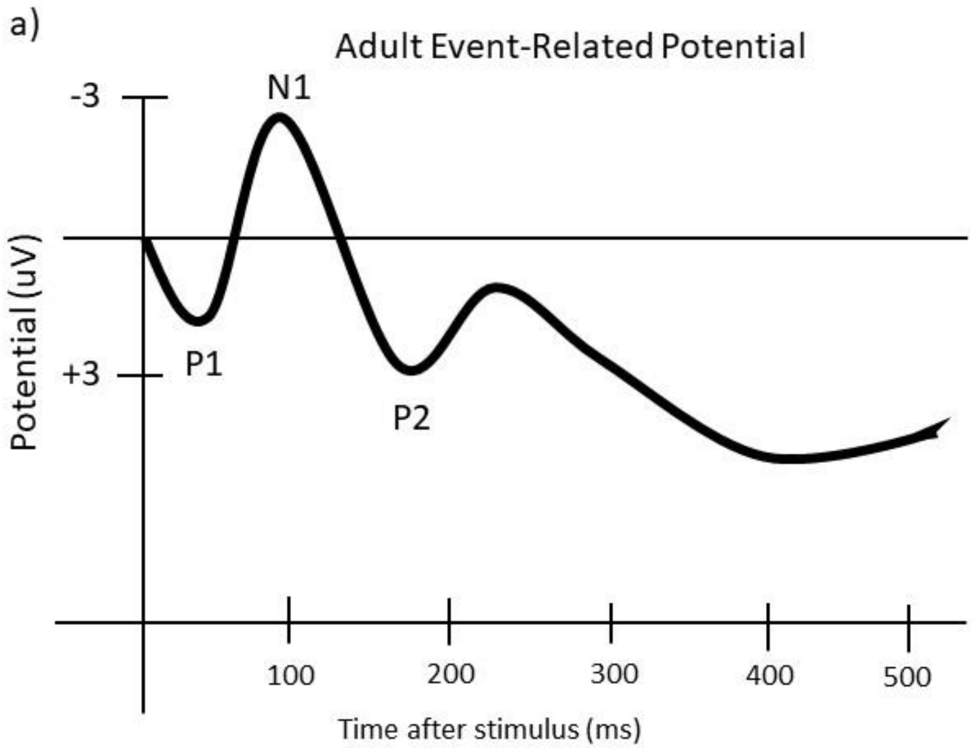
This is followed by the N1 which is an overall negative deflection in the ERP, that consists of three temporally overlapping components at temporal and central sites that peaks at about 80 to 110 milliseconds (ms) post stimulus onset in adults (Lightfoot, 2016). The components of the N1 can be split into 2 components, the N1b that is in the centro-frontal brain area , and the T1 complex over temporal sites that consists of a N1a and N1c components (Giard et al., 1994; Vaughan & Ritter, 1970). The N1b component is part of the complex of components found in the central electrodes shown in **Fig. 1a.** The neural sources have been found bilaterally in the supratemporal primary auditory cortices that lie in the temporal lobe for the N1b (Hari et al., 1982; Knight et al., 1980; Lightfoot, 2016; Picton et al., 1999). The neural generators for the N1a and N1c components have been localized to the auditory association cortex in the superior temporal gyrus (Pang & Taylor, 2000; Scherg & Von Cramon, 1986). The N1 component has also been shown to be positively affected by attention levels, as the amplitude of this component has shown to increase with increasing attentional levels (Picton & Hillyard, 1974).

Lastly, a positive deflection in the ERP between 140 to 170 seconds post stimulus onset represents the P2 component (Ponton et al., 2000), shown in **Fig. 1a**. The neural generators have been localized to the auditory association cortex anterior to the source of the N1 components in this cortex in the temporal lobe (Bosnyak et al., 2004; Lightfoot, 2016; Shahin et al., 2003). The subject's level of attention has also been shown to affect the P2 component, with greater attention towards the stimuli being associated with a substantial increase in this component (Picton & Hillyard, 1974).

Children

In young children, this P1-N1-P2 complex is not fully developed and their corresponding complex is dominated by a P1 component, that is a positive deflection peaking at about 100 ms, followed by the N2 component which is a negative deflection occurring between 220 and 280 ms in their central ERPs (Donkers et al., 2015; Pang & Taylor, 2000; A. Sharma et al., 1997). This P1-N2 complex in children is shown in **Fig. 1b.** Through developmental maturation of the cortex as speech and language function improves, the P1 component in children decreases in latency and amplitude, forming the P1 in adults (Koravand et al., 2012; Ponton et al., 2000; A. Sharma et al., 1997). The P1 has been proposed as a biomarker of auditory cortical maturation and reflects the status of the cortical auditory system (A. Sharma et al., 2015).

The N1b and P2 components that dominate the CAEP in centro-frontal areas in adults are generally not seen in children and adolescents until after 15-16 years of age for speech sounds (Pang & Taylor, 2000). Although, with longer interstimulus intervals (ISIs) between auditory stimuli, the N1-P2 component has been demonstrated in children as these components have been shown to be sensitive to stimulus rate in children (Čeponien≐ et al., 2002; Donkers et al., 2015). The complex of components in children typically has a developmentally specific N2 that occurs between 200-280 ms in the centro-frontal brain regions after the P1 component (Čeponien≐ et al., 2002; Koravand et al., 2012; Takeshita et al., 2002). The neural generators of this component have been localized to the bilateral supratemporal auditory cortex in the temporal lobe (E et al., 2007; Matas et al., 2015). The amplitude of this component has been shown to increase during childhood and thereafter with development decreases in amplitude until adult values are reached (Ponton et al., 2000). The complexity of the tones has been shown to have a positive relationship with the N2, with increasingly complex tones eliciting a larger N2 amplitude than simpler tones (Čeponien≐ et al., 2002; Čeponiené et al., 2001).



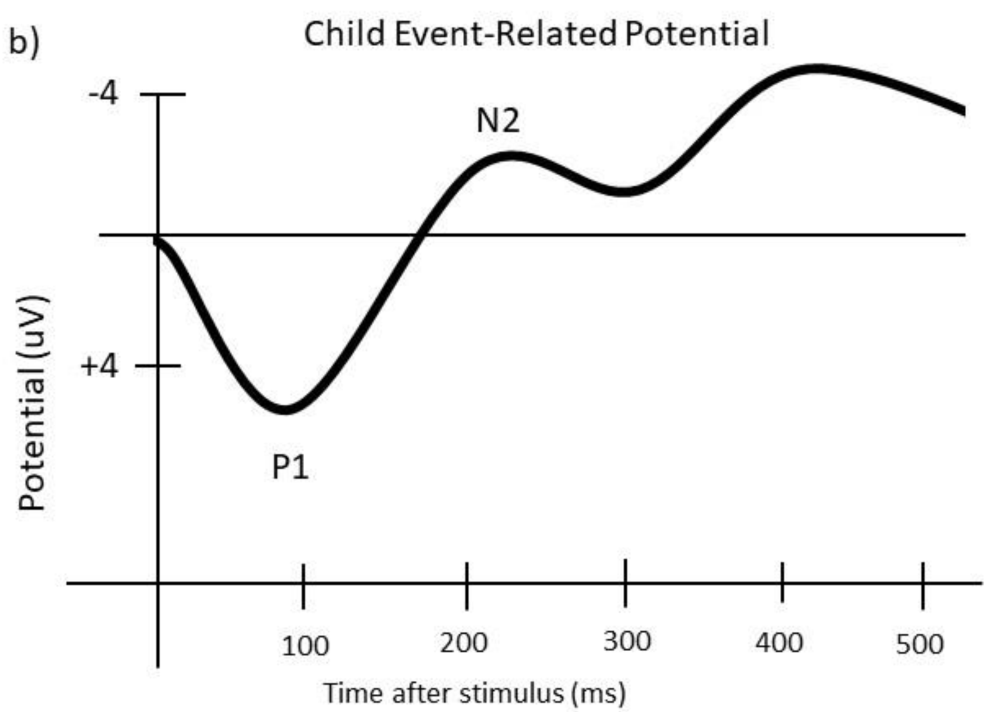


Figure 1. a) P1-N1-P2 complex in the Adult ERP, **b)** P1-N2 complex in the Child ERP

1.4.4. Higher Order (semantic) Processing Component: N400 component

Once cortical auditory processing occurs, higher-order processes such as semantic processing can transpire. Semantic processing is typically investigated using the N400 component of the ERP, which is a relative negative deflection in the ERP that peaks at approximately 400 ms post stimulus onset (M. Kutas & Hillyard, 1980; Marta Kutas & Federmeier, 2011; Rapin & Dunn, 1997). This component is a well-established index for semantic processing and is thought to be associated with assessing preceding context to elucidate to the level of semantic congruency of the stimuli, regardless of modality (Marta Kutas & Federmeier, 2011; Sur & Sinha, 2009). Neural generators of the N400 component have been identified in different areas in the temporal lobes and prefrontal lobes by neuroimaging techniques including intracranial recordings, MEG and event-related optical signals (Marta Kutas & Federmeier, 2011; Lau et al., 2008).

It was first discovered by Kutas and Hillyard in 1980 (M. Kutas & Hillyard, 1980; Marta Kutas & Federmeier, 2011). In this study, participants read seven-word sentences one word at a time in their head, and the last word was occasionally semantically incongruent. The incongruent words elicited a negative component that peaked at 400 ms post stimulus onset that is known as the N400 component. Additionally, the greater the level of incongruency introduced, the larger the amplitude of the N400 (M. Kutas & Hillyard, 1980; Marta Kutas & Federmeier, 2011). The ease of accessing and integrating words with mental representations, given the preceding context is inversely associated with the amplitude of the N400 component (Holcomb, 1993; Kutas & Federmeier, 2011; Kutas & Hillyard, 1980, 1984). For example, a sentence that ends in a semantically incongruent word, such as “She was eating at the dinner table using a *ball”* would elicit an N400 component with a larger amplitude in comparison to a component elicited by a sentence that ended with a semantically congruent word such as “She was eating at the dinner table using a *fork”*.

The N400 component to spoken language has been shown to be evoked in adults and children, however, there are age-related differences in the duration, latency, amplitude and distribution of the component (Atchley et al., 2006; P. J. Holcomb, 1993). In children, the N400 component in comparison to adults is typically at a later latency, of longer duration, and larger in amplitude (Atchley et al., 2006; P. J. Holcomb, 1993). Furthermore, this component to spoken words in children may look like one large negative wave consisting of the initial N400 component between 300 to 500 ms, followed by a large negativity that follows persisting through 500 to 900 ms (Holcomb, 1993; Silva-Pereyra et al., 2005). This negative slow wave can be considered the late negative component (LNC) in children. Research suggests that the initial N400 component indexes preliminary access and retrieval of the word and the LNC is associated with integration of the word with mental representations (Silva-Pereyra et al., 2005). These components undergo developmental modulations, as the amplitude and latency decreases with increasing efficiency in semantic processing, which starts in childhood and reaches adult levels in late adolescence/early adulthood (Phillip J. Holcomb et al., 1992). Additionally, the brain distribution changes with age, with the N400 component typically being found in central-parietal brain regions in adults, that is slightly more frontal distribution for auditory compared to visual stimuli (Atchley et al., 2006; Marta Kutas & Federmeier, 2011), while in children, it is more broadly and frontally distributed (Atchley et al., 2006).

1.4.5. Semantic Processing ERP Studies: N400 effect

Studies have however, focused on the N400 effect as a measure of semantic processing, which is a difference in amplitude between semantically congruent and incongruent stimuli (Coderre et al., 2017; Marta Kutas & Federmeier, 2011). When the sentence, "The oven is …", ends with a semantically congruent word such as "*hot"*, the amplitude evoked is smaller than the amplitude evoked when the sentence ends with a semantically incongruent word such as "*hairy"* and this difference in amplitude called the N400 effect is shown in **Fig. 2**.

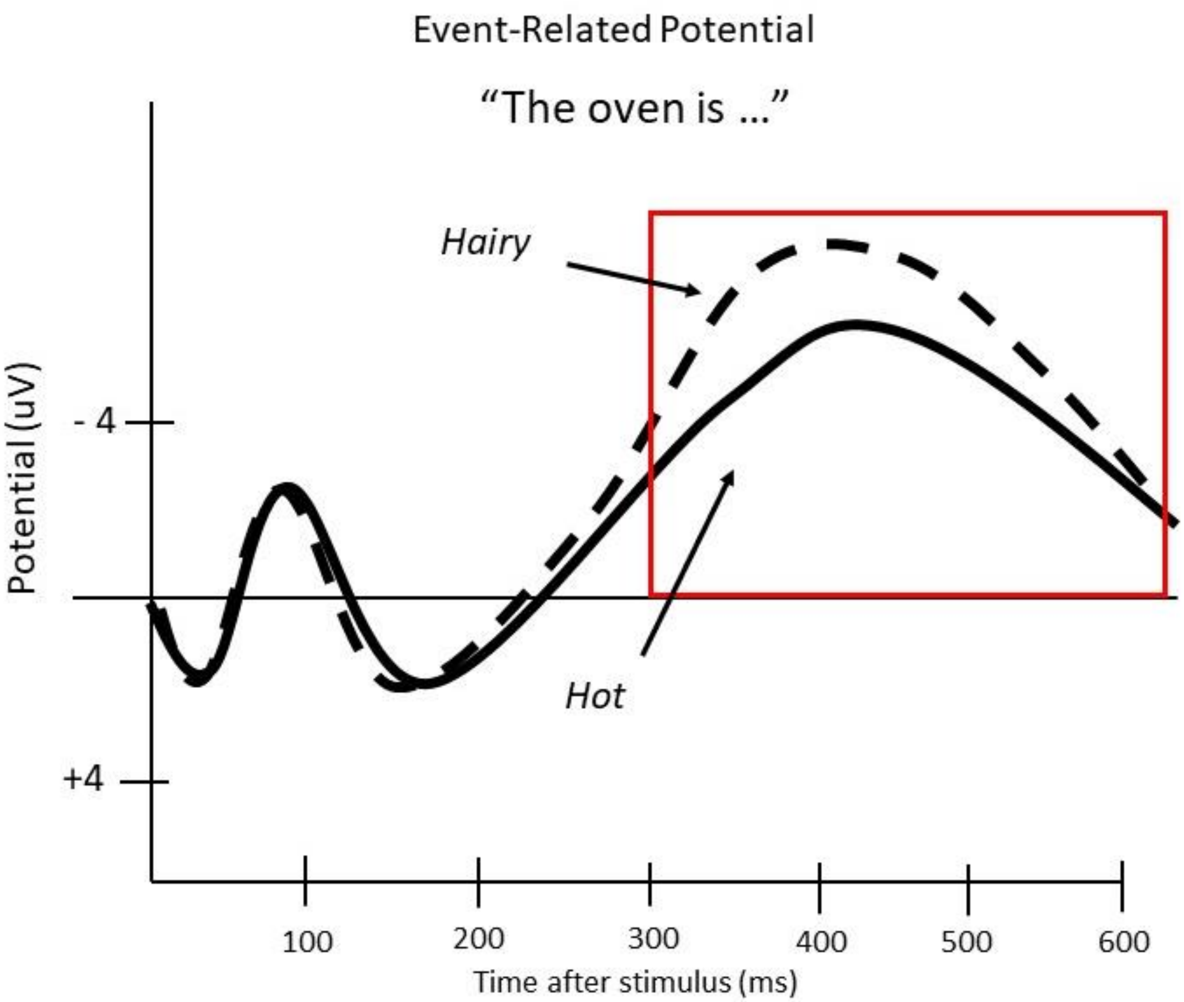


Figure 2. The N400 effect. This difference in amplitude to congruent ('hot') and incongruent ('hairy') stimuli is highlighted by the red box.

N400 effect in Adults

The N400 effect in adults reflects a sensitivity of the N400 component to semantic incongruency, that facilitates an increase in amplitude with increasing semantic incongruency. In a study done by Benau et al. (2011), adults were presented with sentences, one word at a time, and each sentence ended in a word that was either congruent (e.g., "We put syrup on the pancakes"), moderately incongruent (e.g., "The parking lot was filled with lobsters") or strongly incongruent (e.g., "My dad takes pictures with a flute") with the preceding context. ERP results revealed a graded N400 response, with greater incongruency resulting in an N400 component with a larger amplitude in adults (Benau et al., 2011), thus showing sensitivity to semantic incongruencies.

N400 effect in children

The N400 component does not show the same level of sensitivity to incongruency in children as it does in adults. More specifically the absence of the N400 effect in children is not always associated impairments in semantic processing abilities.

