Statistical Analysis of Altered Habituation to Sensory Stimuli In Young Adults with Genetically Inherited Autism

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Introduction

Autism Spectrum disorder (ASD) is a developmental disorder that is defined by social and communication challenges and restrictive and repetitive behaviors. These symptoms are often accompanied by sensory, motor, perceptual, and cognitive abnormalities as well. Many individuals with ASD have different ways of learning, paying attention, or reacting to things.These signs tend to develop early in childhood, but remain present throughout the individual's lifetime. Although the diagnostic protocol is well established via cognitive tests administered by trained professionals, ASD is highly heterogeneous in its presentation, from varying symptoms, to differing developmental capabilities. Once considered a rare disease, ASD may be affecting nearly 1% of the world's population.

Prior to the fifth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM), the American Psychiatric Association (APA) distinguished between three syndromes: Autistic, Aspergers (AS), and Pervasive Developmental Disorder not Otherwise Specified (PDD-NOS) (1). In the 1990s, as Asperger's studied his patients, he often focused on their positive attributes of original thought and intellectual interests (2). The idea that autism and AS were different syndromes was mainly decided by the fact that individuals with AS have good cognitive and language skills in the beginning years of life. Children with AS also seemed more interested in social interactions than those with Autistic Disorder, however they approach it in an impaired fashion. Asperger himself notes that AS can be distinguished from Autistic Disorder based individuals with AS strong cognitive abilities, language skills, and high intelligence to the point that they have a vocabulary before they even walk. In 1994, AS was officially introduced into the DSM-IV. In order to be diagnosed with AS, the child was not able to qualify for Autistic Disorder; if the child qualified for a diagnosis of Autistic Disorder that took precedence over

Asperger's Syndrome. The issue with AS diagnoses is the aforementioned idea of precedence for Autisitc disorder. The core features of AS were not excluded from all cases of Autisitic disorder making the diagnosis even more difficult. Using the DSM-IV guidelines to diagnose AS became virtually impossible, which led to its removal in the DSM-V. As for PDD, this diagnosis applies when an individual fails to meet the criteria of Autistic Disorder in the DSM-IV, but has similar diagnostic features including stereotype behavior, and social limitations (3). The diagnostic criteria for PDD-NOS are not explicit, without any specific scoring or testing methods. Due to the ambiguous nature of PDD-NOS, this too disappeared from the DSM-IV. With the publication of the DSM-V AS and PDD-NOS were folded into a new category called Autism Spectrum Disorder to acknowledge the similarities across the syndromes, to allow for diagnosis to be acquired more readily, and to remove distinctions made by clinicians based on instinct. The diagnosis of ASD recognizes that autism presents in a variety of ways among individuals leading to an extremely heterogeneous spectrum. Despite the removal of boundaries between the disorders and now being diagnosed with a broad disorder, clinicians should still tailor and individualize their treatment plans accordingly.

Autism Spectrum Disorder is considered to be a lifelong syndrome. The symptoms associated with the disorder can range from minor to major. The symptoms may be so major that they completely impair all daily function for the individual, and they can only engage in daily activities through intensive support from caregivers. The symptomatology that is suggested to be diagnostic of ASD includes socialization issues, receptive and expressive language difficulties, abnormal sensory motor movements, repetitive and stereotyped behaviors, and compulsions and rituals. These symptoms are not present across all individuals diagnosed with ASD and they often present in very individualized fashions (4).

The vast symptomology of ASD is further compounded by the common comorbidities of the syndrome. The psychological comorbidities that flood the ASD population include intellectual disabilities, anxiety, depression, and ADHD.. The medical comorbidities include seizures, sleep difficulties, gastrointestinal issues, mitochondrial dysfunction, and immune system abnormalities (5).

Intellectual disabilities (ID) consist of social, cognitive, and adaptive skill deficits (6). ASD is one of the most common comorbidities found with ID and the trend has been found that greater intellectual disability will correlate with increased severity of autism spectrum disorder. The prevalence of the comorbidity is estimated to be around 40% and has been associated by common symptomatology such as social and communication deficits, restrictive and repetitive behaviors, and increasingly challenging behavioral issues. Cervantes and Matson conducted a study to analyze the effects of having both ID and ASD. They concluded that when a patient presented with both ASD and ID, not only were their symptoms of ASD more pronounced, but also they had higher levels on the on the Anxiety, Schizophrenia, Stereotypies/Tics, SIB, Eating Disorders, and Impulse Control subscales. While ID is very often associated with ASD, this co-occuring diagnosis increases the risk for many other psychopathological syndromes.

Anxiety has been acknowledged as a coexisting issue within the ASD population since the 1940s and possibly even before. Although anxiety is not considered diagnostic of ASD, the social deficits of preferred isolation and lack of social contact, may be the root of anxiety issues (7). The awareness of social impairment may foster anxiety in children with ASD which ultimately increases the manifestation of social disabilities. White et al. developed a literature review of the published articles from August 2008 containing a combination of the key words *"autism, asperger(s),* or *pervasive developmental disorder"* and "*anxiety* or *anxious".* The population

researched were children from the ages of 6-18 diagnosed with ASD, Aspergers, or pervasive developmental disorder and the diagnosis of anxiety were assessed using direct observation or report from the teacher, parent, or child themselves. The studies reviewed yielded results indicating that 11-84% of children with ASD contain some element of anxiety. Some of the most common anxiety disorders found in this population included phobias, generalized anxiety disorder, separation anxiety disorder, obsessive compulsive disorder, and social phobia. The anxiety diagnosis was found across individuals with various levels of cognitive functioning. Depression in individuals with ASD leads to greater deficits in the core diagnostic features and higher rates of suicide. Based on a reveiew of suicidal ideation, and attempts in ASD preformed by Legers and Rawana, it was found that 11-50% of this population engages in suicidal behaviors (8).

While symptoms of depression and the core symptoms of ASD have significant overlap, it is estimated that 42-56% of individuals with ASD have a comorbidity of depression (9). The presentation in these individuals is often complicated by the social, communicative, and cognitive impairments in ASD that are often present. Symptoms of ASD such as social withdrawal and abnormal speech patterns are misinterpreted as depression and vice versa. An additional issue with the diagnosis of depression in ASD are the tools used for diagnosis specifically in children and young adults with ASD. With an increasing need to understand depression in ASD, Kim and Lecavalier conducted a literature review using PubMed and psycINFO in February 2020. The key words indicated were "autism OR autistic OR ASD", or "Asperger OR Asperger's", or "PDD OR "pervasive developmental disorder" and "child OR adolescent OR youth" and "depress* OR mood OR MDD OR dysthymia". The goal was to study the themes and quality of studies in depressive youth with ASD and to establish gaps in

knowledge on the topic. Depending on assessment tools used in the analyzed articles, the prevalence of depression ranged from 8-26%. Individuals with ASD who were also suffering from anxiety had an increased prevalence of depression as well, estimated at 35%. When compared to age matched typically developing children, the children with ASD showed increased rates of depression. Depressive symptoms are not identical across individuals due to many factors. Evidently, depression is a major issue in children with ASD.