This was shown in the child cohort in Benau et al. (2011)'s study that exhibited no gradation in their N400 component response. Both levels of incongruency evoked N400 components with negligible differences between their amplitudes, but both were however larger than the response elicited by the congruent condition. This contrasts with the graded response shown in the adult cohort in this study. Moreover, there was no difference between the two cohorts regarding their behavioural performance, which involved rating on a scale of 1-5 if the sentences presented "made sense", with 1 suggesting that the sentence presented made no sense (Benau et al., 2011). These results implied that adults and children were both exhibiting semantic processing, but the neural processing underlying this language process was different, that is, their N400 components were not exhibiting the same level of sensitivity to incongruency.

This lack of sensitivity to incongruency was also found by Kallioinen et al. (2016), who had three cohorts of children (between 5 to 7 years of age): 1) those with normal hearing (NH), 2) those with hearing aids (HA), and 3) those with cochlear implants (CI) in their study. Participants were presented with a spoken word (prime) that was followed by an image (target). There were three conditions in this paradigm: 1) the congruent condition, where the word perfectly matched the picture (e.g., heard the word 'wolf' and presented with picture of a wolf), 2) the moderately incongruent condition (e.g., heard the word 'wolf' and presented with picture of a bear), and 3) completely incongruent (e.g., heard the word 'wolf' and presented with a picture of a sock). Results revealed that all three cohorts had an N400 component response that was larger for the moderately and completely incongruent conditions in comparison to the congruent condition, which implied that an N400 effect occurred in all cohorts. However, the amplitude of the N400 component for the moderately incongruent in comparison to the completely incongruent conditions in children with NH and children with HA did not significantly differ, indicating a lack of sensitivity to differing levels of incongruency in thesechild cohorts. In children with CI on the other hand, the completely incongruent condition evoked the largest response, revealing a graded response with increasing incongruency eliciting a larger response. The presence of the graded N400 component response would typically indicate better semantic processing performance, but it was not associated with better semantic processing skills in children with CI. These results were interpreted as possible differences in processing strategies between children with NH and children with CI. It was speculated that children with CI may be engaging with predictive processing, which is a top-down strategy used by this group, where they are predicting the outcome, while NH children may be engaging with a more passive bottom-up approach that does not involve predictions (Kallioinen et al., 2016.)

1.4.6. Semantic Processing ERP Studies in Children with ASD: N400 effect

Studies thus far examining the neural processing underlying semantic processing in children with ASD have nonetheless viewed the N400 effect as a measure of semantic processing.

Various studies primarily have focused on children with ASD with high language capabilities and results have shown no N400 effect in these children (Dunn & Bates, 2005; McCleery et al., 2010; Ribeiro et al., 2013). For example, this was the result reported by Dunn and Bates (2005), who had children with ASD between 8 to 12 years old with language scores within normal range, auditorily attend to a list of spoken words and listen for “animal” words. Words that were not semantically categorized as “animals”, indicating that they were semantically incongruent with the preceding context, did not elicit a larger response than congruent words for the category in children with ASD (Dunn & Bates, 2005). This was also the result found in young children with ASD between 4 to 7 years of age with language scores within normal range, who were presented with an image (prime), which was followed by an auditorily presented word or environmental sound (target) that matched or mismatched the image. The children with ASD in this study did not elicit an

N400 effect for spoken words, but they did for the environmental sounds, reflected by a larger response when the target environmental sound was incongruent to the prime (image) (McCleery et al., 2010). Additionally, Riberio et al. (2013) reported no N400 effect in older children with ASD (mean age: 13 years±4) who were matched for verbal intelligence quotients (VIQ) with age-matched TD children, using not just spoken words, but also music excerpts. In one condition in their study, participants listened to sentences that ended with a picture (target image) that was congruent or incongruent with the preceding sentence context and in the other condition, a musical excerpt was used as the priming cue for the target image. The participants were instructed to evaluate whether the target cue was related to the prime (sentence or music excerpt) and results revealed no difference in the N400 component between congruent and incongruent targets for both the spoken word and music condition in children with ASD (Ribeiro et al., 2013). These aforementioned studies interpreted the absence of the N400 effect as neural processing deficits associated with semantic processing deficits observed in children with ASD.

However, language capabilities in children with ASD is a highly heterogenous, ranging from those presenting with verbal fluency that possess pragmatic deficits to those who are minimally verbal (MV) to NV (Brignell et al., 2018; Mody & Belliveau, 2013; Rapin & Dunn, 1997). Primarily focusing on children with higher language capabilities when investigating semantic processing limits knowledge regarding children with lower language abilities who can also possess these language difficulties. Cantiani et al. (2016) were one of the few studies to include young MV and NV children with ASD (3 to 7 years of age) in their study investigating semantic processing in children with ASD. These participants were presented with an image (prime) that was followed by an auditorily presented word that either matched the image (congruent) or mismatched the image (incongruent), with no behavioural response required. Results revealed that the MV and NV children with ASD did not evoke an N400 effect when comparing responses for the incongruent and congruent conditions (Cantiani et al., 2016), that falls in line with research on children with ASD with higher language capabilities.

Nonetheless, there is evidence that contradicts these results in children with ASD with lower language capabilities as well. This was reported in a recent study by Distefano et al. (2019), which involved a passive picture-word matching paradigm that was conducted on verbal children with ASD, MV children with ASD, and TD children as the control group. Both verbal and MV children with ASD in this study showed an N400 effect that was significantly temporally delayed in comparison to children in the control group. This informed researchers that there were delays in neural processing underlying semantic processing that could be associated with deficits in this language domain (DiStefano et al., 2019), rather than the absence of the N400 effect. Additionally, in a study done recently by Manfredi et al. (2020), older children with ASD (mean age: 12.6, SE=1.9) with significantly lower VIQ than age-matched TD children exhibited an N400 effect that was either not significantly different or was present but, was significantly attenuated in comparison to TD children, depending on the modality of the stimuli. This study involved two conditions: 1) listening to three-word sentences (verbal condition) and 2) watching 3 panel comic strips (visual condition), with half of the trials in each condition containing an incongruent word for the last word or an incongruent panel for the last comic panel, respectively. For the visual condition, children with ASD showed no difference in response compared to TD children, with both child cohorts showing an N400 effect when incongruency was introduced to the last panel of the comic strip. For the verbal condition however, in comparison to the TD children who exhibited an N400 effect, the children with ASD had an N400 effect that was significantly smaller (Manfredi et al., 2020).

These ERP studies investigating the neural basis of semantic processing in children with ASD have primarily used the N400 effect as their measure of semantic processing and there remains no consensus regarding the alterations in the N400 effect in children with ASD. Therefore, the neural processing underlying these deficits remains unknown.

In addition, very few of these studies mentioned above examined the CAEPs, although the N400 component can be affected by the CAEPs (DiStefano et al., 2019; Manfredi et al., 2020; McCleery et al., 2010; Ribeiro et al., 2013). In the few studies that did examine CAEPs, their results regarding cortical auditory processing in children with ASD were opposing, thus the association remains unknown. The study by Cantiani et al. (2016) was one of the two studies that involved an analysis of the P1 component as a measure of cortical auditory processing. Their results revealed that children with ASD had relatively intact cortical auditory processing indexed by similar amplitudes for the P1 component in comparison to the control group, however there were latency delays. The association between the latency delays shown and higher order processing such as semantic processing however, were not further examined (Cantiani et al., 2016). In the study by Dunn and Bates (2005) that included the N1b component as a measure of corticall auditory processing, they found no differences in latency or amplitude between TD children in the control group and children with ASD for the N1b component, suggesting no cortical auditory processing deficits in children with ASD (Dunn & Bates, 2005).

Chapter Two: Rationale, Aims and Hypotheses

Since the discovery of ASD, research has been done to enhance the understanding of the neurobiology underlying receptive language processing in children with ASD. However, deficits in semantic processing, which is one of the processes that are essential for receptive language processing, have been shown in children with ASD and the neural underpinnings related to semantic processing impairments remains unknown.

Research surrounding semantic processing in children with ASD has focused on the N400 effect and this is misleading because in TD children the sensitivity of the N400 *component*/LNC to differing levels of semantic incongruencies is not the same as adults and the N400 *effect* may be absent without any associated impairments in semantic processing abilities in children. The study described in this thesis will focus on investigating the N400 component itself and the associated LNC in children with ASD because these components themselves reveal information regarding the ease of accessing and integration of words with their mental representation given the semantic context. The results reported by Cantiani et al. (2016) prompted the investigation of the N400 component/LNC itself, as they reported lower overall neural strength during the N400 component in children with ASD with low language ability in comparison to TD children. However, the focus of their study was on the N400 effect and there were limitations to their study such as their small sample size number for both TD children and children with ASD with low language ability. Additionally, they interpolated approximately 25% of their EEG channels and only focused on children with ASD with low language ability (Cantiani et al., 2016). This precludes children with ASD with high language abilities, thus not encapsulating the full range of individuals with language deficits in ASD. The aim of this project was to expand on and extend the study done by Cantiani et al. (2016) by including young children with ASD who have a variation in their language capabilities to better represent the variability seen in real life and to include a focus on the auditory modality.

2.1. Primary Aim and Hypotheses

Primary Aim

We firstly aimed to evaluate the neural processing underlying semantic processing in TD children to characterize the N400 component and LNC in TD children. These responses would be compared to children with ASD with a variation on language ability in the ASD cohort, determining potential differences and/or similarities in the timing and amplitude of the N400 component and LNC to identify differences in semantic processing in children with ASD.

Primary Hypotheses

We hypothesize that:

1) Children with ASD in comparison to TD children will have an N400 component and LNC with a delayed onset and smaller amplitude.
2) The N400 component/LNC amplitude will predict the language test scores in children.

2.2. Secondary Aim and Hypothesis

Secondary Aim

Our second aim was to confirm age-related differences in semantic processing by investigating the N400 effect and N400 component (and LNC in children) in TD children compared to adults.

Secondary Hypothesis

We hypothesized that TD children show less sensitivity to semantic incongruencies compared to adults, reflected by a negligible difference between their N400 component amplitude for the congruent and incongruent condition.

Chapter Three: Methods

This chapter provides a description of recruitment criteria for participants, study design, the experimental setup for collecting EEG data, the experimental procedure as well data analyses and statistical comparisons. The study was approved by the Research Ethics Boards (REB) at the Hospital for Sick Children and testing was done at the Behavioural Assessment Unit at the Peter Gilgan Centre for Research and Learning, Hospital for Sick Children in Toronto.

3.1. Participants

A total of 59 participants were recruited for this study, including young adults, TD children and children diagnosed with ASD. More detailed participant descriptions are contained in the following sections. Written informed consent was obtained from adult participants and parents of children. As well, informed verbal assent was obtained from the children. Medication, parent reported language ability/use and handedness were obtained from both TD children and children with ASD. At the end of testing, adult participants received a small gift card and child participants received a small gift card and toys. Additionally, children and parents were provided lunch and parents were reimbursed for travel.

Target recruitment numbers were 30 for each of the child cohorts and 15 for the adult cohort. Due to COVID-19, recruitment was halted due to safety in March 2020.

3.1.1. Adult cohort

Sixteen adults that were recruited for the Adult cohort through word of mouth and through flyers posted at the Hospital for Sick Children for neuroimaging studies. Inclusion criteria were as follows: 1) >=18 years of age, 2) ability to provide consent, 3) can press buttons with fingers.

Exclusion criteria were as follows: 1) self-reported history of neurological, psychological, and/or psychiatric disorders, 2) self-reported peripheral hearing impairments, 3) self-reported visual, academic, language, reading or cognitive deficits, 4) inability to sit for a minimum of 5-min intervals for 15 mins.

3.1.2. Typically Developing (TD) cohort

Twenty-four TD children that were recruited for the TD cohort through flyers posted on social media such as Facebook and Twitter, bulletin boards in community centers, hospitals, schools in Toronto area, summer camps and through word of mouth. Posters provided a short description of the study, inclusion criteria and contact information. A member of the team would follow up with interested families of possible participants via an initial telephone call and/or email to provide further details regarding the study and inclusion criteria, as well as screen for exclusions.

Inclusion criteria were as follows: 1) 4 to 8 years of age, 2) ability to provide assent, 3) can press buttons with fingers, 4) academic performance at appropriate grade-level.

Exclusion criteria were: 1) parent reported history of neurological, psychological, and/or psychiatric disorders, 2) parent reported impairments in peripheral hearing, 3) parent reported visual, academic, language, reading or cognitive deficits, 4) inability to sit for a minimum of 5-minute intervals for approximately 15 mins.

3.1.3. ASD cohort

Nineteen children diagnosed with ASD were recruited through various ways. Children with ASD were initially identified and recruited through the Province of Ontario Neurodevelopmental Disorder Network (POND) database from Holland Bloorview Kids Rehabilitation Hospital in Toronto, where data were acquired from a large-scale study on neurodevelopmental disorders. Additionally, participants were recruited from flyers posted on social media such as Facebook and Twitter, on bulletin boards in the community centers, other hospitals, ASD research centres and through word of mouth. A member of the team conducted a before-study email or telephone screening with interested families of possible participants to provide additional information regarding the study and inclusion criteria, as well as screen for exclusion.

Inclusion criteria were as follows: 1) a clinical diagnosis of ASD based on clinical judgement by a professional and/or supported by the results of the Autism Diagnostic Observational Schedule - Generic (ADOS-G)/Autism Diagnostic Observational Schedule–2 (ADOS-2) or the Autism Diagnostic Interview-Revised, 2) 4 to 8 years old, 3) ability to provide assent.

Exclusion criteria were as follows: 1) head sensitivity that resulted in inability to wear EEG cap, 3) inability to sit for a minimum of 5-minute intervals for approximately 15 mins, 4) parent reported impairments in peripheral hearing.

3.2. Assessments

Neuropsychological assessments of language and cognitive function were administered to children the day of EEG acquisition. This was done to characterize the children with ASD in the ASD cohort as well as ascertain normal range in children in the TD cohort. The two tests that were administered by examiners the day of EEG acquisition were the Peabody Picture Vocabulary Test, Fourth Edition (PPVT) and the Subtests of the Wechsler Preschool & Primary Scale of Intelligence (WPPSI) – 2nd edition. These tests were used to evaluate receptive language skills and cognitive function. All neuropsychological assessments took approximately an hour to administer. Other previously administered neuropsychological assessments that measure language and cognitive skills such as the Preschool Language Scale – Fifth Edition (PLS-5) and the Stanford Binet Test - Fifth edition (BINET) were obtained from participants with ASD who were unable to compete the PPVT and WPPSI due to their cognitive and language capabilities.

3.2.1. Language Assessments

Peabody Picture Vocabulary Test – Fourth edition (PPVT-IV) (*Peabody Picture Vocabulary Test | Fourth Edition*, n.d.) (Dunn & Dunn, 2007)

The PPVT-IV is a test that measures receptive vocabulary and does not require reading or writing abilities (Eigsti, 2013; *Peabody Picture Vocabulary Test | Fourth Edition*, n.d.). The child is presented with a set of four pictures, the examiner verbally pronounces a stimulus word that names one of the pictures, and the child is instructed to choose what picture best matches the stimulus word.

Preschool Language Scale—Fifth Edition (PLS-5)(*PLS-5 Preschool Language Scales 5th Edition*, n.d.) *(Zimmerman, Steiner, Pond & Evatt, 2011).*

The PLS-5 is a language assessment that is designed to assess developmental language in children aged form birth through to 7 years and 11 months old and assess possible language delays or deficits. This test involves interactive, play-based assessments that evaluate receptive and expressive language skills in children.

3.2.2. Cognitive Function Assessments

Weschler Preschool & Primary Scale of Intelligence – Second Edition (WPPSI-II) (Weschler, 1967)

The WPPSI-II is a standardized test that measures a child's IQ. There are sets of subtests that measure different cognitive functions such as verbal comprehension, visual spatial skills, fluid reasoning, working memory and processing speed to make up the full-scale IQ score. With the focus of study being on receptive language processing and the nature of the paradigm, subtests that focused on verbal comprehension, fluid reasoning and working memory were selected. The subtests used to obtain the verbal comprehension score were the Information subtest, which consists of general knowledge questions and the Similarities subtest, that requires identification of similarities between common objects. For language measures, two participants with ASD were unable to complete the PPVT-IV, therefore their verbal comprehension standard score on the WPPSI-II was used as their language score. As for fluid reasoning, the Matrix Reasoning subtest which consisted of participants choosing a picture to complete patterns, and the Picture Concepts test, that required choosing two or two pictures with common trait between them, were used. Lastly, working memory was measured by the Picture Memory subtest that required memorizing pictures and identifying them on the following page and Zoo Locations subtest that involved memorizing the location of animal cards and placing them in the same location following a short delay. Fluid reasoning and working memory scores were used for cognitive measure scores.

Stanford-Binet Intelligence Scales - Fifth edition (SB-5) (*(SB-5) Stanford-Binet Intelligence Scales, Fifth Edition | WPS*, n.d.) *(Roid, 2003).*

The SB-5 test is a standardized test that measures intellectual function in participants that are two years of age and older. It obtains a single composite or full-scale intelligence quotient (IQ) score as well as both non-verbal IQ and VIQ. This is gauged by five components of cognitive ability: fluid reasoning, knowledge, quantitative reasoning, visual-spatial processing and working memory. Fluid reasoning and working memory scores on the SB-5 were the focus for the present study to coincide with the cognitive domains captured by the WPPSI-IV. Additionally, the VIQ score was used for one child with ASD (N=1) in the ASD group as their language measure due to their inability to complete the assessments conducted the day of EEG acquisition, and scores from other previous language assessments were unavailable.

The standard scores for all assessments used in this study were matched for age to achieve age-equivalent scores and had a mean of 100 and SD ± 15. **Table 1** describes the which assessments were used for language and cognitive function in TD children and children with ASD in this study.

Table 1. Language and Cognitive Assessments used to evaluate language and cognitive function in the TD cohort and the ASD cohort

	TD cohort (N=21)	ASD cohort (N=14)
Language Assessment	• PPVT (N=21)	• PPVT (N=10) • PLS-5(N=1) • WPPSI-IV – Verbal comprehension index score (N=2) • SB-5 – verbal IQ (VIQ) (N=1)
Cognitive Assessment	• WPPSI-IV- working memory scores and fluid reasoning (N=21)	• WPPSI-IV–working memory scores and fluid reasoning (N=7) • SB-5 – fluid reasoning and working memory scores (N=1)

3.2.3. Hearing Assessment

All participants' hearing sensitivity for calibrated pure tones was screened across the speech spectrum (250 to 4,000 Hz). This was used to determine if the participant's hearing levels fell within normal limits at baseline. Participants sat in a quiet room and insert earphones were placed in each ear. An examiner instructed participants to lift their hand every time they heard a sound. Stimuli were pure tones of 250 Hz, 500 Hz, 1000, 2000 Hz and 4000 Hz that were each presented 3 times with varying interstimulus intervals (ISIs) (1 to 5 sec) between the three tones. All pure tones were delivered at 15 dB HL. If there was no response, the level was increased to 20 dB HL. If there was no response at this level for any of the pure tone stimuli, the participant would have been excluded from data analysis. This did not occur in any participants, indicating that all had normal hearing sensitivity.

3.3. Data Acquisition

EEG was recorded in a quiet room in Behavioural Assessment Unit at the Peter Gilgan Centre for Research and Learning in Toronto.

3.3.1. Stimuli

Stimuli were single syllable words spoken by a female native English speaker and delivered at 65 ± 3 dB HL, binaurally through insert earphones. These words were recorded on a digital system, digitized at a rate of 44.1 kHZ (16-bit resolution; mono). Additionally, stimuli were between 400-700 ms in duration and were vocabulary highly familiar to young children, derived from the MacArthur-Bates Communicative Development Inventories: Words and Sentences Vocabulary checklist (CDI) (*MacArthur-Bates Communicative Development Inventories*, n.d.). The words in the CDI are classified into semantic categories and four categories were selected for this paradigm: Animal, Body, Food & Drinks and Action Words. Twelve single syllable words were chosen per category. The list of words used in their corresponding categories is in Appendix A.

3.3.2. EEG Task

A category (prime word) was provided to participants at the beginning of each block verbally. Following that, participants listened to a list of words and indicated after every word in the block, if the words was in the category (semantically congruent condition) or out of category (semantically incongruent condition). To indicate their choice, participants responded by pressing a computer mouse button labelled "Yes", for semantic congruency with the given prime, or "No" for semantic incongruency, or "I don't know", if participants did not know. Words were presented one at a time and to proceed to the next word, the behavioural response of a button press indicating their choice with the computer mouse was required. Additionally, after every behavioural response, a two sec ISI occurred before the onset of the next word. This occurred until the participant finished 24 words, reaching the end of the block, and this was repeated for each block. An example of the task is shown in **Fig. 3**. Their button responses as well as their RT was recorded as behavioural responses.

The prime word was only provided to participants at the beginning of the block, however a computer screen that was situated 60 cm in front of them displayed the category in the middle of a black screen, along with corresponding pictures for that category (not included in the words used in the tasks) beside the word as a reminder of the prime word (category) during the entire block on the screen. Additionally, a "Yes" and a "No" was displayed on the bottom of the screen, corresponding to positions of the buttons on the mouse that would be used to capture the behavioural response. This was used as a reminder of what the button responses were. These visuals remained the same during the entire block and ended once the block was over (see **Fig. 4**).

There were four blocks in total, corresponding to the 4 categories selected from the CDI (Animals, Body, Food & Drinks and Action Words). Each block consisted of 12 semantically congruent and 12 semantically incongruent words. From the 4 blocks, there were 48 words per condition, which translated to 48 trials per condition and 96 trials all together. The words within each block were randomized once and this order was used for each participant. The order of blocks, however, was randomized for each participant.

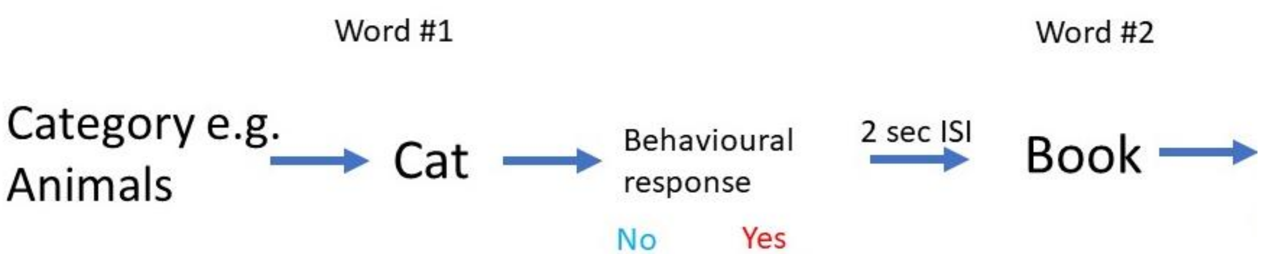


Figure 3. EEG Paradigm Task

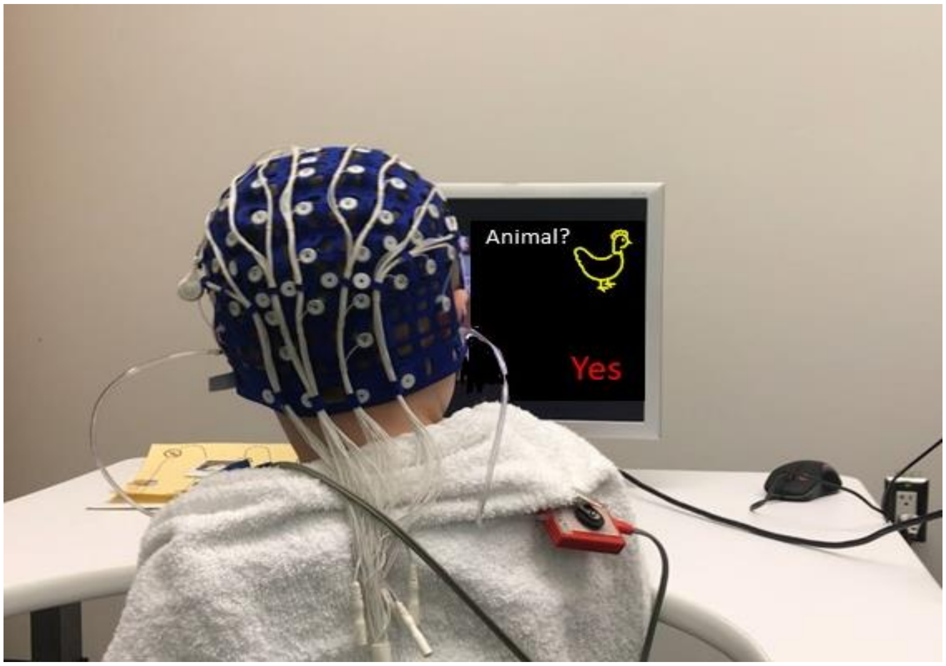


Figure 4. EEG Task Set Up

3.3.3. Stimuli and Task Development

The task was based on the paradigm used by Dunn and Bates (2005), where participants were required to do semantic categorization of spoken words (Dunn & Bates, 2005). The categories and words chosen for the task used in this study were selected from the CDI. The CDI contains commonly acquired words by children, which are already separated into semantic categories. It is the most widely used parent-report instrument of early language development in children with ASD (Tager-Flusberg et al., 2009).

The criteria for selection of words were: 1) had to be single syllable words, 2) be non-plural, and 3) did not end in an /**s**/ sound. The categories had to be distinct from one another and have a minimum of 12 words for each category. That resulted in the four categories selected for the paradigm being "Animals", "Body Parts", "Food & Drinks", and "Action Words", which are verbs. These categories created the four blocks for the task. The semantically congruent words block were the 12 words selected from each semantic category as listed by the CDI. The semantically incongruent words for each category were randomly selected from the remaining words from the other three categories.

The stimuli were recorded using a microphone in a quiet room in the BAU at the PGCRL, on the version 2.4.2 of Audacity (R) recording and editing software (https://audacityteam.org/). The female English speaker rehearsed all 48 words prior to recording to ensure that the words were clearly and fluently pronounced. The speaker was also instructed to speak in a natural and emotionally neutral manner while words were being recorded. The words were edited to 400-700 ms in duration, silent spaces before and after the words were removed and the leveling the intensity of each word was done using the audio editing software: Goldwave version 6.36 (Goldwave Inc, http://www.goldwave.ca). Stimuli levels were calibrated using a sound level meter. Additionally, Goldwave was used to insert trigger codes at the beginning of each word, which was necessary for the alignment of the stimuli and EEG data. After the stimuli were recorded and edited, the actual task was programmed in the stimulus presentation software (Presentation, Neurobehavioral System Inc.) and presented on a laptop. Behavioral responses were measured using this software.

3.3.4. EEG Acquisition Parameters

EEG data were recorded continuously from a high density 64-channel Quick-Cap Neo Net cap using a NeuroScan v4.5 Synamps2 amplifier system (Compumedics, El Paso, TX, United States), with electrode placement determined by the International 10-20 system (Klem et al., 1999), shown in **Fig. 5**. The sampling rate was 1000 Hz and filtered from DC-100 Hz. The recordings were referenced to an electrode between Cz and CPz electrodes for acquisition and impedance was kept below 10 KΩ. In effort to ensure that there were not large impedance differences between participants, examiners tried to keep the range for impedance between 5-10 Hz KΩ for all participants. The EEG cap captured eye movements using four integrated bipolar leads for vertical and horizontal electrooculography. EEG was synchronized with the onset of each word by trigger codes that were sent from the stimulus presentation software (Presentation, Neurobehavioral systems, Berkeley, CA, United States) to the EEG data acquisition system via a parallel port.

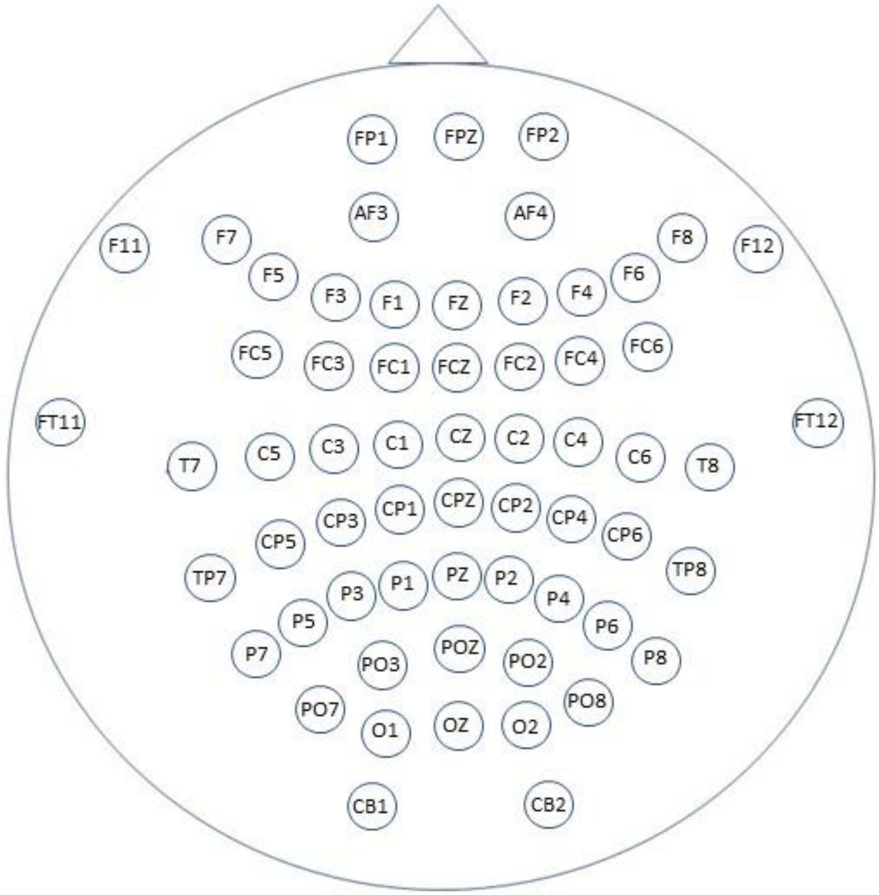


Figure 5. Head Map of EEG Recording Electrodes

3.3.5. Procedure

For child participants, some arrived for testing with a sibling or friend. In this case, one child would be administered the battery of neuropsychological tests, while the other child would complete the EEG tasks, and they would switch tasks after a lunch break. For adult participants, only the EEG task was administered.

During the EEG recording, participants were seated comfortably 60 cm away from a computer screen where the visual stimuli of the paradigm would be presented (shown in **Fig. 4**). Insert earphones were placed in each of the participant's ears and the hearing assessment was administered before EEG acquisition. All participants were fitted with caps according to the circumference of their head and conducting gel was applied to the electrodes on the cap. Additionally, the four blocks of the paradigm were randomized for the participant. After the EEG cap was properly fitted and prepared, participants were reminded to try to sit at still as possible during the EEG recording.

The task would begin, and participants were verbally instructed as to the category by an examiner, which was their prime word (e.g., Animals). Following that, participants were instructed that they were going to be listening to a list of words and after every word they would have to indicate if the words were in the category given (semantically congruent) or out of category (semantically incongruent), as fast as possible. To indicate their choice, they held a computer mouse the entire time with two hands and were instructed to respond using a button press labelled "Yes", for when the word was in the category (semantically congruent) or "No" for when the word was out of category (semantically incongruent), or "I don't know", if participants did not know. Behavioural responses were recorded.

A practice trial was administered at the beginning of each block where participants would get feedback after every response if they were correct or not. This occurred for six trials to ensure that participants understood the behavioural response required. In these trials, words that were not part of the blocks were used for the practice trials.

An examiner sat next to the child throughout EEG recording to ensure as minimal movement as possible, as well monitoring the participant's attention and arousal during the task. After every block, examiners asked participants if they needed a break, or electively chose to take a break to lower fatigue and attrition rates in child cohorts. Additionally, another examiner monitored the continuous EEG recording for all channels so that issues arising from electrodes could be recorded and corrected offline.

Participants in the ASD cohort who were unable to do the behavioural portion of the task did the same experimental procedure, however they just listened to the list of words while an examiner clicked the response button every 2-3 secs. The behavioural responses were omitted for these individuals.

Two other tasks were administered during the EEG acquisition. One consisted of an auditory brainstem response task that investigated auditory processing, while the other task required participants to watch a 15 min clip from a movie to investigate speech tracking. These tasks will not be explored in this paper. EEG testing time took approximately 2 hours per participant and neuropsychological assessments took approximately 1 hour per participant, which in total was 3 hours of testing per individual.

3.4. Data Analyses

3.4.1. Data Analysis for Language Assessments in the TD cohort and the ASD cohort

A one-way ANOVA was run in R (version 4.03) to analyze the differences between TD children in the TD cohort and children with ASD in the ASD cohort on language ability indexed by their language scores. The independent variable was cohort (TD cohort, ASD cohort).

Furthermore, due to the variation in language scores in children with ASD and due to a portion of children in the ASD cohort having language scores falling 1.5 SD below the standard mean, children in the ASD cohort were divided by their language ability. Children with ASD in the ASD cohort with scores below 1.5 SD of the standard mean were considered the "ASD cohort with low language ability" (ASD cohort: low language) because children with other language disorders such as dyslexia have shown similar scores and this cut off has been used in multitude of studies to identify language impairments in children (Loucas et al., 2008; Snowling et al., 2020; Spaulding Tammie J. et al., 2006). In total there were six children in the ASD cohort: low language (N=6) and eight children with ASD considered the "ASD cohort with high language ability" (ASD cohort: high language) (N=8) indicated by scores that were within normal (100 ±1 SD) or above the standard mean. A one-way ANOVA was used to assess differences in language ability between children in the ASD cohort: high language and ASD cohort: low language indexed by their language scores.