As with depression, Attention Deficit Hyperactivity Disorder (ADHD) has many of the same core symptoms seen in ASD. This goes so far as when the Diagnostic and Statistical Manual of Mental Disorders published their fifth edition, ADHD was not diagnosed if ASD was present (10). The resulting studies assessing comorbidity of ASD and ADHD were conducted on small clinical samples. Stevens et al. gathered data from the Survey of Pathways to Diagnosis and Services (Pathways), from a 2011 survey that surveyed families nationwide with children under age 18 (11). The goal of this survey was to establish a sample population of each state of children with special healthcare needs. They found that In the ASD sample, 40.60% (n = 717) of children were found to also have a confirmed ADHD diagnosis and an additional 13.25% were found to also have both ADHD and ID diagnoses. When a sample weight was applied to this data, the prevalence increased to 59% indicating that more than half of children diagnosed with ASD have a comorbidity for ADHD. Evidently, ADHD is one of the highest comorbid syndromes diagnosed with ASD.

Although ASD diagnosis may seem difficult since there are no medical tests available to diagnose the disorder, there are trained professionals capable of assessing the condition. These professionals include psychologists, psychiatrists, pediatric neurologists, developmental pediatricians, and more. With proper training and experience, the diagnosis can be made

following an evaluation using APA guidelines and various other cognitive tests. The APA consistently reviews and modifies their diagnostic guidelines as new discoveries are made in the ever evolving field of neurodevelopmental disorders. The DSM-5, containing the most up to date diagnostic criteria for ASD, delineates the following guidelines (9): a child must have persistent deficits in each of three areas of social communication and interaction plus at least two of four types of restricted, repetitive behaviors. The three areas of social communication and interactions include social and emotional reciprocity, nonverbal communication, and developing, maintaining, and understanding relationships. Restricted interests and repetitive behaviors examples include stereotyped or repetitive motor movements, use of objects, or speech, insistence on sameness, inflexible adherence to routines, or ritualized patterns of verbal or nonverbal behavior, highly restricted, fixated interests that are abnormal in intensity or focus, hyper- or hypo reactivity to sensory input or unusual interest in sensory aspects of the environment. Additionally, to be diagnosed, these symptoms must be present in early development even if they are not fully manifested until later in life and these symptoms must cause significant impairment in multiple spheres of life, whether that be social, occupational, or any other important area needed for normal functioning. Based on the individual's needs in the various diagnostic domains, the DSM-5 assigns a severity level based on how much caregiver support is necessary. The levels range from level one which is "requiring support" to level two which is "requiring substantial support" to level three which is "requiring very substantial support". The level of social communication deficits and restricted and repetitive behaviors necessary to wind up in each level are elucidated in the manual. To receive an ASD diagnosis, the condition of the individual cannot be better described by another disorder such as intellectual disability or global developmental delay. Although, as noted above, these syndromes are often

comorbid, it is important to confirm that the individual is not simply experiencing ID in isolation.

Many other diagnostic tools diagnose autism in terms of language delay, cognitive functioning, and behavioral issues, however, these are not specific enough to warrant the ASD diagnosis. Rather, what would be more useful would be developing a tool to measure the severity of core autism symptoms which more accurately describe the specific features of autism as opposed to other similar syndromes. The Autism Diagnostic Observation Schedule originally published by Lord et al. in 1989, has been a useful tool in assessing the severity of autism that each individual presents with (12). A trained examiner administers the test picking the best module for the age and level of functioning of the child. The administrator scores the test items based on a scale from 0-3, zero being no abnormality in that field to 3 being moderate to severe abnormality. The different modules have been modified since the original test in 1989, to make the scores from each one collapsable and comparable across the participants. ADOS-2 has been found to be more accurate in determining the ASD diagnosis than various other diagnostic methods. The ASD diagnosis received from an ADOS test is vital for the individual to receive the proper therapies and services they need. Ultimately, what ADOS does is it provides a methodical and standardized method for identifying children with ASD eliminating issues of subjectivity from clinicians (13-14).

It has been recognized to date that a combination of environmental factors and genetic factors cause the autism phenotype. The various environmental factors thought to have an effect on the causation of autism are parental age, teratogenic compounds, perinatal risks, medication, smoking and alcohol use, nutrition, vaccination, toxic exposures, and the role of extreme psychosocial factors (15).

Advanced parental age is well established in being a risk factor for chromosomal aberrations. There is also evidence to suggest that advanced parental age may increase the risk of neurodevelopmental disorders and psychiatric syndromes, ASD included (16). This is likely due to de novo mutations, specifically during spermatogenesis, making the paternal age increasingly of concern when compared with maternal age when considering ASD. This risk is believed to be the greatest when both parents are at an advanced age.

Maternal weight has been in question as to whether obesity could affect neurodevelopment of the fetus or not. Studies indicate the children born to mothers that were both extremely underweight and extremely overweight were at increased risk of developing ASD, signifying both extremes are harmful to the development of the fetus (17). Further studies interested in obesity specifically have found that offspring of overweight parents when compared with mothers of normal weight have a 36% increased risk of ASD. Maternal weight does seem to be involved in fetal neural development.

Maternal diabetes has indicated an additional risk of ASD development in the fetus (18). The odds increase by about 62% as compared to non diabetic mothers. Moreover, hypertension, which affects nearly 10% of all pregnant women, has also been associated with an increased risk of ASD development (19).

There are a number of viral and bacterial infections associated with an increased risk of ASD (20). The first infection discovered to have an association with ASD was rubella, and now maternal influenza is thought to increase the risk of ASD progression. The timing of the infection, namely which trimester the mother contracted the infection, did not affect ASD development, rather it was simply the presence of the infection that was influential. The development of ASD may not be directly related to the infection, but rather be a consequence of

the increased work needed of the maternal immune system to fight the infection. All things considered, maternal infection can increase the risk of fetal ASD by at least two fold. Antidepressant and anticonvulsant medications surfaced as medications of concern when considering healthy fetal development. Valproic acid, an antiepileptic and mood stabilizer, has suggested multiple risk factors, with developmental delay and cognitive impairment of interest here (21). Selective serotonin reuptake inhibitors (SSRIs) are often used to treat depression, but they are able to cross over the placental barrier with the possibility of affecting the fetus (22). The reported risk of autism and SSRI use has been small but present. More harmful substance use such as cigarette use and alcoholism are associated with neurological, psychiatric, and neurodevelopmental disorders (22-24). The research with regards to ASD in specific has elicited mixed findings, but this is still something to bear in mind when considering ASD risk factors. Maternal nutrition directly affects the trajectory of fetal development since the fetal growth requires many nutrients as does placental growth. Malnourishment negatively impacts fetal development and increases the risk of autism (26). The deficiency of essential nutrients such as vitamin D, iron and zinc and copper are of specific interest. Vitamin D receptors and enzymes are present operating in neuronal cells indicating a role in vitamin D and neurodevelopment in utero. Studies have pointed to a role of vitamin D deficiencies contributing to the etiology of ASD (27). Furthermore, iron is essential for neural function in all individuals, but with a developing fetus in specific. Although findings regarding iron deficiency and ASD development have been conflicting, there has been evidence to suggest that mothers with iron deficiency had double the chance of producing children with ASD than mothers with normal iron levels (28). Maternal zinc deficiency has been associated with neural tube defects and may contribute to

ASD as well (29). Both elevated and decreased levels of copper have been correlated with brain development issues such as ASD progression (30).

Although it was originally believed that vaccinations, specifically the measles mumps and rubella (MMR) vaccine contributed to ASD development through a proposed pathway of bowel inflammation developing into ASD, this theory has subsequently been falsified (31). Claiming vaccines are a causal factor in ASD development has little to no support from scientific literature (32).

Moreover, in recent years, genome sequencing has revealed that there are hundreds of genes involved with the development of ASD (33). In some cases, genetic mutations are thought to be causational allowing for a genetic explanation of the clinical manifestations of ASD along with genetic counseling for family members at risk. Technological advancements including microarrays have allowed the study of copy number variants (CNVs) within the genome allowing for the identification of recurring locations of CNVs in the genome. These CNVs are often de novo variants, meaning they occur spontaneously during paternal germline meiosis, and are not present in all paternal cells (34). It has been suggested that these de novo CNVs alter the expression of synaptic genes which may suggest that dysfunctional synapses may account for the neurological findings in ASD. Further technological advancements have promoted the identification of genetic variants and single nucleotide assessment of rare genetic variants that had been undetectable until this point. For one, Whole Exome Sequencing (WES) has allowed for the detection of variants that transform protein coding regions and many variants that are associated with ASD (35). WES has allowed for the identification of rare de novo variants in the coding regions of the genome at the single nucleotide level. It has been found that many individuals with ASD have protein truncating variants, including nonsense, frameshift, or splice

sites. Beyond this, Whole Genome Sequencing (WGS), has enabled the analysis of the genome beyond that of microarray or WES (36). WGS can detect that which was undetectable in the previous analysis tools such as the noncoding regions of the genome. This is valuable when studying ASD, as there may be an association between mutations in the non-coding regions of the genome which contains many regulatory regions, which may specifically affect neurobiology, and the ASD phenotype. Understanding the genetic architecture of ASD is central to understanding the genotype and phenotype while simultaneously providing the most accurate diagnosis and treatment plan.

Neurophysiological data measurements may also be useful in serving as biomarkers for differences between ASD and typically developing individuals. This would be specifically of use when measuring processing discrepancies between groups. Electrophysiological Brain Response (EEG) scalp recordings provide a non-invasive readout of neural processing with time accuracy down to the millisecond (37). Nerve cells constantly produce electrical signals in distinct lobes of the brain during the various daily activities. Conductive electrodes are attached to the various lobes externally, and these electrical signals are recorded. EEG can be of specific use when measured during presentation of a sensory stimulus and analyzing the brain's response. This is readout also known as an event related potential (ERP) and is the most useful data point when analyzing information processing at specific time points (38). This is further useful in the case of ASD since EEG/ERP can indicate when and where information processing may be altered (39). Variance in sensory ERPs among individuals with ASD and typically developing individuals may serve as evidence for a biomarker marking clinical evidence of sensory processing differences. This may be seen across various sensory modalities, including auditory and visual. The peaks retrieved from EEG measurements may be useful in assessing response time to stimuli

while also measuring adaptation when presented with repetitive stimuli. EEG/ERP can be a useful tool in indexing brain function in ASD and characterizing their sensory processing deficits, a core diagnostic feature of ASD.

The symptomatology of individuals with ASD of interest for this study are sensory issues. A recent hypothesis has suggested that those with ASD are unable to utilize contextual information from their environment to predict future events (40). Furthermore, it has been postulated that abnormal sensitivities may not be due to instantaneous differences in sensory processing between ASD and typically developing (TD) individuals, but rather because of how stimuli are processed over periods of time (41). More specifically, autism may be associated with a reduction in sensory habituation, namely those with autism are unable to decrease their responses to ongoing or repetitive stimuli. This compromise of habituation may lead to hyposensitivity in ASD, as individuals with ASD have less likelihood of recognizing different stimuli in the presence of repetitive ones (42). In direct contrast, reduced habituation may lead to hypersensitivity in ASD due to the fact that every single stimulus seems novel to them (43). There have been previous studies indicating that those with ASD did not show a progressive decreasing response to repetitive auditory stimuli and simple visual stimuli, indicating a lack of habituation (44). Measures of neuronal data would only strengthen the claim that individuals with ASD are less able to habituate to sensory stimuli when compared with TD counterparts. Neuronal data suggesting habituation would show a lower cortical response with ongoing repetition of stimuli, whether auditory or visual (45-46). Various studies utilizing fMRI and EEG have indicated decreased habituation in individuals with ASD (47-49). There are several studies that have found evidence counter to this as well; there is a lack of difference in habituation between ASD and TD groups.

Due to the mixed findings in the field of sensory habitation in ASD, my study further investigates whether there is reduced habituation in autism. The hypothesis of the study is that young adults with autism habituate differently to sensory stimuli than typically developing young adults. This hypothesis was tested by administering a sensory entrainment study while collecting EEG measurements and analyzing these data to compare these two experimental groups. The entrainment study consisted of conditions that would be valuable when considering the issue of habituation. The latter part of the study utilized cognitive test scores and neuronal data to attempt to understand the relationship between neuronal habituation patterns and the clinical phenotype.

Methods

Participants

21 young adults diagnosed with ASD and 22 IQ and age matched (TD) controls participated in the study. (Table 1)

	Age	Sex	ADOS Severity Score	IQ (Full Scale)	IQ (Verbal)	IQ (Non Verbal)	RBSR	SRS (Total Score)	Vineland (ABC Score)
ASD (21)	22.5±5.7	19 (M) 2 (F)	8±2.5	100.5±17.3	100.5±27.4	103.3±23.6	20±16.2	57.6±5.8	71.9±11.4
TD (22)	21.9±2.8	14 (M) 8 (F)	N/A	109.6±13.9	109.5±22.6	103.1±22.5	3±5.5	47.2±3	115.75±15.9

Table 1: Mean ± SD of Age, sex and Cognitive Test Scores of the two groups.