3.4.2. Data Analysis for Cognitive Assessments: Working Memory in the TD cohort and the ASD cohort

Working memory is a component of executive function that can affect semantic processing and plays a large role in the task used in this study, thus was the domain selected for further investigation in children (Blijd-Hoogewys et al., 2014; Dawson & Guare, 2004; Gathercole & Baddeley, 1993). A one-way ANOVA was used to analyze the differences between children in the TD group and in the ASD group on working memory indexed by their working memory scores.

3.4.3. Data Analysis for Behavioural Results (Accuracy and Reaction (RT)) in the Adult cohort, TD cohort and ASD cohort

Two separate 3x2 ANOVAS were used to analyze behavioural data and evaluate differences between adults, children in the TD cohort and children in the ASD cohort on accuracy and RT on the paradigm. The independent variables were cohort (Adult cohort, TD cohort, ASD cohort) and condition (congruent, incongruent) for both tests. Effect size was calculated using partial eta squared due to the unequal sample sizes of the three groups.

3.4.4. EEG Data Preprocessing

EEG data were imported and processed offline using the Fieldtrip toolbox in Matlab (Oostenveld et al., 2011). Data were low pass filtered at 30 Hz for all participants, with a notch filter implemented only for children. The data were referenced to a common average for adults and to an average of the mastoid electrodes (M1 + M2) for all children in the TD cohort and ASD cohort. Data for all cohorts were epoched into individual trials relative to the onset of the word (-1.5 to 2 s) and downsampled to 500 Hz. Acquisition records and visual inspection were used to identify consistently noisy channels that were replaced with interpolated proximal channels. An independent component analysis was used to identify and remove eye artifacts. An automatic artifact rejection of trials was initially carried out for electrical activity over 250 uV, however visual inspection of the rejected trials revealed a high number of misclassified epochs. Therefore, trial-by-trial examination was done by examiners to identify individual trials for exclusion. Exclusion criterion was > 3 channels with electrical activity over 250 uV. The data for each participant were averaged and baseline corrected from 500 ms preceding the word to the onset of the word at 0 ms. These trials were then grand averaged for across participants by condition for each cohort.

3.4.5. Data Analysis for EEG Data

The CAEPs and N400 component/LNC in adults and the TD children were first identified to investigate normative cortical auditory and semantic processing and to examine age-related differences. The CAEPs were explored to assess cortical auditory processing of the spoken words that can affect higher order processing such as semantic processing. The results of children in the TD cohort were then compared to children in the ASD cohort: high language and in the ASD cohort: low language to identify similarities and differences between all three child cohorts.

3.4.5.1. Examining the CAEPs, N400 component/LNC and N400 effect the Adult cohort and TD cohort

Visually identifying CAEPs and N400 component/LNC: Global Field Power (GFP)

Global field power (GFP) is a measure that characterizes the strength of the neural responses recorded from all electrodes simultaneously distributed over time, that is reference-independent (Skrandies, 1990). It represents the change in overall level of activation in the brain and is measured by the standard deviation of amplitude across all electrodes at each sampling point (Skrandies, 1990). The GFP plot can illustrate temporal changes in the overall strength of the electric potential as a function of cohort and condition. The maxima of the GFPs can be used to interpret overall ERP components in multichannel EEG data and thus the GFP for each condition for each participant was calculated. In the Adult cohort and the TD cohort, the GFPs were grand averaged for each condition.

Based on visual inspection of the maxima of the GFPs during the first 300 ms in both adults and TD children, CAEPs in both cohorts were identified to investigate cortical auditory processing of the stimuli. Topographical plots corresponding to TWs identified for the CAEPs from the GFP, were plotted for each condition to visualize the distribution of electrical activity across the scalp during the components.

Additionally, the maximum of the GFP waveforms after 300 ms was used to visually identify higher order components such as the N400 component in adults and TD children, as well as the LNC for the children.

Statistically identifying the N400 component/LNC and N400 effect scalp distribution in Adults and TD children: cluster-based permutations

To statistically identify where the N400 component/LNC in each condition was occurring and if there was an N400 effect in adults and TD children, cluster-based permutations were used. FieldTrip's cluster-based permutation tests are statistical analyses that can determine if there are significant differences in electrical activity between two conditions in electrode clusters, allowing for spatial analyses (Maris & Oostenveld, 2007). Additionally, it controls for the type 1 errors encountered in EEG research with multiple comparisons by clustering together spatiotemporally adjacent electrodes that all exhibit a similar difference (in polarity and magnitude).

At every electrode two-tailed t-tests are used to compare electrical potential between conditions at the time-sample selected. For within-subject analyses, paired t-tests are used, while between-subjects analyses require unpaired t-tests. Clusters are then formed from spatiotemporally adjacent electrodes that had exceeded a threshold that is specified (usually significance level), requiring at least two electrodes to construct a cluster. The t-values are summated within each cluster. These values are compared to a null distribution from however many permutations specified, using the Monte Carlo method, that assumes no difference between conditions. Any cluster-level t statistics that falls in the ± 2.5th percentile are considered significant. Both the cluster-based permutations tests to identify the N400 component/LNC and the N400 effect were done on adults and children separately.

Cluster-based permutation: N400 Component/LNC

To investigate where the N400 component/LNC itself occurred spatially across the scalp, cluster-based permutations ($p<0.05$, 1000 permutations) were applied to investigate differences in electrodes between the baseline period (-500 to 0 ms) and two-time windows (TW) of 300-700 ms and 700-1100 ms identified from the GFP, in each condition (congruent, incongruent) separately. Any electrode clusters would indicate an increase in activity after stimulus onset. Positive clusters would reflect greater positive activity, while negative clusters would reflect greater negative activity, with the latter indicating an N400 component/LNC.

Cluster based permutation: N400 effect

FieldTrip's cluster-based permutation tests ($p<0.05$, 1000 permutations) using paired t-tests were done to determine if there were significant differences between the semantically congruent and incongruent conditions in electrode clusters. Permutation testing was applied to data averaged within two TWs: 300-700 ms and 700-1100 ms that were determined from the GFP. Significant positive clusters represent greater positive activity for semantically incongruent condition and significant negative clusters represent greater negative activity for the semantically incongruent condition. The latter would indicate an N400 effect is occurring, as the incongruent condition would have a larger amplitude than the congruent condition response.

3.4.5.2. Examining Differences in the CAEPs and N400 component/LNC between the TD cohort, the ASD cohort: high language and the ASD cohort: low language

With the neural correlates of cortical auditory and semantic processing identified in TD children, this allowed for comparison with children with ASD in the ASD cohort: high language and in the ASD cohort: low language.

Visual inspection of differences in the CAEPs and N400/LNC: TD cohort, the ASD cohort: high language and the ASD cohort: low language

For children in the ASD cohort: high language and in the ASD cohort: low language, the GFPs were calculated for each participant and grand averaged for each condition in both cohorts. The GFPs for each condition for children in the TD cohort (in blue), the ASD cohort: high language (in purple), and the ASD cohort: low language (in green) were plotted on the same axes to visualize differences between groups in cortical auditory processing indexed by the CAEPs and semantic processing indexed by the N400 component/LNC**.** The TWs selected for examination of the CAEPs (80-120 ms, 130-180 ms, and 200-260 ms) and N400 component/LNC (300-1100ms) were based on the results of children in the TD cohort. Topographical plots corresponding to TWs identified for the CAEPs from the GFP were plotted for each condition to visualize the distribution of electrical activity across the scalp during the components in both ASD cohorts separately. This was also done for the TW identified for the N400 component/LNC for both cohorts.

Statistically identifying the N400 component/LNC scalp distribution in children in the ASD cohort: high language and in the ASD cohort: low language*: cluster-based permutations*

To statistically identify where the N400 component/LNC in children in the ASD cohort: high language and ASD cohort: low language was occurring, cluster-based permutations ($p<0.05$, 1000 permutations) using paired t-tests were applied to investigate differences between the baseline period and the two TWs of 300-700 ms and 700-1100 ms post stimulus onset, in each condition separately for both ASD cohorts.

Statistical differences in the P1 Component and N400/LNC: TD cohort, ASD cohort: high language, and ASD cohort: low language

The GFP amplitude of the P1 component and the N400 component/LNC were used to investigate differences between child cohorts because the GFP represents the change in overall level of activation in the brain and is not subject to electrode choice or reference dependent (Skrandies, 1990). Additionally, the CAEP that was examined was the P1 component between 80-120 ms, as this is earliest and the most reliably identified CAEP dominating the complex in children (Donkers et al., 2015; Pang & Taylor, 2000; A. Sharma et al., 1997). Furthermore, it reflects the start of cortical auditory processing of spoken words and has been associated with the maturation status of the cortical auditory system (A. Sharma et al., 2015).

For all children in child groups (N=35), the GFP amplitude of P1 component for the selected two TWs specified below for the congruent and incongruent condition were calculated and submitted to the linear mixed model shown below for the P1 component. Additionally, what cohort the participants were in, their age, and sex were also submitted to this linear mixed model as main effects. These values were analyzed using a repeated measures ANOVA using a linear mixed effects model analysis in R (version 3.6.1.) using the lme4 package. This was done to investigate if there were effects of cohort, condition, TW or any interactions on the amplitude of this component while also considering the effects of age, and sex. Additionally, the effect size was determined using partial eta squared in R.

P1 component linear mixed model

GFP Amplitude ~ cohort x condition x TW + age + sex + (1|participant)

For this linear mixed model, cohort (TD cohort, ASD cohort: high language, ASD cohort: low language), condition (congruent, incongruent), TW (TW1: 80-100 ms, 100-120 ms), age (in years) and sex (male, female) were submitted as fixed effects. The random effect of "participant" was included to account for the individual differences in baseline measurements by assuming different random intercepts for each participant.

The GFP amplitude of the N400 component for the selected five TWs specified below for both conditions were calculated and submitted to a new linear mixed model shown below for the N400 component. Additionally, what cohort participants were in, their age, and sex were submitted to the linear mixed model as main effects to investigate if there were effects of cohort, condition, TW or any interactions on the amplitude of the N400 component, while considering the effects of age and sex. The effect size was also calculated using partial eta squared.

N400 component/LNC linear mixed model

GFP Amplitude ~ cohort x condition x TW + age + sex + (1|participant)

For this linear mixed model, cohort (TD cohort, ASD cohort: high language, ASD cohort: low language), condition (congruent, incongruent) and TW (TW: TW1 (300-450 ms), TW2 (450-600 ms), TW3 (600-750 ms), TW4 (750-900 ms), TW5 (900-1050 ms)), age (in years) and sex (male, female) were submitted as fixed effects. The random effect of "participant" was included to account for the individual differences in baseline measurements by assuming different random intercepts for each participant.

3.4.5.3. Multiple Regression Analyses

P1 component GFP Amplitude and LNC GFP Amplitude in Children

A multiple linear regression analysis was done to produce a statistical model that predicts how P1 component GFP amplitude causes the LNC GFP amplitude to change in children. For this analysis, the GFP amplitude for both conditions between 600-1050 ms were combined and averaged for each child participant for the LNC component. This TW was when semantic processing was occurring in all participants identified by the cluster-based permutations results and the results of the linear mixed model for the N400 component/LNC (300-1050 ms) GFP amplitude indicated no differences between conditions for the amplitude of this component. Thus, the two conditions were collapsed, and these values were submitted as the dependent variable (outcome) to a multiple linear regression analysis using the nmle package in R to develop a model for predicting the LNC GFP amplitude. The GFP for both conditions between 80-120 ms were combined and averaged for each child participant for the P1 component. The P1 component GFP amplitude of each child participant as well as their age, sex (male, female) and cohort (TD cohort, ASD cohort: high language, ASD cohort: low language) were submitted to the prediction model as the independent variables referred to as the predictors. An ANOVA was run on the model to test whether any of the variables were significant predictors of the LNC GFP amplitude. The effect size was also calculated using partial eta squared. The model is shown below:

LNC ~ P1 component *Cohort (TD cohort, ASD cohort: high language, ASD cohort: low language) + age (in years) + sex (male, female)

LNC GFP Amplitude and Language score in Children

A multiple linear regression was calculated to predict language score (outcome variable) based on average GFP amplitude of the LNC in children while accounting for what cohort (TD cohort, ASD cohort) participants were in, their age and their sex, using the nmle package in R. An ANOVA was run on the model to test if any of the variables were significant predictors of the outcome variable. The GFP for both conditions between 600-1050 ms were combined and averaged for each child participant for the LNC. This average LNC GFP amplitude of each child participant as well as their age, sex (male, female) and cohort (TD cohort, ASD cohort: high language, ASD cohort: low language) were submitted to the prediction model as the independent variables referred to as the predictors. An ANOVA was run on the model to test whether any of the variables were significant predictors of language in children. The effect size was also calculated using partial eta squared. The model is shown below:

Language score ~ LNC * cohort (TD cohort, ASD cohort) + age (in years) + sex (male, female)

Chapter Four: Results

4.1. Participant Cohort Characteristics

Fifty-nine participants were recruited for this study, which included 16 adults in the Adult cohort, 24 TD children in the TD cohort and 19 children diagnosed with ASD in the ASD cohort. One adult participant was excluded from EEG data analyses due to an ill-fitting EEG cap, therefore 15 adults were included for EEG data analyses. As for TD cohort, three children were excluded due to excessive muscle movements and insufficient high-quality EEG data, therefore 21 children were included for analyses in the TD cohort. For the ASD cohort, two of the 19 children with ASD were unable to complete the EEG study protocol as well as the neuropsychological assessments due to their cognitive function and/or motor abilities and therefore were excluded from the study. Thus, 17 participants with ASD completed the study. However, three participants with ASD were excluded from analyses of EEG because of excess motor movements; thus, EEG data from a total of 14 children with ASD were included for analyses in the ASD cohort (see **Table 2**).

4.1.1. Adult cohort

The mean age of participants in the Adult cohort was 22.8 ± 4.37 years. The group consisted of six males and nine females. All adults in this cohort reported having normal or corrected-to-normal vision, reported having no peripheral hearing impairments and results from their hearing assessment indicated hearing levels were within normal limits at baseline.

4.1.2. TD cohort

Twenty-one TD children in the cohort that were included for analyses had a mean age of 6.3 ± 0.93 years, with no significant difference between ages with children in the ASD cohort (p=0.69). This cohort included 10 males and 11 females, and all participants were right-handed. None of the children were taking any medications at the time. Nineteen of the 21 children spoke English as their primary language; two children spoke English as their second language. These participants with English as a second language however, fluently spoke, understood, and used vocabulary in English as indicated by their parents. Additionally, eight of the 21 TD children in the cohort received extra help for school for a variation of skills such as mathematics, speech therapy, resources/small groups, and reading. For all children in this cohort, parents reported that children had normal or corrected-to-normal vision and no peripheral hearing impairments. Results from their hearing assessments that indicated hearing levels within normal limits at baseline. Details regarding the demographics on the children in the TD group are on **Table 2**.

4.1.3. ASD cohort

The mean age of children with ASD in the ASD cohort was 6.4 ± 1.1 years and this cohort consisted of two females and twelve males (see **Table 2**). Eleven of the participants were right-handed and three participants were left-handed. Four of the children with ASD in this cohort were taking medications, with details listed in **Table 2.** All children with ASD in the ASD cohort spoke English as their primary language except for 2 participants who spoke English as their secondary language. For latter two participants, they began speaking English before the age of three however and were attending an English-speaking school. Twelve of the 14 children with ASD in this cohort were receiving on-going extra help for school, including in areas such as developmental/social skills and speech/language. For all children in this cohort, parents reported that the children had normal or corrected-to-normal vision and had no peripheral hearing impairments/sensitivities. Results from their hearing assessments that indicated hearing levels within normal limits at baseline.

Table 2. Participant Demographics

	Adult cohort	TD cohort	ASD cohort
Sample size	15	21	14
Male: Female	9:6	10:11	12:2
Age (Mean±SD) Range	22.9 ± 4.3 18-35	6.3 ± 0.93 5-8	6.4 ± 1.1 5-8
Handedness	N/A	21 right-handed	11 right-handed: 3 left-handed
Medication	N/A	None were taking medications	• Ritalin and Clonidine (N=1) • Arbaclofen (N=1) • Valtrex, Tenox, Difluxan and Celexa (N=1) • Montelukast (N=1)

4.2. Language and Working Memory Assessment Results in the TD cohort and the ASD cohort

4.2.1. Language Assessment Results

On the day of EEG acquisition, all 21 children in the TD group completed the PPVT. The cohort of participants with ASD however, had varying language and cognitive skills that resulted in a portion of participants not being able to complete the PPVT (N=4) the day of acquisition. Language scores for these four participants were derived from other neuropsychological assessments shown in **Table 1**, which describes which assessments were used to measure language and cognitive function in the TD children and children with ASD that were included for analyses.