The age range for the ASD group was 17.4-37.6 with a mean age of 22.5. For TD, the range was 17.2-26.6 with a mean age of 21.9. These ages are based on the time of their entrainment study and EEG collection ASD participants were recruited without regard to sex, race or ethnicity; TD

participants were recruited to match the ASD group on age (two-tailed t-test for unpaired samples with unequal variances and α=0.05; t=-0.21; p=0.83). IQ quotients for verbal (VIQ), performance (PIQ), and full scale (FSIQ) were calculated for 18 ASD participants and 14 TD participants using the Wechsler Adult Intelligence Scale (WAIS). To be considered a participant in the ASD cohort, participants had to meet diagnostic criteria for ASD using the following guidelines: 1. Autism Diagnostic Observation Scale (ADOS-2); 2. Diagnostic criteria for autism spectrum disorder as delineated in the DSM-5; 3. Clinical diagnosis from a licensed clinicain with considerable experience in diagnosing and treating ASD.Repetitive Behavior Scale-revised (RBSR), was selected for inclusion in this study because it measures how far the repetitive behaviors in autism span. The specific sections included within this test are "Ritualistic/Sameness Behavior," "Stereotypic Behavior," "Self-injurious Behavior," "Compulsive Behavior," and "Restricted Interests." all of which seem relevant in a study on habituation. Next, the Social Responsiveness scale test (SRS) which measures social impairment was performed.. Sections included within this test are social awareness, social cognition, social communication, social motivation, and restrictive interest and repetitive behaviors. Restrictive interest and repetitive behaviors is of specific interest here as it shows an inability to adjust. Lastly, the vineland which is a measurement of independence was performed. It measures how much participants are doing compared to a TD person in their age range. To be considered a participant for the TD cohort, participants had to have no history of neurological, developmental, or psychological syndromes or first degree relatives with ASD. All participants were required to pass a vision and hearing test on the day of testing.

Stimuli and Task

The entrainment study consisted of six distinct conditions with both auditory and visual stimuli. The following analysis was performed on auditory data so that will be described at length. Auditory stimuli were delivered at an intensity of 75 dB SPL via a single, centrally-located loudspeaker (JBL Duet Speaker System, Harman Multimedia). The task was designed to test the hypothesis that young adults with ASD habituate differently to sensory stimuli than TD young adults. The task included six conditions. The differences between them was mainly the time between the beeps. The isochronous condition had equal time intervals of 666 ms between each beep. The interchanging condition had four beeps with time intervals of 333 ms and then the subsequent three beeps had time intervals of 999 ms. The gradual condition had time intervals that increased between beeps. The random condition had a random time interval between each beep ranging from 170 ms to 1150 ms. The small jitter condition has a few beeps very close together but then the next cluster does not repeat for a set amount of time. The large jitter is analogous to the small jitter, but the cluster of beeps has a slightly larger time interval between each one. Each condition is composed of 60 trials, and they are presented three times in a random order in each session. Each participant completed 8 sessions. The order of the blocks presented to each participant was selected randomly prior to the study. The instructions provided to each participant were to listen to the activity and react by hitting a button for each target. The target was a beep in a different pitch than all the others. The appearance of the target condition appeared in about 10% of the trials.. Responses between 150-1500ms post stimulus onset were considered correct, while anything outside of that range were not. Breaks were provided to generate maximal concentration across participants. The following analysis was performed on the isochronous condition to best assess the notion of habituation.

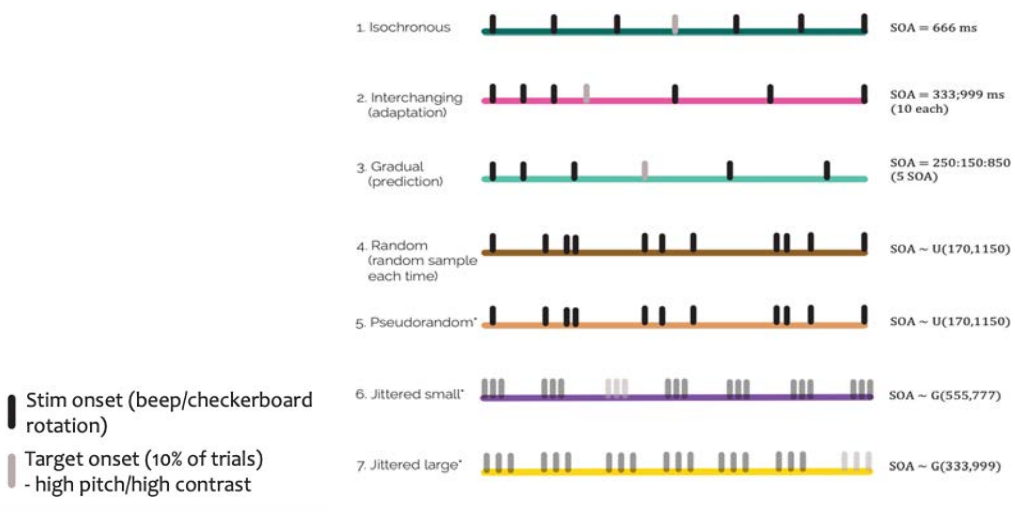


Figure 1: Schematic of Experimental Paradigm. The paradigm shows the six different conditions with varying ratios and time intervals between stimuli. Participants underwent auditory entrainment tasks under these six conditions.

The following data collection and processing followed the protocol of Beker et al. and have been taken from their previous work.

Data acquisition

Response times were recorded with the Presentation© software. EEG recordings were collected from 70 active channels (64 scalp channels and 6 external electrodes) at a digitization rate of 512 Hz, using ActiView (BioSemiTM, Amsterdam, The Netherlands). Analog triggers indicating the latencies of stimulus onsets and button presses were sent to the acquisition PC via Presentation® and stored digitally at a sampling rate of 512 Hz, in a separate channel of the EEG data file.

Data processing

Data were processed and analyzed using custom MATLAB scripts (MATLAB r2017a, MathWorks, Natick, MA), and the FieldTrip toolbox (Oostenveld et al., 2011, Donders Institute

for Brain, Cognition and Behavior, Radboud University, the Netherlands). A minimum number of 50 EEG trials per condition per analysis was set as a criterion for a participant to be included in the analysis, however, most participants had more than 100 trials in each condition (mean±sem TD: 168±37; ASD: 136±24). Due to occasionally long reaction times in some of the participants, only responses within 1000ms after stimulus presentation were considered as valid for further analysis of the behavior data.

EEG Processing

To tailor analyses to each of our research questions, data processing involved four different sub-pipelines, as elaborated below. All epochs, except for those used for measuring entrainment (see 3 below) were down-sampled to 256 Hz, epoched and band-pass filtered between 0.1 and 55 Hz using Butterworth Infinite Impulse Response (IIR) windowing, with filter order of 5. Epochs were then demeaned to normalize for DC shifts, and baseline-corrected, as specified below.

1. Visual Evoked Response (VEP): continuous data were epoched with respect to each visual stimulus: 200 ms before and 850 ms after stimulus onset, and baselined to the 100ms window before the onset of each visual stimulus. Two averaging approaches were taken, so that we could measure and compare between groups on a) the overall VEP, and b) sequence effects on the CNV, as follows:

    1. VEP: To compare sensory-evoked potentials between the groups, trials were averaged across all stimuli, regardless of their placement in the sequence. Data were referenced to a frontal channel (AFz) to optimize visualization and measurement of the VEP over occipital scalp at channels O1, O2, and Oz.

Comparison between the groups on the VEP was calculated on the voltage at the peak of the P1 component (~100ms) for each participant.

2. CNV: To find differential patterns of anticipation with respect to the place of a cue in the sequence, trials were averaged separately for visual stimuli 1 through 4 in the trial. ERP analyses were focused on preparatory effects (the CNV) that are commonly seen over fronto-central sites (**Walter *et al.*, 1964**; **Gaillard, 1976**; **Breska & Deouell, 2017**), and were measured here at frontal channels (AFz, FP1, AF3, and AF4). To optimize visualization and measurement, data were referenced to the two ground electrodes: Common Sense Mode (CMS), and Driven Right Leg (DRL), used in BioSemi as active and passive electrodes respectively. CNV activity was measured prior to the upcoming stimulus in the sequence, at −200 to 0 ms relative to stimulus (visual or auditory) onset.

2. Entrainment: to measure entrainment of neural oscillations to the event timing, the EEG data were epoched at 3100ms before and 300ms after the auditory event, to encompass the full sequence of stimuli comprising a trial, and trials were baselined to the 100ms before the onset of the first visual stimulus in the sequence. Data were referenced to channel AFz. Analyses of frequency and phase were performed on epochs that were low-pass filtered at 55Hz, and high pass filtered at 0.1Hz. To visualize entrainment of oscillatory activity to the rhythm of visual events (1.5 Hz), data were low-passed filtered at 1.9 Hz. For this, a Finite Impulse Response (FIR) filter, with normalized passband frequency of 1.9 Hz, stopband frequency of 4 Hz, passband Ripple of 1Hz and stopband attenuation of 60 dB, was applied.

3. Auditory Evoked Response (AEP): To analyze evoked responses to the auditory target stimulus and possible differences between the groups with respect to condition, data were epoched 300ms Sec. before and 850ms Sec. after the auditory stimulus onset, and baselined to 100ms before auditory stimulus onset, and as per convention, referenced to a channel near the left mastoid (TP7). Statistical analysis was performed on data from fronto-central channels at the maximal peak of each participant's P1 (~50ms), N1 (~100ms) and P2 (~200ms) components.

For all pipelines, after epoching, a two-stage automatic artifact rejection was applied at the single trial level. First, channels that varied from the mean voltage across all channels and from the auto-covariance by 1 standard deviation were classified as bad. A maximum of six bad channels was set as an inclusion criterion for trials to be analyzed. For these trials, channels were interpolated using the nearest neighbor spline (**Perrin *et al.*, 1987**; **Perrin *et al.*, 1989**). Second, a criterion of ±120μV was applied. Electrodes that exceeded this criterion were considered bad. Participants that had less than 50 trials per condition were excluded from further analysis.

Results

I investigated the influence of an auditory isochronous and repeating stimulus on neural entrainment and anticipation processes in both typically developing young adults and young adults with ASD using measurements obtained from EEG data. The neuronal data was then correlated with cognitive test scores explore

Figure 2a illustrates the grand average of all the isochronous trials across all participants from both the TD and ASD groups. This includes about 1,000 trials per participant that underwent the entrainment study, specifically eighteen TD participants and seventeen ASD participants. The peaks originally considered for analysis were p1, n1, and p2 respectively, but the p1 group was barely visible, and the n1 peak did not provide significant results. Consequently, the p2 peak was utilized for analysis both here and with the remaining analyses. This grand average utilized the C1, C2, Cz channels from the EEG electrodes. Figure 2a suggests that the ASD group has a higher average voltage for the P2 peak which would show a lack of habituation in comparison to the TD but this is not enough information to make that conclusion. The next step was running a T-test (figure 2b) which compared the difference between the TD groups and ASD groups across all of the channels to see if there was a significant difference between experimental groups. Even though in figure 2a it seemed to show a neurological distinction between the groups in channels C1 C2 and CZ, the data were not statistically significant.