Mean language scores for both the TD group and the ASD group are shown in **Fig. 6a**. Although means scores for both groups were within the average range, there was a significant between-group difference (p=0.008), with the ASD group having a lower mean score (95±24.5 vs 112± 11.1) compared to the TD group.

Differences in Language Ability in the ASD cohort: A Subset with High Language Ability and A Subset with Low Language Ability

Due to the variability in language scores in children in the ASD cohort and because a portion of children in the cohort had scores 1.5 SD below the standard mean of 100 indicating language deficits, children in the ASD cohort were divided into different language groups. Six children had scores that fell below 1.5 SD of the standard mean were considered the participants in the ASD cohort with low language abilities (ASD cohort: low language). The eight other children in the ASD cohort had scores that were within (100 ± 1 SD) or above the standard mean and were considered the participants in the ASD cohort with high language abilities (ASD cohort: high language). This can be seen in **Fig. 6b**. The mean scores were significantly different (p<0.001) between these two ASD groups, with a mean score (± SD) of 114.5 ± 6.6 for the ASD cohort: high language (N=8) and 67.8 ± 9.9 for the ASD cohort: low language(N=6).

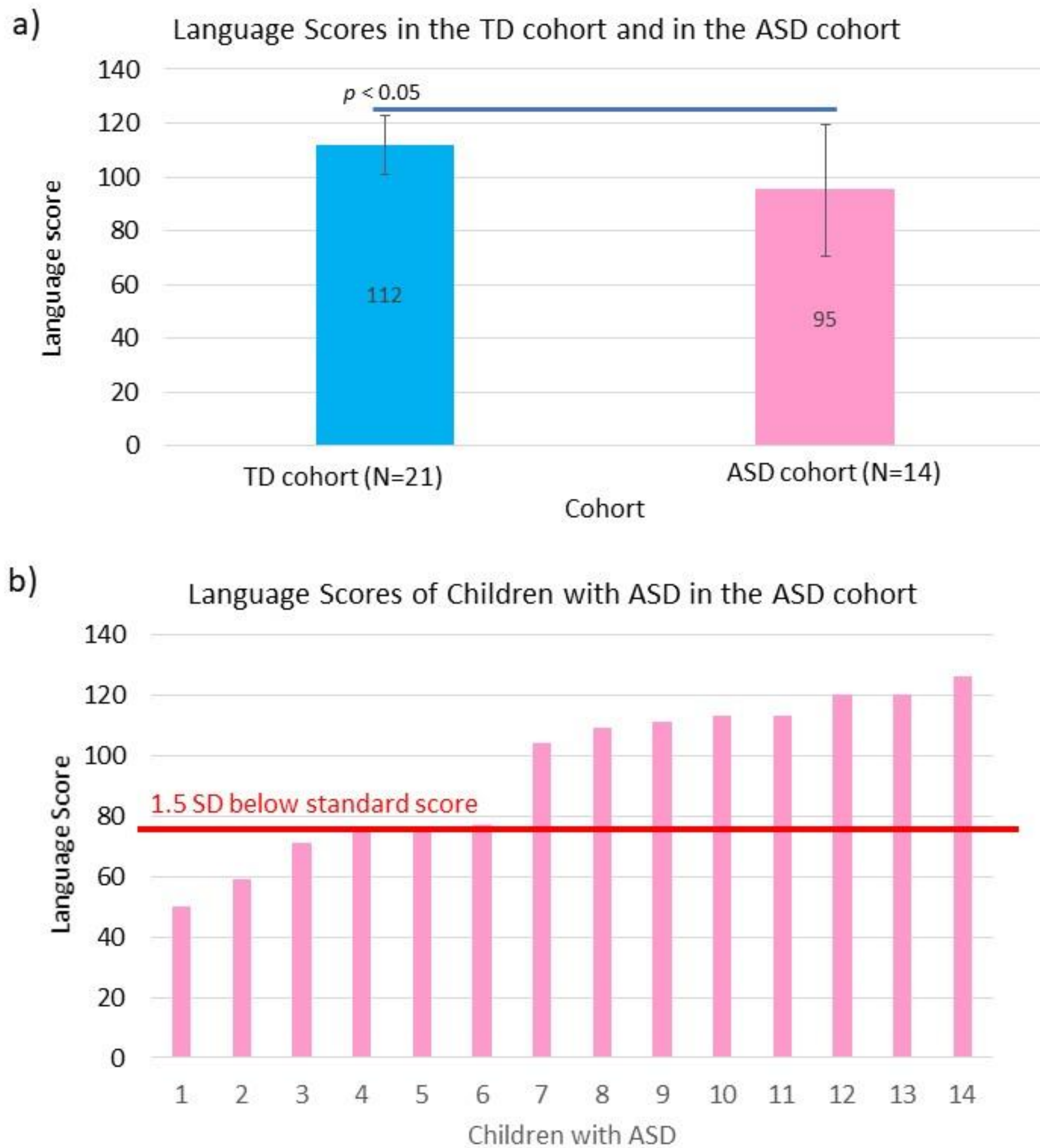


Figure 6. a) Language Scores in the TD cohort and ASD cohort, **b)** Language Scores in Children in the ASD Cohort

4.2.2. Cognitive Function Results: Working Memory

All children in the TD cohort (N=21) were able to complete the WPPSI-IV that measured their working memory abilities. However, for the ASD group, a subset of children with ASD in this group were unable to complete the WPPSI-IV assessment (N=6) and results from other assessments for cognitive function (including a measure of working memory) were unable to be obtained. All subsequent results on working memory only contain data from the N=6 subset. **Table 1** describes which assessments were used for working memory scores in children in the TD group and the subset of children in the ASD cohort.

There was no significant difference in working memory scores between children in the TD group (M=102, SD=11.9) and in the subset of children in the ASD group (M=91.4, SD=21), p=0.0885.

4.3. Behavioural Results in the Adult cohort, TD cohort and ASD cohort

All adults (N=15) and TD children (N=21) completed the behavioural portion of the EEG task. For children in the ASD cohort, a subset of them (N=9) completed the task while the remainder (N=5) did not complete the button press. All subsequent behavioural responses for the ASD cohort only contain data from the N=9 subset.

4.3.1. Accuracy

There was a significant main effect of cohort on accuracy, $F(2,84)=21.759$, $p<0.0001$, $\eta^2=0.34$. There was no main effect of condition on the accuracy, $F(1,84)=0.234$, $p=0.630$, $\eta^2=0.002$. The interaction effect was not significant, $F(2,84)=0.232$, $p=0.794$, $\eta^2=0.004$.

Tukey post hoc tests revealed that adults (M=98.5%, SE=0.608) had a higher accuracy than both TD children (M=86.5%, SE=1.66), $p<0.001$, and children with ASD (M=72.9%, SE=4.58), $p<0.001$. Furthermore, children in the TD cohort had a higher accuracy than children in the ASD group, $p<0.001$. These differences in accuracy are shown in **Fig. 7a**.

4.3.2. Reaction Time (RT)

There was a significant main effect of cohort on RT, $F(2,4214)=494.4834$, $p<0.0001$, $\eta^2=0.19$. There was also a main effect of condition on the RT, $F(1,4214)=11.648$, $p=0.0006$, $\eta^2=0.0028$. There was no significant interaction between the two factors, $F(2,4214)=1.0583$, $p=0.3471$, $\eta^2=0.0005$.

Tukey post hoc tests revealed that adults (M=1, SE=0.0127) had faster RT compared to both TD children (M=1.92, SE=0.022), $p=0$, and to children with ASD (M=1.84, SE=0.038), $p=0$. However, there was no significant difference between TD children and children with ASD, $p=0.072$, regarding their RT. The differences in RT are shown in **Fig. 7b**.

Additionally, the congruent condition (M=1.5, SE=0.021) evoked a faster RT than the incongruent condition (M=1.6, SE=0.022), $p=0.00065$, but the effect size is extremely small ($\eta^2=0.0028$).

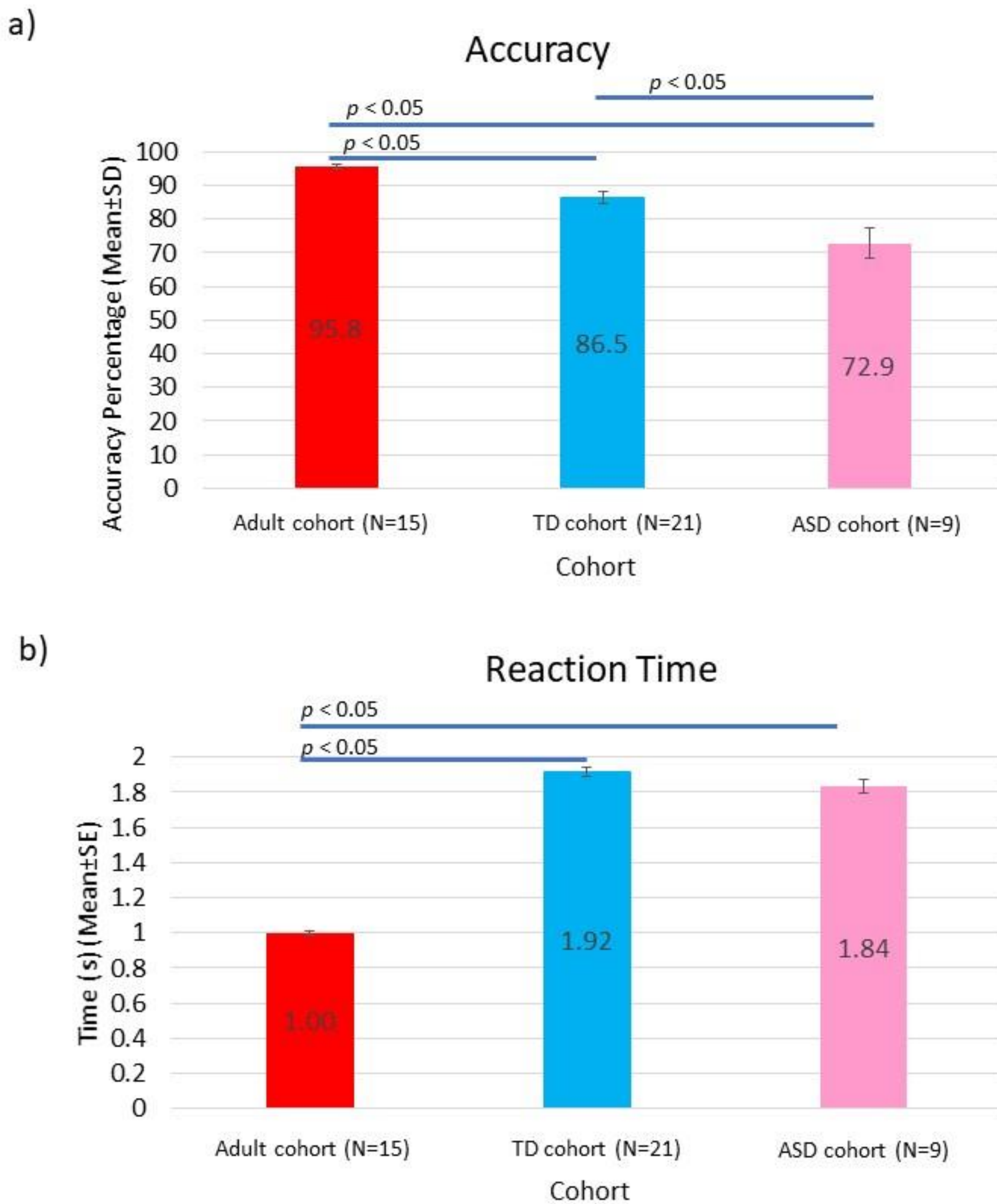


Figure 7. a) Accuracy Results in the Adult cohort, TD cohort, and ASD cohort, **b)** RT Results in the Adult cohort, TD cohort, and ASD cohort

4.4. EEG Results

After preprocessing the EEG data, an average of 1±0.86 channels were interpolated for cohorts, with no significant differences between participant groups for their number of interpolated channels (p=0.2).

For trial numbers per condition, there were an average of 46 trials (SD=2.86) for the Adult cohort, 46 trials (SD=4) for TD cohort and 44 trials (SD=5.6) for ASD cohort, with no significant difference between cohorts for number of trials (p=0.21) for the congruent condition. The incongruent condition also had no significant difference (p=0.27) between cohorts for number of trials with 47 trials (SD=3) in the Adult cohort, 46 trials (SD=4.1) in the TD cohort and 44 trials (SD=6) in the ASD cohort. There was no significant difference in number of trials between the congruent and incongruent conditions for any of the cohorts (p>0.05).

4.4.1. EEG Results in Adults: CAEPs, N400 component and N400 effect

The grand averaged GFP for the adult cohort for each condition were plotted on the same axes and these waveforms are shown in **Fig. 8a**.

Visually identifying CAEPs and N400 component in Adults: GFPs and topographical plots

Three CAEPs were identified from visual inspection of the GFP for each condition within 0 to 300 ms. Between 50 ms to 80 ms, there was a maximum in the GFP that was identified as the P1 component. Following the P1 component, between 90 to 150 ms there was a maximum in the GFP that was identified as the N1b component. Lastly, between 160 ms to 260 ms, there was a maximum in the GFP that was identified as the P2 component. This complex of components (P1-N1-P2) is shown in **Fig. 8a**, with each component highlighted in by the dotted black boxes. The distribution of activity of the P1 component, N1b component and P2 component are shown in topographical plots in **Fig. 8b**.

Visual identifying the N400 component in Adults: GFP and topographical plots

Between 300 ms to 1100 ms, there was a large maximum in the GFP. This was identified as the N400 component in adults and is highlighted in by the yellow dotted box in **Fig. 8a**. During this TW, the incongruent condition response also appeared larger in comparison to the response to the congruent condition, indicating an N400 effect occurred in adults. The distribution of electrical activity of the N400 component for the congruent and incongruent condition is shown in **Fig. 8b.**

Statistically identifying the N400 component scalp distribution in Adults: cluster-based permutations

Results from the cluster-based permutation tests comparing baseline activity to the two TWs revealed one significant negative centro-frontal cluster for each condition. The negative response was significant over a central-frontal cluster of the electrodes between 300-700 ms (max sum=-26.5858, $p<0.024$), and between 700-1100 ms (max sum=-25.3984, $p=0.022$) for the congruent condition. For the incongruent condition, a significant negative cluster of electrodes was also found between 300-700 ms (max sum=-36.933, $p=0.006$) and between 700-1100 ms (max sum=-26.1939, $p=0.018$) in the centro-frontal electrodes. This is shown in **Fig. 8b**, with red dots showing electrodes with significantly negative activity in comparison to baseline. A large negativity was evoked for the congruent and incongruent condition in adults.

Statistically identifying the N400 effect in Adults: cluster-based permutations

Cluster-based permutations revealed one significant negative cluster (max sum =-28.1235, $p=0.01$) with a centro-frontal scalp distribution between 300-700 ms, shown in topographical plots displaying the scalp distribution of activity occurring only in the incongruent condition in **Fig. 8c**, with red dots showing electrodes with significantly more negative activity in the incongruent condition. No significant effects were observed between 700-1100 ms (max sum= -12.1368, $p=0.0310$). This suggests that an N400 effect occurred in adults in this cohort between 300-700 ms with a centro-frontal scalp distribution.