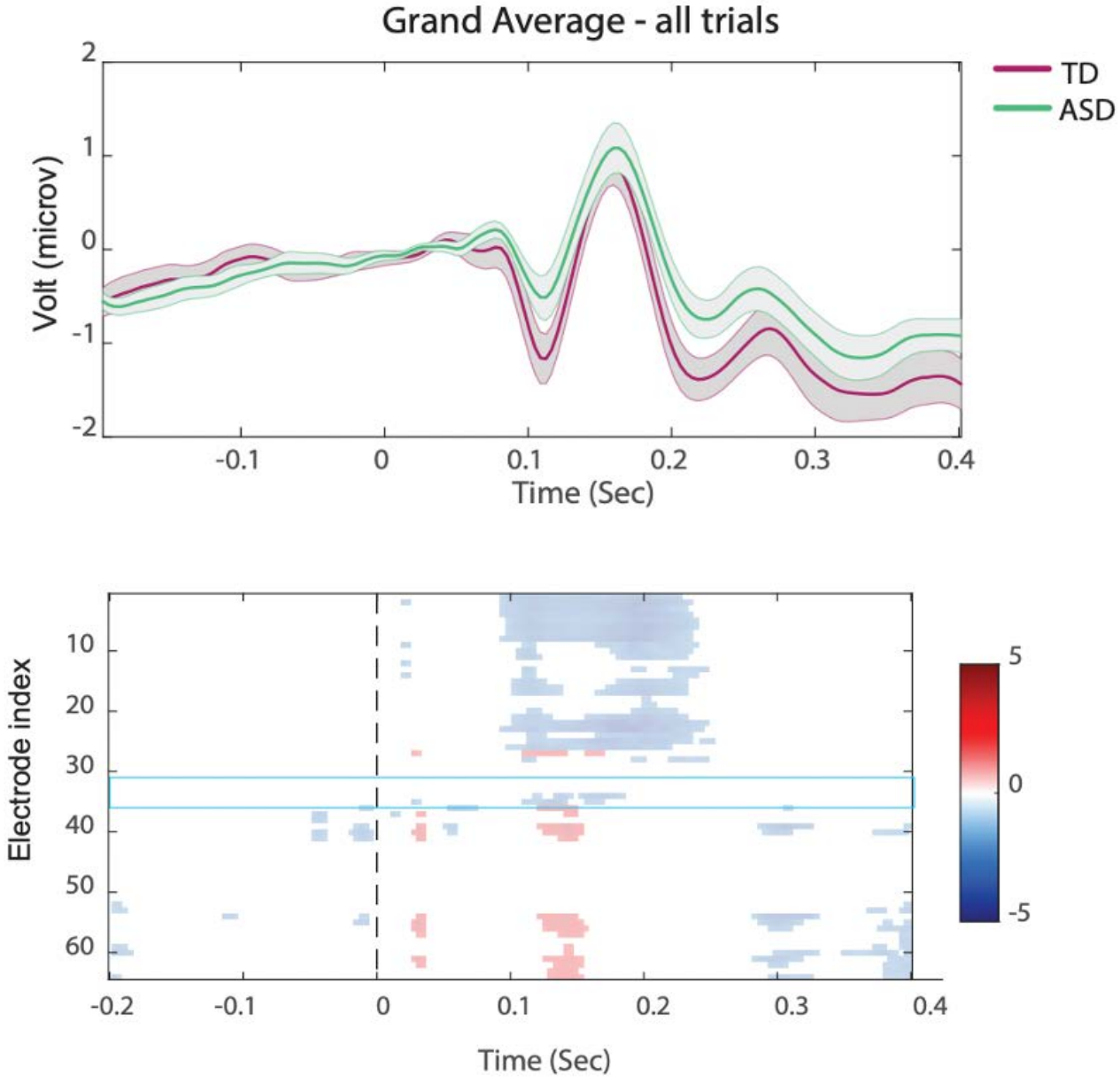


Figure 2: Patterns in Sensory Habituation measured using EEG for ASD vs TD, for all Trials Conducted A. EEG pattern using Grand average of all trials conducted utilized the C1, C2, Cz channels from the EEG electrodes. Volts, a measure of brain electrical activity is measured on the y axis over time in seconds measured on the x-axis. The ASD group is represented by the green line while TD is represented by the pink line. B. T-test using the data of all trials from EEG data collected during entrainment study. Significance was measured with p value ≤0.05. The light blue box is around electrodes of interest.

Figure 3 represents the average of the first and last twenty trials for the TD and ASD groups respectively. The goal of this figure is not to make any statistical assertions, but rather to reveal to the naked eye that there is a difference between the first and last twenty trials for each experimental group. Figure 3 seems to indicate that the voltage of the TD participants in the first twenty trials is higher than that of the same groups last twenty trials, signifying habituation. This is in direct contrast to the ASD group, whose voltage of the first twenty trials appears lower than the voltage of the last twenty trials, signifying a lack of habituation. Although not meant for mathematical affirmations, Figure 3 is invaluable in furthering my hypothesis.

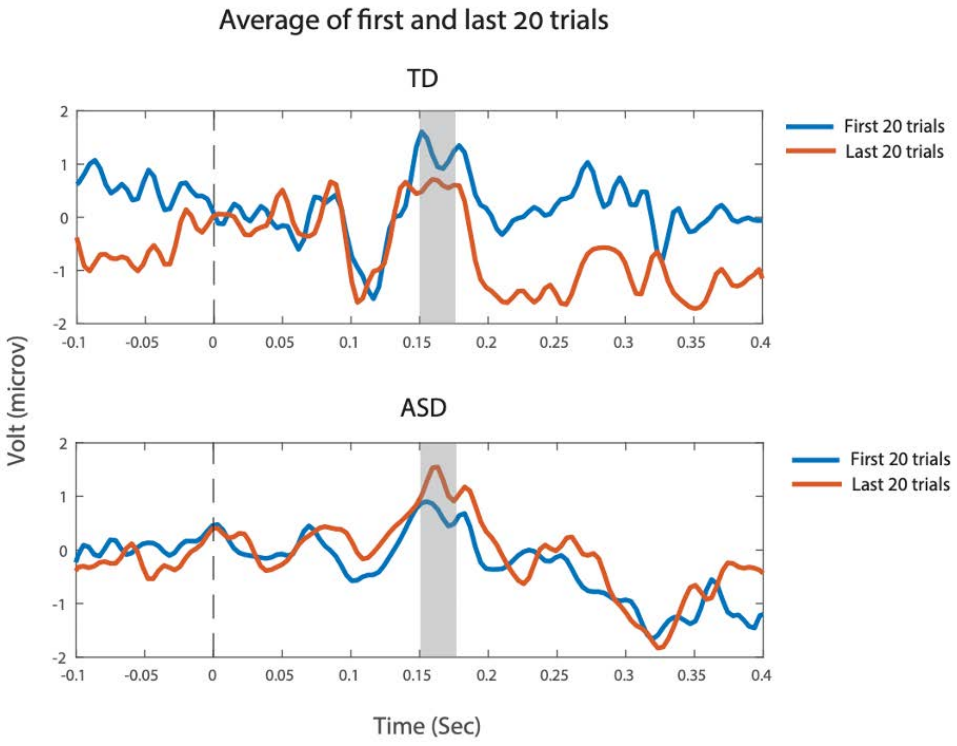
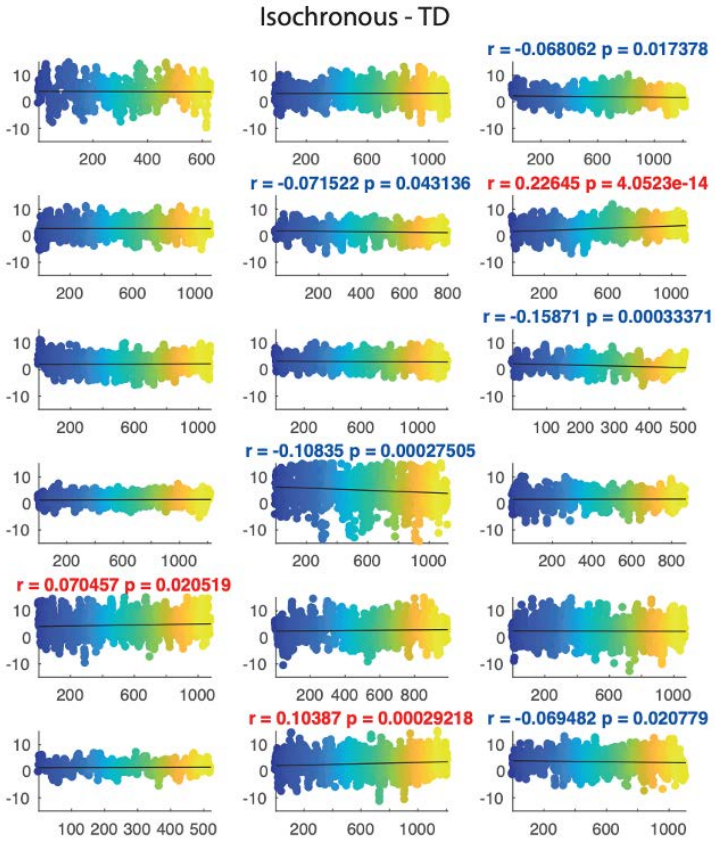


Figure 3: Assessing Trends in Habituation using EEG measurements from the First 20 vs Last 20 Trials For TD (above) and ASD (below) groups. EEG channels utilized for creation of the figures are C1, C2, Cz. Volts, a measure of brain electrical activity is measured on the y axis over time in seconds measured on the x-axis. The blue line represents the first 20 trials and the orange line represents the last 20 trials. The gray rectangle signifies the peak of interest.

The next point of consideration is the voltage trends of each individual participant across all of their isochronous trials. If habituation was observed, the r value, representing the slope, would be negative, meaning voltage went down for that participant across repeated trials. P values less than .05 were considered significant and labeled in the figure. Significant negative slopes are labeled in blue, while statistically significant positive slopes are labeled in red. The hope was to observe positive slopes for the ASD participants and negative slopes for the TD participants. However, as seen in figure 4, only five out of seventeen ASD participants exhibited a positive slope of statistically significance and three out of seventeen actually had a negative slope. With regard to the TD group, only four out of eighteen had a negative voltage slope while three out of eighteen participants had positive slopes. The various other individuals had voltage slopes around zero, revealing nothing about the experimental groups. The desired trend was seen slightly but not to the extent needed.



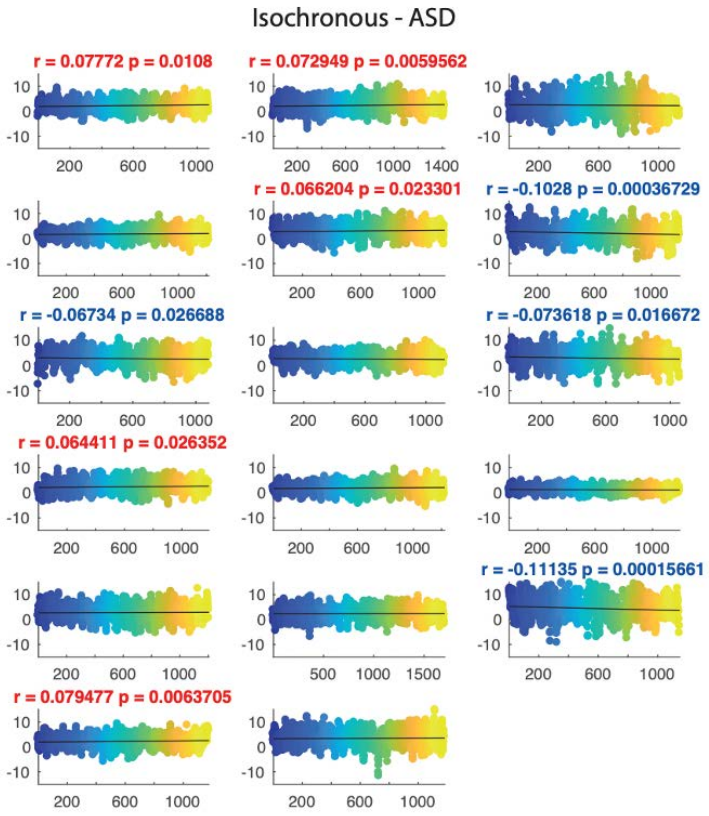


Figure 4: Slopes of P2 peaks from EEG measurements provided during all Isochronous trials in both ASD (left) and TD (right). Volts, a measure of brain electrical activity is measured on the y axis over time in seconds measured on the x-axis. Significance was measured with p value ≤0.05. Significant r and p values are indicated by red (positive slope) and blue (negative slope)

When considering the transformed R scores from all the isochronous trials, figure 5 shows that the median R score between the two experimental groups are pretty similar. Interestingly, the TD group contained the most positive R score, which is the opposite from the expected results. When a permutation test with n=10,000 iterations on the above data was run, the p value equaled 0.9, indicating that there is no significant difference between the two groups' R scores.

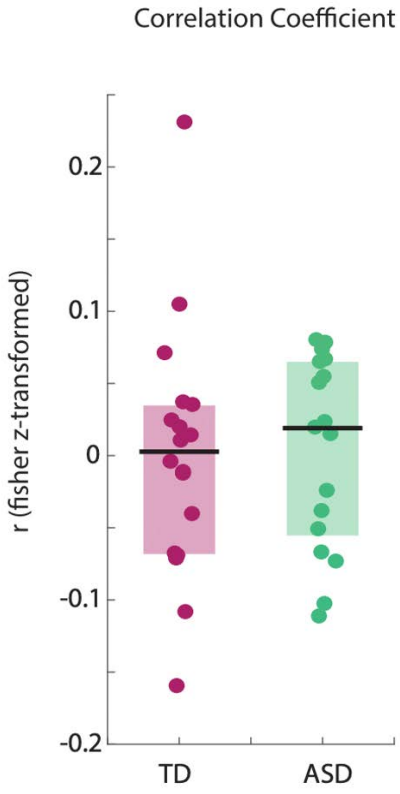


Figure 5: Transformed R-scores from all isochronous trials measured in TD vs ASD. Transformed R scores are plotted on the Y axis. The Pink box and dots represent the TD group values and the green box and dots represent the TD group values.

In conclusion, when analyzing the neuronal data from all the isochronous trials across all participants was analyzed, the hypothesis stating that ASD participants have reduced sensory habituation when compared to TD individuals was not verifiable.

Instead of stopping there when the results seemed discouraging, the first sixty trials of the isochronous condition were analyzed. Just as when all isochronous trials were analyzed, the goal in analyzing the first 60 trials was to observe negative r values for the TD group and positive R values for the ASD group. This would signify habituation in TD young adults and a lack thereof in the ASD group. When analyzing just sixty trials, many of the TD participants, eight out of eighteen, exhibited negative voltage slopes of statistical significance (figure 6). Many of the other TD participants demonstrated negative slopes that were not statistically significant, but appeared negative to the naked eye. This result confirms that there was habituation for the TD group within this first block of trials. This is in direct contrast to the ASD group. Six out of seventeen participants exhibited a statistically significant voltage slope while many other participants had positive slopes that were not statistically significant but appeared positive on the graph.This result confirms that were was not habituation for the ASD group within the first block of trials Although these results only account for about half of the participants in the experimental groups respectively, the overall trend is exactly as had been expected.