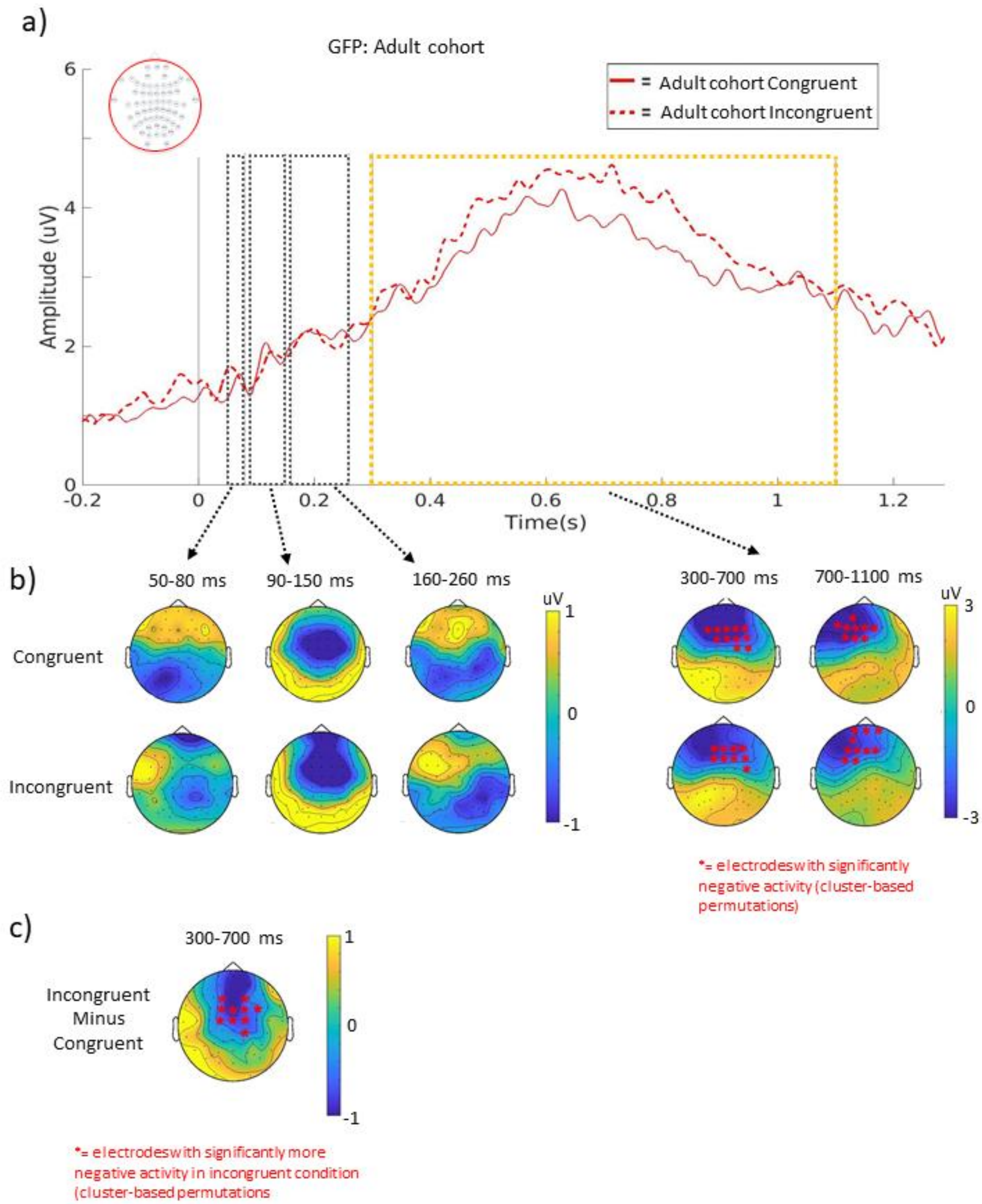


Figure 8. a) GFPs for the Congruent (solid line) and Incongruent (dotted line) condition in Adults, **b)** Topographical head maps showing activity during the CAEPs and N400 component in each condition in Adults. *= electrodes with significantly negative activity in

comparison to baseline during the N400 component**, c)** Topographical head map showing activity only in the Difference condition in Adults. *=electrodes with significantly more negative activity in incongruent condition

4.4.2. EEG Results in TD children: CAEPs, N400 component/LNC and N400 effect

The grand averaged GFP for each condition was plotted for children in the TD group on the same axes shown in **Fig. 9a** with activity of this cohort shown in blue.

Visually identifying the CAEPs in TD children: GFP and topographical plots

Visually, in the first 300 ms of GFP in children in the TD group, three components could be identified highlighted by the dotted black boxes in **Fig. 9a**. The first maximum of GFP was between 80-120 ms and this response was identified as the P1 component in children in response to spoken words, with the scalp distribution shown in **Fig. 9b**. The second maximum of the GFP was visually identified between 130-180 ms and was identified as the N1b component in children, with the distribution displayed in **Fig. 9b**. The third maximum of the GFP was between 200-260 ms that was identified as the P2 component in children, with the distribution displayed in **Fig.9b**. This complex can be seen in **Fig. 9a**, with each component highlighted by the dotted black boxes.

Visually inspection the N400 component/LNC in TD children: GFP and topographical plots

Visual inspection of the grand averaged GFPs revealed a large maximum that spanned across 300-1100 ms, highlighted by the yellow dotted box in **Fig. 9a.** This was identified as the N400 component between 300-700 ms and LNC between 700-1100 ms that follows in children, with the scalp distribution of the components shown in topographical plots in **Fig 9b**. Although, the amplitude of the peaks did not differ between conditions, the amplitudes during this TW were visually significantly different from baseline in TD children.

Statistically Identifying the N400 component/LNC in TD children: cluster-based permutations

Results from the cluster-based permutation tests in the TD group comparing baseline activity to the two active TWs, revealed a significant negative electrode cluster with a broad scalp distribution between 300-700 ms (max sum=-178.9688, $p<0.0001$), that shifted more frontally between 700-1100 ms (max sum=-253.0493, $p<0.0001$) for the congruent condition. This also occurred for the incongruent condition, as a broadly distributed significant negative cluster was also found between 300-700 ms (max sum=-149.235, $p<0.0001$), that shifted more frontally between 700-1100 ms (max sum=-292.5243, $p<0.0001$). This is shown the topographical plots in **Fig. 9b** displaying the scalp distribution for both conditions with the red dots showing electrodes with significantly negative activity in comparison to baseline. A large negativity was elicited to both congruent and incongruent conditions in TD children.

Statistically identifying the N400 effect in TD children: cluster-based permutations

Cluster-based permutations revealed there was no significant negative clusters within the 300-700 ms, and 700-1100 ms TWs ($p>0.05$) in any electrodes, when comparing congruent and incongruent conditions. This indicated that there was no N400 effect in the children in the TD cohort.

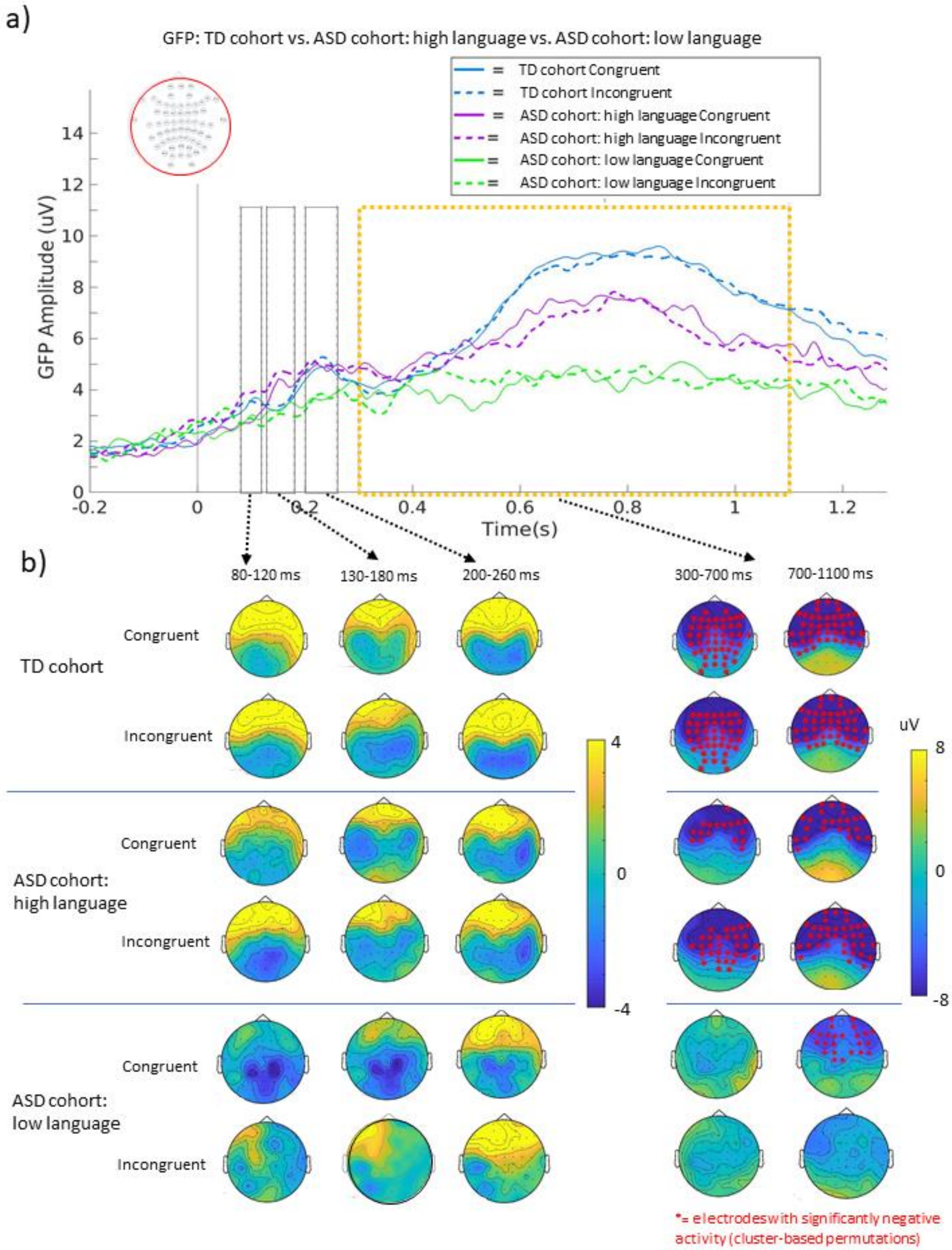


Figure 9. a) GFPs for the Congruent (solid line) and Incongruent (dotted line) condition in the TD cohort (blue), the ASD cohort: high language (purple) and the ASD cohort: low

language (green). Black dotted boxes identify the CAEPs and yellow dotted box identifies the N400 component/LNC, **b)** Topographical head maps showing activity during the CAEPs and N400 component/LNC in each condition for all child cohorts (top to bottom: TD cohort, ASD cohort: high language, ASD cohort: low language). ***=** electrodes with significantly negative activity in comparison to baseline during the N400 component/LNC

4.4.3. Comparing the EEG Results in the TD Cohort, ASD cohort: high language and ASD cohort: low language: CAEPs and N400 component/LNC

The GFPs for both the congruent and incongruent condition for the TD cohort (in blue), the ASD cohort: high language (in purple) and the ASD cohort: low language (in green) were overplotted in **Fig. 9a**.

Visual inspection of the GFP between 80-120 ms for the P1 component, between 130-130 ms for the N1b component, and between 200-260 ms for the P2 component suggested potential differences between the child groups for cortical auditory processing shown in **Fig. 9a** highlighted in the black dotted boxes. The scalp distribution of the components in both ASD cohorts are shown in **Fig. 9b**. For the P1 component in the first box, which is the component further investigated, the ASD cohort: low language displayed a smaller response for responses for both conditions in comparison to the TD cohort and the ASD cohort: high language.

Additionally, visual inspection of the GFPs suggested that there were differences in the overall activity during the N400 component/LNC response between 300-1100 ms between all three child cohorts. The TD cohort had the largest response, followed by the ASD cohort: high language with the second largest response and the ASD cohort: low language with the smallest response in **Fig. 9a** highlighted by dotted yellow box.

Statistically identifying the N400 component/LNC scale distribution in the ASD cohort: high language and ASD cohort: low language: cluster-based permutations

ASD cohort: high language

Results from the cluster-based permutation tests comparing baseline activity to the two TWs revealed one significant negative centro-frontal cluster of electrodes between 300-700 ms (max sum=-51.5772, p=0.02) and 700-1100 ms (max sum=-78. 5920, p<0.001) for the congruent condition. For the incongruent condition, significant negative clusters were also found between 300-700 ms (max sum=-96.3537, p=0.01) and 700-1100 ms (max sum=-174.244, p<0.001) with a centro-frontal scalp distribution. This is shown in **Fig. 9b,** with red dots showing electrodes with significantly negative activity in comparison to baseline. A large negativity was elicited to both congruent and conditions in children in the ASD cohort: high language.

ASD cohort: low language

Results from the cluster-based permutation tests comparing baseline activity to the activity during the two TWs for the N400 component/LNC revealed one significant negative centro-frontal cluster for only the congruent condition. The negative response was significant over a broad central-frontal cluster of electrodes between 700-1100 ms (max sum=-92.5032, p<0.001) for the congruent condition. For the incongruent condition, there was no significant negative clusters found between 300-700 ms and 700-1100 ms, (p>0.05). This is shown in **Fig. 9b,** with red dots showing electrodes with significantly negative activity in comparison to baseline**.** A negativity was elicited in the congruent condition, between 700-1100 ms in children in the ASD cohort: low language.

Statistical differences in the GFP for the P1 component: TD cohort vs. ASD cohort: high language vs. ASD cohort: low language

Results from the linear mixed model for the P1 component are shown in **Table 3**. There was a significant main effect of age, $F(1,30)=4.2425$, $p=0.04819$, $\eta^2=0.12$, as well as a significant interaction between cohort and condition, $F(2,96)=3.7366$, $p=0.02737$, $\eta^2=0.07$.

Table 3. Linear mixed model results for the P1 component

Factor	F-value	Numerator Degrees of Freedom	Denominator Degrees of Freedom	*p-value*	Effect size
Cohort	0.5076	2	30	0.60702	0.03
TW	2.6756	1	96	0.10517	0.03
Condition	0.318	1	96	0.57414	3.30e-03
Age	4.2425	1	30	0.04819	0.12
Sex	1.9781	1	30	0.16986	0.06
Cohort:condition	3.7366	2	96	0.02737	0.07
Cohort:TW	0.1409	2	96	0.86874	2.93e-03
TW:condition	0.2878	1	96	0.59288	2.99e-03
Cohort:TW:condition	0.0060	2	96	0.99404	1.25e-04

Post-hoc tests for P1 component

For age, there were decreases in the P1 component GFP amplitude with increasing age for participants, shown in **Fig. 10a**. An ANOVA was done to test if there any significant differences in age between the cohorts and results revealed no significant differences in age between the TD cohort (M=6.3 years ±0.91), the ASD cohort: high language (M=6.4 years ±1.7) and ASD cohort: low language (M=6.5 years ±1.1), p=0.9043, with the distribution of age for each cohort displayed in **Fig. 10b.**

Additionally, post-hoc comparisons were done on the interaction effect between cohort and condition on the amplitude of the P1 component between 80-120 ms shown in **Table 4**. Results revealed that the ASD cohort: high language had a larger response in the incongruent condition compared to the congruent condition, with a medium effect size of 0.07(Maher et al., 2013)..

Fig. 11a shows the average GFP amplitude during the 2 TWs in the child cohorts averaged over conditions revealed the from the linear mixed model. Qq plots confirm normal distribution of the GFP amplitude of the P1 component (80-120 ms) in children in **Fig. 11a** as well.

Figure 10. a) Age and P1 component GFP amplitude relationship in the child cohorts revealed the from the linear mixed model for the P1 component, **b)** Age distribution in child cohorts

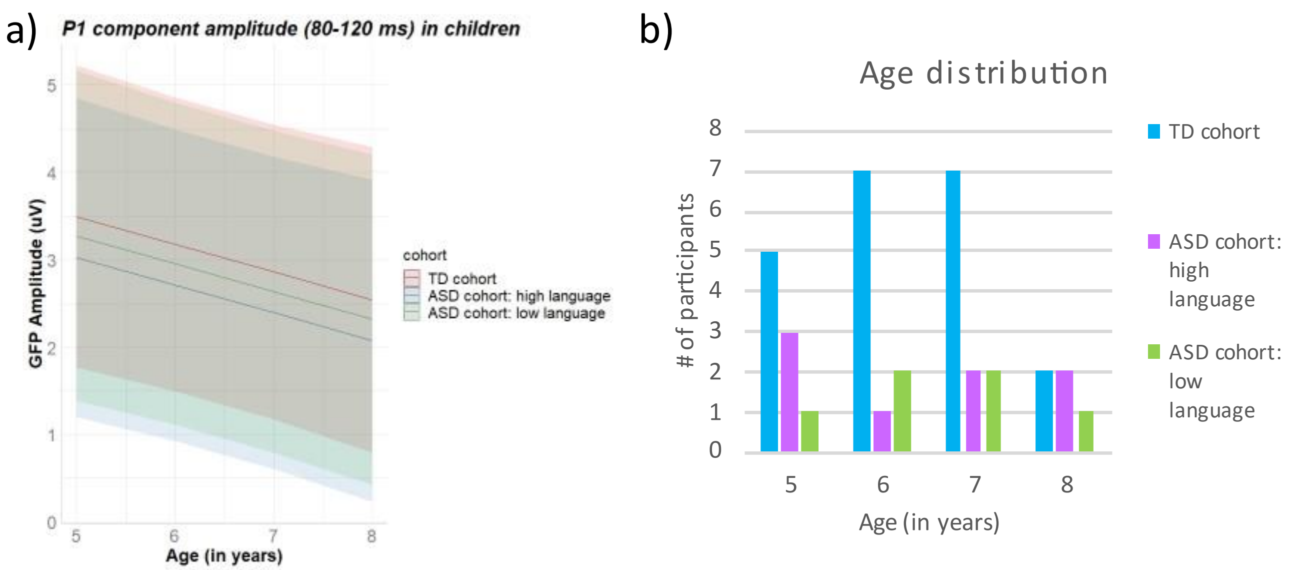


Table 4. Post-hoc Comparison Results for the P1 component in child cohorts

	Significant	**Not significant**
Cohort x condition	ASD cohort: high language: • Incongruent (M=3.95, SE=0.385) > Congruent (M=2.94, SE=3.95), *p*=0.003	TD: • Congruent (M=3.49, SE=0.239) = Incongruent (M=3.52, SE=0.239), *p*=0.9055 ASD cohort: low language: • Congruent (M=3.15, SE=0.466) = Incongruent (M=3.01, SE=0.466), *p*=0.7144

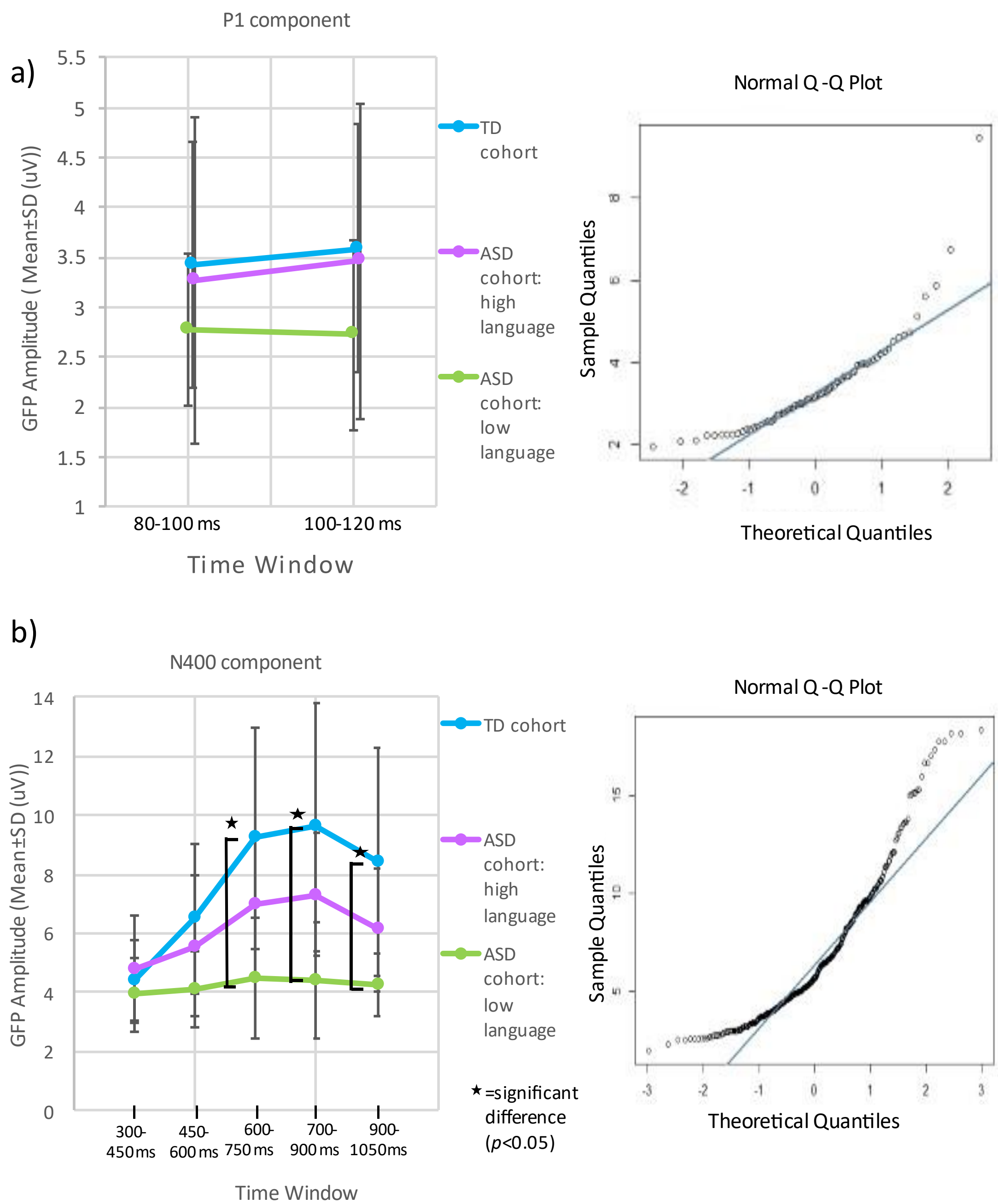


Figure 11. a) Average P1 component GFP amplitude in the child cohorts revealed the from the linear mixed model for the P1 component on the left. The qq plot for the P1 component is on the right, **b)** Average N400 component/LNC GFP amplitude in the child cohorts revealed from the linear mixed model for the N400 component/LNC on the left. The qq plot for the N400 component/LNC is on the right

Statistical differences in the GFP for the N400 component/LNC: TD cohort vs. ASD cohort: high language vs. ASD cohort: low language

Results from the linear mixed model are shown in **Table 5.** There was a significant main effect of cohort on amplitude, F(2,30)=5.8582, p=0.007, η^2=0.28, as well as a main effect of TW on amplitude of the N400/LNC, F(4,288)=20.0061, p <0.001, η^2=0.22. More importantly, the cohort by TW interaction was significant, F(8,288)=6.4772, p <0.001, η^2=0.15

Table 5. Linear mixed model results for the N400 component

Factor	F value	Numerator Degrees of Freedom	Denominator Degrees of Freedom	P value	Effect size
Cohort	5.8582	2	30	0.007116	0.28
TW	20.0061	4	288	0.00000000000001499	0.22
Condition	0.0348	1	288	0.852165	1.21e-04
Age	0.1463	1	30	0.704759	4.85e-03
Sex	1.6346	1	30	0.210875	0.05
Cohort:TW	6.4772	8	288	0.00000009300874372	0.15
Cohort:condition	0.7106	2	288	0.492218	4.91e-03
TW:condition	0.1131	4	288	0.977846	1.57e-03
Cohort:TW:condition	0.0614	8	288	0.999871	1.70e-03

Post-hoc test for N400 component/LNC

Post-hoc comparisons were done on the interaction effect on the amplitude of the N400 component/LNC between 300-1050 ms, shown in **Table 6**. Results revealed that there was a significant interaction, with the TD group having a larger amplitude between 600-1050 ms in comparison to the ASD cohort: low language, but there were no differences when compared to the ASD cohort: high language during any TWs. The TWs that were different between the TD cohort and ASD cohort: low language reflect the TW for the LNC. **Fig. 11b** shows the average GFP amplitude during the five TWs in the child cohorts revealed from the linear mixed model. Qq plots confirm normal distribution of the GFP amplitude of the N400 component (300-600ms) and the LNC (600-1050ms) in children in **Fig. 11b** as well.

Table 6. Post-hoc Comparison results for the N400 component/LNC in child cohorts

	Significant	**Not significant**
TW1		• TD cohort (M=4.42, SE=0.572) = ASD cohort: high language (M=4.51, SE=0.953), *p*=0.9962 • TD cohort = ASD cohort: low language (M=3.39, SE=1.161), *p* =0.7097 • ASD cohort: high language = ASD cohort: low language, *p*= 0.7140
TW2		• TD cohort (M=6.52, SE=0.572) = ASD cohort: high language (M=5.30, SE=0.953), *p*=0.5231 • TD cohort = ASD cohort: low language (M=3.55, SE=1.161), *p* =0.0697 • ASD cohort: high language = ASD cohort: low language, *p*=0.4476
TW3	• TD cohort (M=9.24, SE=0.572) > ASD cohort: low language (M=3.92, SE=1.161), *p*=0.0005	• TD cohort = ASD cohort: high language (M=6.66, SE=0.953), *p*=0.0652 • ASD cohort: high language = ASD cohort: low language, *p*=0.1452
TW4	• TD cohort (M=9.63, SE=0.572)> ASD cohort: low language (M=3.84, SE=1.161), *p*=0.0002	• TD cohort = ASD cohort: high language (M=7.02, SE=0.953), p=0.0600 • ASD cohort: high language = ASD cohort: low language , *p*=0.0779
TW5	• TD cohort (M=8.43, SE=0.572)> ASD cohort: low language (M=3.70, SE=1.161), *p*=0.0021	• TD cohort = ASD cohort: high language (M=5.85, SE=0.953), *p*=0.00645 • ASD cohort: high language = ASD cohort: low language, *p*=0.3017

4.4.5. Multiple Regressions Analyses

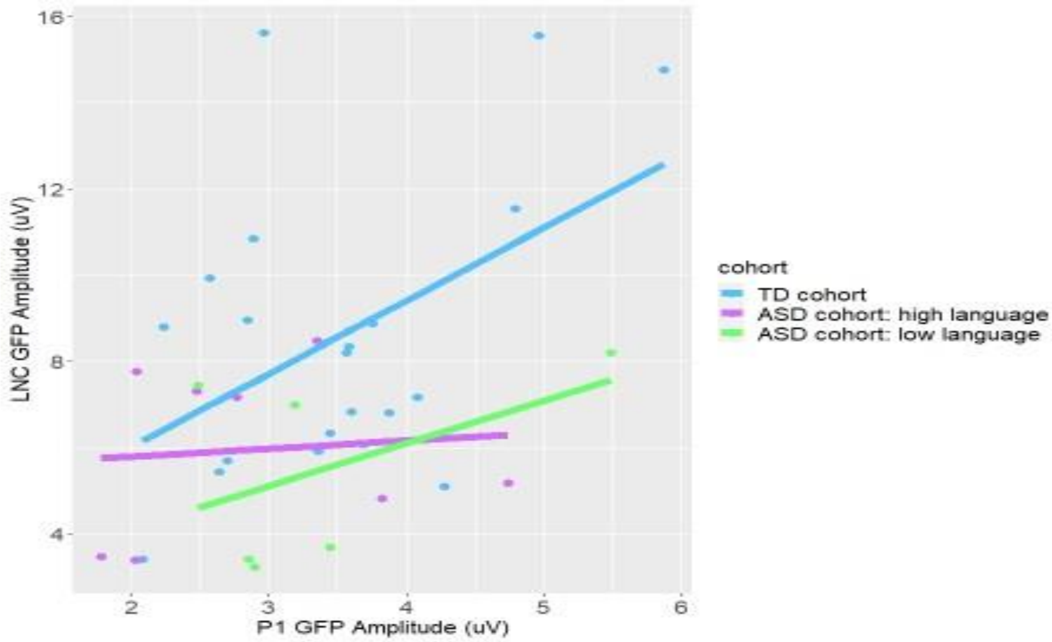
P1 component GFP amplitude and LNC GFP amplitude in Children

There was a significant regression equation found for the multiple regression analysis that had P1 component GFP amplitude, cohort, age and sex as predictors for the LNC GFP amplitude, (F (7,27) = 2.623, *p*= 0.03334, with an R^2 of 0.4047. The results of the ANOVA indicated that the P1 component GFP amplitude was a significant predictor (p=0.008349, η^2=0.23) of the LNC GFP amplitude. However, cohort (*p*= 0.063572), age (*p*= 0.128450), and sex (*p*= 0.722729) or any interaction between cohort and condition (*p*= 0.469291) were not significant predictors of the LNC GFP amplitude. The resulting multiple regression lines are shown in **Fig. 12a**, that indicate that a larger P1 predicts a larger LNC in children.

LNC GFP amplitude and Language Score in Children

A significant regression equation was found (F(5,291)= 3.593, p= 0.01191), with an R^2 of 0.3825. The results of the ANOVA indicated that the LNC component GFP amplitude and cohort (p=0.03180, η^2=0.15) were significant predictors (p=0.02996, η^2=0.15) of language score in children. Moreover, there was a significant interaction between the LNC component GFP amplitude and cohort (p=0.01215, η^2=0.2), with the LNC GFP amplitude predicting the language score in each cohort with differing predictive equations for the TD cohort and ASD cohort. The resulting multiple linear regression lines are shown in **Fig. 12b** and indicate that the LNC GFP amplitude in children with ASD predicts their language score, with increases in LNC GFP amplitude predicting increases in the language score. However, for TD children, the plot suggests that the LNC GFP amplitude does not predict changes in language score, as there were minimal changes to language score with increasing LNC GFP amplitudes.

a)



b)

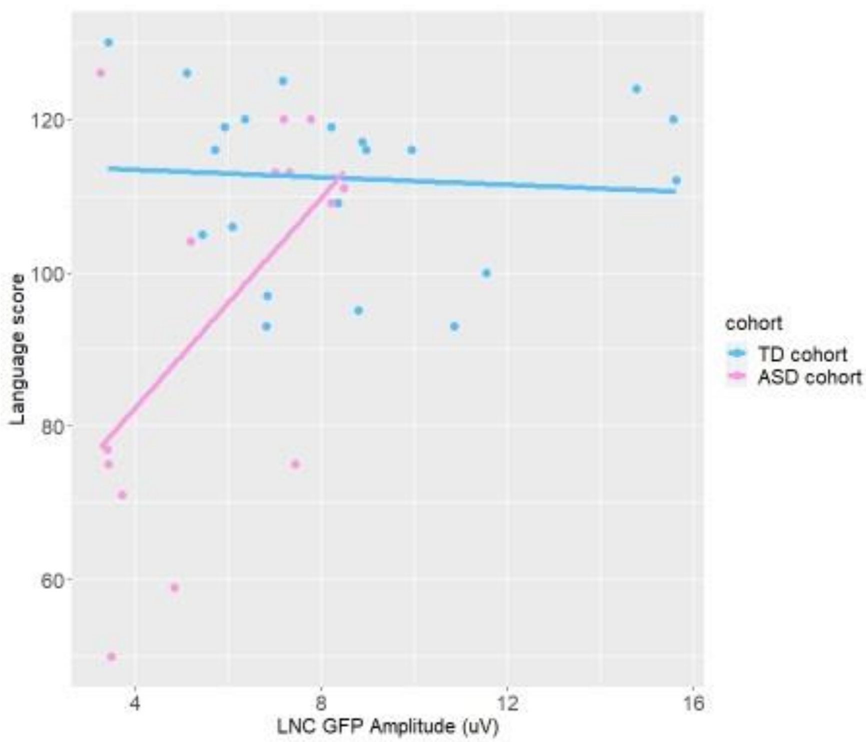


Figure 12. a) Multiple linear regression lines modeling how changes in the P1 GFP Amplitude affects the LNC in TD children and children with ASD with high and low language scores**, b)** Multiple linear regression lines modeling how changes in the LNC GFP amplitude affects language score in TD children and children with ASD

Chapter Five: Discussion

5.1. Summary of the Main Findings

The current study evaluated neural processing underlying semantic processing of spoken words in children with ASD with a variability in language capabilities.

Participants did an auditory semantic categorization paradigm while their brain waves were recorded. The neural responses evoked by adults and children in the TD cohort were evaluated first and this served to validate the semantic categorization paradigm, illustrate the normative neural processing underlying semantic processing and examine age-related differences. The children in the TD group were then compared to children in the ASD cohort with high language scores and children in the ASD cohort with low language scores to identify differences in neural processing in the clinical population.

The current study demonstrated semantic neural processing was more efficient in adults than TD children, which supported the secondary hypothesis regarding age-related differences in semantic processing.

More importantly, results demonstrated significant abnormalities in neural processing underlying semantic processing in children with ASD with low language ability indexed by low language scores compared to TD children. However, there were no differences between TD children and children with ASD with high language ability indexed by language scores within normal range. This supported the first primary hypothesis, as children with ASD with language impairments had neural processing deficits. In children with ASD, decreasing LNC GFP amplitude predicted decreasing language scores, supporting the second primary hypothesis. Furthermore, the lower LNC GFP amplitude was predicted by a lower P1 component GFP amplitude, indicating cortical auditory and semantic processing deficits are affecting children with ASD with language impairments.

5.1.1. Cortical Auditory Processing in Adults and TD Children

Neural findings indicate that cortical auditory processing of the stimuli did occur in both adults and TD children, reflected by a similar complex of components evoked during the first 300 ms post stimulus onset, however, at different latencies.

In adults, their cortical auditory processing was reflected by three CAEPs. These were identified as the P1 component between 50-80 ms, followed by the N1b component between 90-150 ms, as well as the P2 component between 160-250 ms. This complex of components (P1-N1-P2) was expected for cortical auditory processing in adults and has extensively been reported (Beres, 2017; Ceponiene et al., 2002; Pang & Taylor, 2000; Picton et al., 2000).

For TD children, their complex was dominated by similar CAEPs, but at different latencies. The P1 component was between 80-120 ms, the N1b component was 130-180 ms and the P2 component was between 200-260 ms. The P1 component was expected as it is the most identifiable CAEP in children (Campbell et al., 2011; Pang & Taylor, 2000), and indicates when cortical auditory processing began. The N1b and P2 components are not the predominant CAEPs evoked by children in response to auditory stimuli, as it is typically dominated by a P1-N2 complex (Ceponiene et al., 2002; Ponton et al., 2000). The N1b and P2 component have been shown to be sensitive to ISI in children and due to the long ISI (2 sec ISI between words) used in our paradigm, this may evoked these components to be observed in the TD children (Ceponiene et al., 2002; Donkers et al., 2015). In a study done by Gilley et al. (2005), at their longest ISI (2000 ms), which was equivalent to the ISI used in the auditory semantic categorization paradigm in this study, children between 7-8 years of age began to elicit a small P1-N1-P2 complex to a speech syllable (Gilley et al., 2005). A portion of TD children in the TD cohort were 7 to 8 years old, therefore these changes in cortical auditory processing may have been captured.

The complex of CAEPs found in both adults and children indicates that cortical auditory processing of the stimuli transpired and the differences in latency of components in the complex, confirms the age-related changes in cortical ,auditory processing that has been established in previous literature (Beres, 2017; Donkers et al., 2015; Pang & Taylor, 2000; S. Sharma et al., 2012).

5.1.2. Semantic Processing in Adults and TD Children

Behaviourally, both adults and TD children engaged in high levels of semantic processing reflected by their accuracy rates on the paradigm, which were 95.8% and 86.5% respectively. Their results suggested that participants in both cohorts could differentiate between congruent and incongruent words in a category, with no differences in ability to correctly identify between congruent and incongruent words. However, semantic processing skills in adults were better than TD children, indicated by higher accuracy rates in adults. They also had a faster reaction time than TD children, emphasizing the efficiency of semantic processing in adults in comparison to children.

Differences in neural processing also complemented the differences in behavioural results seen in adults and children. In adults, there was a negative deflection in the ERPs for both congruent and incongruent conditions between 300-1100 ms. This was identified as the N400 component between 300-700 ms with a centro-frontal scalp distribution and a sustained negativity that follows between 700-1100 ms in adults. For the N400 component, there was larger response for the incongruent condition between 300-700 ms in the centro-frontal electrodes, that reflected an N400 effect in adults and indicated that the N400 component was sensitive to semantic incongruencies. This corroborates with literature that has shown the N400 effect as an electrophysiological marker for the detection of semantic incongruencies in adults (Benau et al., 2011; M. Kutas & Hillyard, 1980; Marta Kutas & Federmeier, 2011). The sustained negativity shown in the left fronto-central area may be explained by the nature of the stimuli, as auditory N400 components have been shown to be longer in duration (Marta Kutas & Petten, 1994). Moreover, it may indicate post-processing occurred after the initial detection of semantic congruency in adults. This late negativity has been shown in joke and metaphor comprehension paradigms that required semantic reinterpretation or reanalysis of word and preceding context (S. Coulson & Kutas, 2001; Seana Coulson & Williams, 2005; Wlotko & Federmeier, 2012).

In TD children, there was also a large negativity that occurred broadly over the centro-frontal electrodes in both conditions between 300-700 ms, with the negativity shifting more frontally between 700-1100 ms. This was interpreted as the initial N400 component between 300-700 ms and the LNC associated with semantic processing in children between 700-1100 ms. In ERP studies, children have shown a large negativity, that can be divided into an initial negativity referred to as the N400 component that has been thought to index preliminary access and retrieval of the word, followed by a secondary negativity referred to as the LNC that reflects integration of the prime word with mental representations (DiStefano et al., 2019; Silva-Pereyra et al., 2005). Our results suggested that TD children processed semantic aspects of spoken words represented by their N400 component and LNC. In contrast to adults however, the difference between the N400 component and the LNC for congruent and incongruent conditions was negligible, indicating no N400 effect in the TD children. That is, there was no electrophysiological marker of detection of semantic incongruencies in TD children, however, these children had a high accuracy rate (86.5%) on the paradigm, not indicating deficits in this domain. They additionally had language scores within normal range, indicating no language impairments. This all or none response coincides with results from other studies showing an absence of the N400 effect in children that did not correlate with semantic processing deficits (Benau et al., 2011; Kallioinen et al., 2016). Thus, the neural results in TD children suggests that the sensitivity of the N400 component and LNC to semantic incongruencies in TD young children has not fully developed yet. The difference in sensitive may also be due to the different strategies used by adults in comparison to TD children. Adults may be engaging with top down processing through predictive processing, where they are predicting and anticipating the upcoming words (target words), which results in amplitude modulations of the N400 component (Kallioinen et al., 2016; Marta Kutas & Federmeier, 2011; Mantegna et al., 2019). Children on the other hand, may be engaging with bottom up processing that involves integration of words rather than predictive processing, which has been shown to minimize the N400 effect (Kallioinen et al., 2016; Kiang et al., 2013).

Studies that investigate semantic processing in children, should not rely on the N400 effect as the measure of top-down semantic processing.

5.1.3. Differences in Cortical Auditory and Semantic Processing between TD Children, Children with ASD with High Language Ability and Children with ASD with Low Language Ability

Language ability is one of the many aspects that is heterogenous in children with ASD. Studies investigating semantic processing deficits in children with ASD have primarily focused on children with high language capabilities. These findings may not be transferable to children with ASD with greater language impairments, as there has been evidence of differences in brain activation during language processing in individuals with varying language skills (Samson et al., 2015). This study wanted to consider this heterogeneity of language profile in children with ASD and included children with ASD with high and low language capabilities that were assessed separately.

Results revealed that there were no significant differences in cortical auditory processing between TD children, and children with ASD with high language ability and with low language ability, indexed by their overall neural strength during the P1 component. There was, however, an effect of age, as the amplitude of the P1 component decreased with age, which has been reported in literature, but there was no significant difference between the cohorts for their age (Fitzroy et al., 2015; Ponton et al., 2000; A. Sharma et al., 1997). Additionally, the children with ASD with high language ability, did have a larger P1 component response to the incongruent condition in comparison to the congruent condition. There was a medium effect size of this difference, this suggests that there were some slight cortical auditory processing abnormalities in these children with ASD with high language ability, but generally there were no large differences in cortical auditory processing between the three child groups.

After the CAEPs, children with ASD with high language ability, evoked a large negativity identified as the N400 component and LNC that occurred broadly over centro-frontal electrodes between 300-1100 ms for both congruent and incongruent words. There was no difference in neural strength for both the N400 component and LNC between the congruent and incongruent condition, revealing no N400 effect in the children with ASD with high language scores. This response was comparable to TD children, with no differences in their neural strength during the N400 component and LNC. The language ability of these children with ASD, indexed by their language score, was within or above the standard mean, supporting these neural results.

In contrast, children with ASD with low language ability had a small negativity identified as the N400 component that was evoked only in the congruent condition between 700-1100 ms with a centro-frontal brain scalp distribution. This suggests that there were semantic processing impairments in these children with ASD with low language ability because there was no electrophysiological indication of semantic processing in the incongruent condition. Additionally, this response indicated that there was slower neural processing when semantic processing did occur, as it was not present until 700-1100 ms post stimulus onset in the congruent condition. Furthermore, this response was smaller in amplitude in comparison to TD children during this TW emphasizing neural processing impairments in children with ASD with low language ability. Semantic efficiency is related to decreases in the N400 component amplitude, however children not show better semantic processing skills as many studies have shown evidence of deficits in this domain in children with ASD (Battaglia, 2012; Brignell et al., 2018; Henderson et al., 2014; Mody & Belliveau, 2013). Therefore, the smaller amplitude is not related to increased semantic efficiency and indicates restricted processing in children with ASD with low language abilities.

5.1.5. Multiple Regression Analyses

A multiple regression analysis was used to investigate if the LNC GFP amplitude could be predicted by the P1 component GFP amplitude in children, while also considering effects of age, cohort (TD cohort, ASD cohort: high language, ASD cohort: low language), and sex. This model did significantly predict the LNC GFP, with the displayed regression lines shown in **Fig. 12a**, that indicates that larger P1 component GFP amplitudes predict larger LNC component amplitudes in children. However, the *p*-value indicating the significance of cohort as a predictor of the LNC (0.063572) was close to .05, and from the plot in **Fig. 12a**, these results suggest that the P1 component GFP amplitude predicts the LNC component GFP amplitude in TD children and children with ASD in the low language group more strongly than children with ASD with high language ability. To investigate if the LNC GFP amplitude could predict language scores in children, a multiple linear regression analysis was done and the results supported the secondary hypothesis that the N400 component amplitude predicts language score in children, but specifically for children with ASD. For TD children, there were minimal changes in language score predicted by increasing LNC GFP amplitude shown in **Fig. 12b**, therefore the LNC component GFP amplitude does not predict changes in language score in TD children. However, for children with ASD, the LNC component GFP amplitude strongly predicts the language score, with increases in LNC component GFP amplitude predicting increases in the language score.

5.2. Conclusions

Overall, results reveal that TD children successfully processed semantic aspects of auditory stimuli, but their neural correlates reflecting semantic processing lack a sensitive to semantic incongruencies of spoken words that was displayed in adults. This supported the secondary hypothesis regarding age-related differences in semantic processing of spoken words between adults and children.

More importantly, findings suggest that there are neural semantic processing deficits in children with ASD that supports the first primary hypothesis regarding deficits in neural processing in children with ASD. The deficits pertained to children with ASD with language impairments, as their neural results revealed no electrophysiological marker (N400 component/LNC) of semantic processing in the incongruent condition. Additionally, when it did occur in the congruent condition, the onset was delayed and more restricted in comparison to TD children. In contrast, children with ASD with language abilities within normal range had no differences in neural processing in comparison to TD children.

Additionally, findings implicate that low language ability is predicted by deficits in the semantic neural processing in children with ASD, supporting the second primary hypothesis. Furthermore, the deficits in semantic neural processing were predicted by deficits in cortical auditory processing in those children with ASD. Therefore, children with

ASD with language impairment should be monitored for both cortical auditory processing deficits and semantic processing deficits.

5.3. Limitations and Future Studies

There are several limitations to this study. Due to unforeseen circumstances, recruitment and testing was halted due to COVID-19, which resulted in the small sample size for the ASD cohort (N=14). This reduced the power to detect between group differences, which was amplified when the ASD cohort was further divided into two cohorts based on their language ability. These results should be interpreted with caution and should be considered exploratory and descriptive rather than definite confirmations because of the small sample size. Future studies should include larger sample sizes to reduce risk of bias due to a small sample, as there is greater risk of data being driven by a small number of participants that may not be representative of the patient population.

Another possible caveat is that all trials for congruent and incongruent conditions were included, regardless of if the trials were correctly identified or not, which could mask the actual N400 component responses. For example, if all the incongruent words were incorrectly identified as congruent in children, due to the inclusion of all trials for each condition, results may indicate no differences in neural response for congruent and incongruent words. This would be masking the actual response to incongruent words and this could be possible if the participants were not familiar with the target words and their categorization. The accuracy rates of the adults, TD children, and the subset of children with ASD who completed the behavioural portion in this study nonetheless, were relatively high and helps mitigates this possible confounding factor. However, there was a subset of children with ASD who had no behavioural responses recorded, therefore there was no indication of whether they knew the words. However, the words selected for the paradigm were based on the MacArthur-Bates CDI that has been used in other studies investigating semantic processing in young children with ASD (DiStefano et al., 2019). The words on the CDI checklist are used to assess a child's level of comprehension and expressive abilities and this assessment is meant to assess children between 16-30 months of age (Mayor & Mani, 2019). The children with ASD who participated in this study were older than the target age range for the CDI assessment by a two-fold minimum to reduce this potential factor. Additionally, only including correctly identified trials would decrease the number of trials which would increase the noise in the averaged EEG data that could conceal the actual neural responses (Cantiani et al., 2016). Future studies could include pre-baseline assessments of how well the participants knew the target words, that would ensure the target words were known by participants prior to EEG testing.

Another limitation to this study was that the hearing threshold assessment required behavioural responses. No children were excluded due to this assessment, however, children with ASD show deficits in cognitive and language skills that may interfere with the ability to perform the behavioural aspects of hearing threshold test that was administered (Tager-Flusberg, 1999). There is less reliability of the hearing screening especially in children with ASD with lower language scores because behaviour responses on the EEG task for these children were not captured due to their lower capabilities. Therefore, it was possible that normal hearing thresholds were incorrectly identified in these children with ASD and deficits would affect their capabilities to hear spoken words affecting neural results. However, an exclusion criterion for this study was peripheral hearing difficulties reported by parents and no parent reported any hearing difficulties minimizing the possibilities of impaired hearing sensitivities.

Additionally, working memory was not further examined in relation to semantic processing although it is associated with semantic processing and children with ASD have shown deficits in this domain of cognitive function (Friedman & Sterling, 2019; Habib et al., 2019). Baddeley's model of working memory suggests that the phonological loop temporarily stores spoken words that can be used for higher order processing such as semantic processing (Gathercole & Baddeley, 1993). In the semantic categorization paradigm used in this study, the preceding context (category) to words would need to be maintained in working memory to assess the categorization of the words in the list. Additionally, the level of working memory must be higher in this paradigm because the preceding context (category) was only instructed to participants at the beginning of the block, before the list of words was presented. The WPPSI-IV was administered to collect a measure of working memory in child cohorts, however a subset of participants with ASD were unable to complete the task and other working memory assessments from this subset were not received. Therefore, the working memory capabilities of this portion of children with ASD could not be assessed and the effects of working memory were not further examined. Consequently, future studies should capture a measure working memory and minimize the amount of working memory needed by auditorily presenting the category preceding the target word for every word in the block.

Lastly, attention allocation could affect the N400 component but, there was no measure for participants' attention allocation in this study. Evidence has shown that attention modulates the N400 component, with decreasing attention correlating with decreasing N400 component amplitudes (Erlbeck et al., 2014; Marta Kutas & Federmeier, 2011). The behavioural responses of participants in this study however, indicated to their attention was allocated to the spoken words, excluding the subset of children with ASD who could not complete the behavioural portion of the task. For all children however, an experimenter sat with them to ensure that they were attentive towards the stimuli and redirected their attention to the ear inserts to help attenuate this limitation.

This study builds on knowledge on the neural underpinnings of semantic processing in children with ASD, but there are several limitations that future studies should aim to address to better understand the differences in processing. Furthermore, using a longitudinal approach in the future with this methodology would offer a way to monitor the trajectory of impairments in children with ASD. By understanding these neural deficits, it helps identify where therapies should be targeting in children with ASD and a way to monitor the efficacy of therapies allowing for better informed decisions for children with ASD.

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Appendix A.

Categories and Words

Animal	In/Out of Category	Body Parts	In/Out of Category
Arm	Out	Arm	In
Bear	In	Belt	Out
Bee	In	Bread	Out
Belt	Out	Break	Out
Bird	In	Cheek	In
Boot	Out	Chin	In
Cake	Out	Coat	Out
Cat	In	Drive	Out
Cow	In	Duck	Out
Dog	In	Ear	In
Drink	Out	Foot	In
Eye	Out	Frog	Out
Frog	In	Grape	Out
Gum	Out	Head	In
Hen	In	Hen	Out
Hug	Out	Knee	In
Lamb	In	Leg	In
Leg	Out	Mouth	In
Milk	Out	Pull	Out
Pig	In	Sock	Out
Read	Out	Soup	Out
Sheep	In	Toe	In
Shirt	Out	Tongue	In
Wolf	In	Tooth	In

Food & Drinks	In/Out of Category	Action Words (Verbs)	In/Out of Category
Bear	Out	Bee	Out
Bib	Out	Bib	Out
Bread	In	Cake	Out
Cake	In	Cheek	Out
Cat	Out	Climb	In
Chin	Out	Corn	Out
Corn	In	Draw	In
Egg	In	Drive	In
Eye	Out	Hand	Out
Frog	Out	Hat	Out
Grape	In	Jump	In

Gum	**In**	Kick	**In**
Hat	**Out**	Pig	**Out**
Look	**Out**	Pull	**In**
Meat	**In**	Read	**In**
Milk	**In**	Run	**In**
Pop	**In**	Sheep	**Out**
Salt	**In**	Sing	**In**
Shoe	**Out**	Skate	**In**
Sleep	**Out**	Sock	**Out**
Soup	**In**	Soup	**Out**
Toast	**In**	Swim	**In**
Toe	**Out**	Throw	**In**
Wipe	**Out**	Toe	**Out**