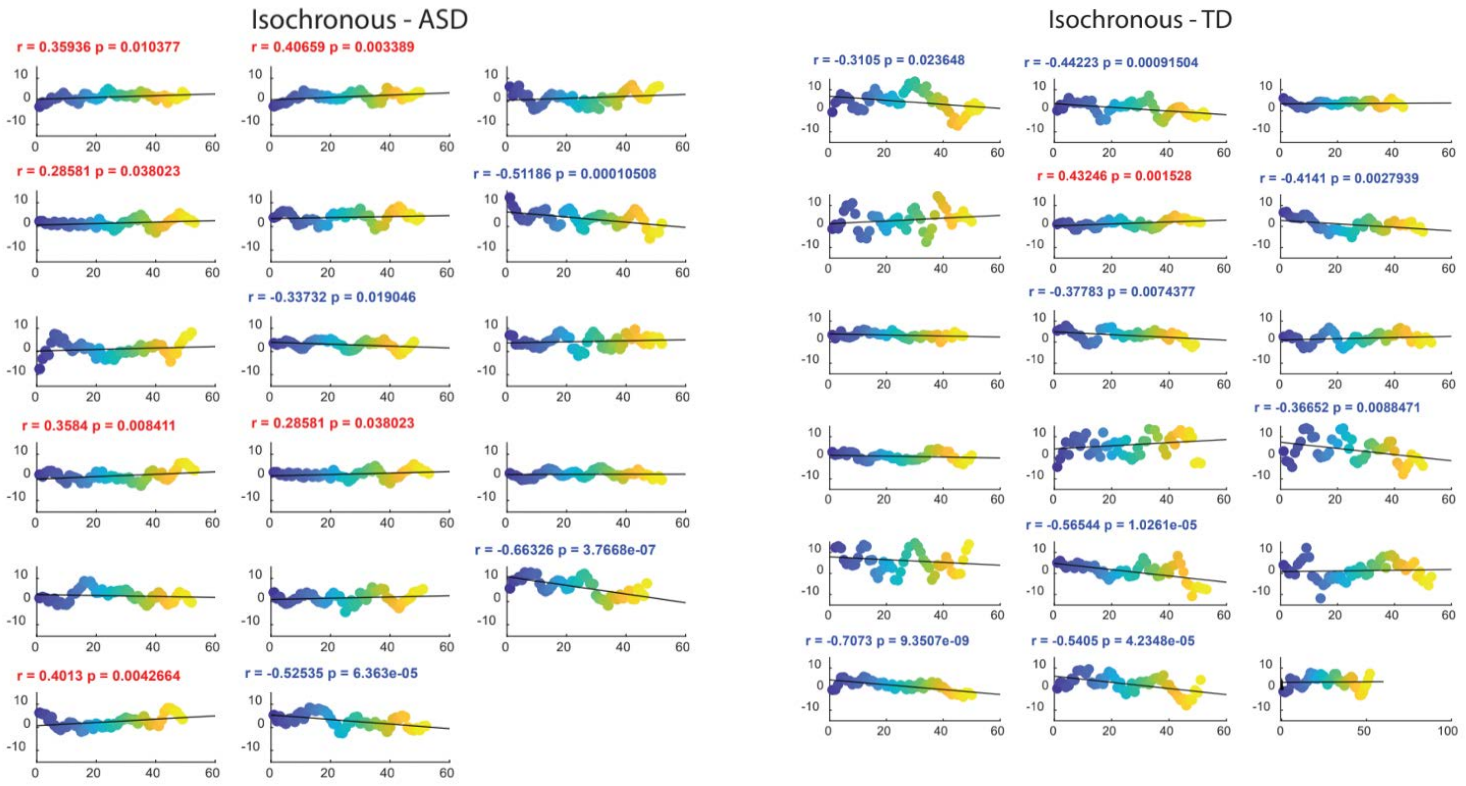


Figure 6: Slopes of P2 peaks from EEG measurements provided during the first 60 Isochronous trials in both ASD (left) and TD (right). Volts, a measure of brain electrical activity is measured on the y axis over time in seconds measured on the x-axis. Significance was measured with p value ≤0.05. Significant r and p values are indicated by red (positive slope) and blue (negative slope).

Figure 7 shows that the median transformed R scores between the two experimental groups are no longer similar. For the TD group, the median R score is negative, while for the ASD group the R score is slightly positive. When the permutation test was run on this data with n=10,000 iterations there is a marginally significant effect. With p=0.05, the resulting p value obtained was 0.051.

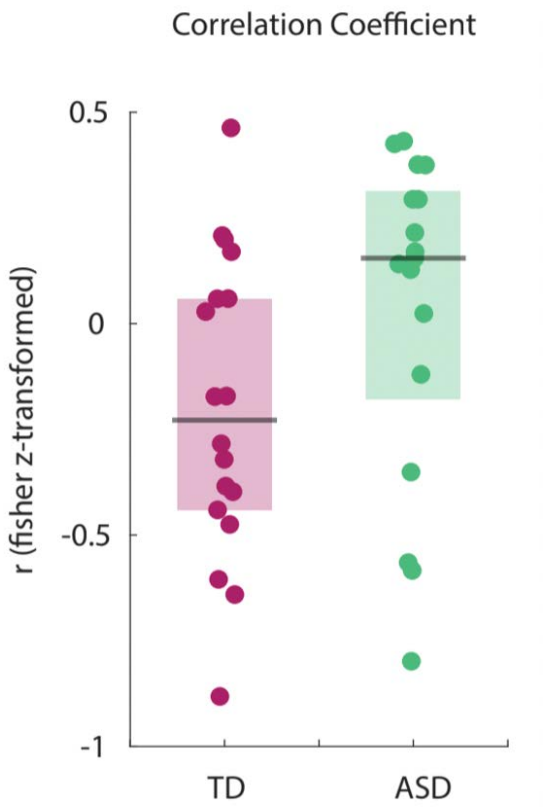


Figure 7: Transformed R-scores from the first 60 isochronous trials measured in TD vs ASD. Transformed R scores are plotted on the Y axis. The Pink box and dots represent the TD group values and the green box and dots represent the TD group values.

With this barely significant finding, it is difficult to confidently assert that my hypothesis was correct, but it lends some support to the hypothesis that young adults with ASD have reduced sensory habituation patterns when compared with TD young adults. It was evident when looking at the first 60 trials that the ASD group had increasing voltage across the trials and therefore did not habituate as surely as the TD group did.

Lastly, a correlation matrix was constructed utilizing the entrainment study EEG data and the cognitive test scores across participants from the TD and ASD groups respectively. The data in

the matrix again made use of the p2 peak voltage values including the R of p2 maximums and the value of the maximum voltage of the P2 peak across the participants. The other items in the matrix include the cognitive tests scores collected across participants including FSIQ, VIQ, PIQ, RBSR, SRS-2 total score, and SRS-2 Restrictive interests and Repetitive Behaviors score. Figure 8 was produced using matlab by uploading all the cognitive and neuronal data from 18 TD participants and then created a matrix. A correlation matrix was created which shows the correlation between two variables in all possible combinations. The colors of each of the boxes corresponds to the r value. The closer to 1 a slope is, the stronger the correlation between the two items. The lighter blue color means that the slope was closer to one. P scores were then added. A p score below 0.05 was considered significant. There were multiple correlations with a p score in this range including 6 and 8, 7 and 8, 6 and 7 and a few more. The last step in completing this matrix was to run a correction for multiple comparisons. Some of the correlations that had p values in the correct range did not survive the threshold. Those that did survive this correction were plotted with a white dot. The correlations that survived were 4 and 5 which translates to FSIQ and VIQ and 7 and 8 which translates to SRS-total and the subscore of Restrictive interests and Repetitive Behaviors . This result confirmed that these cognitive tests are designed properly in that their subscores correlate well with their overall test. There was no correlation between the neuronal data and the cognitive test scores.

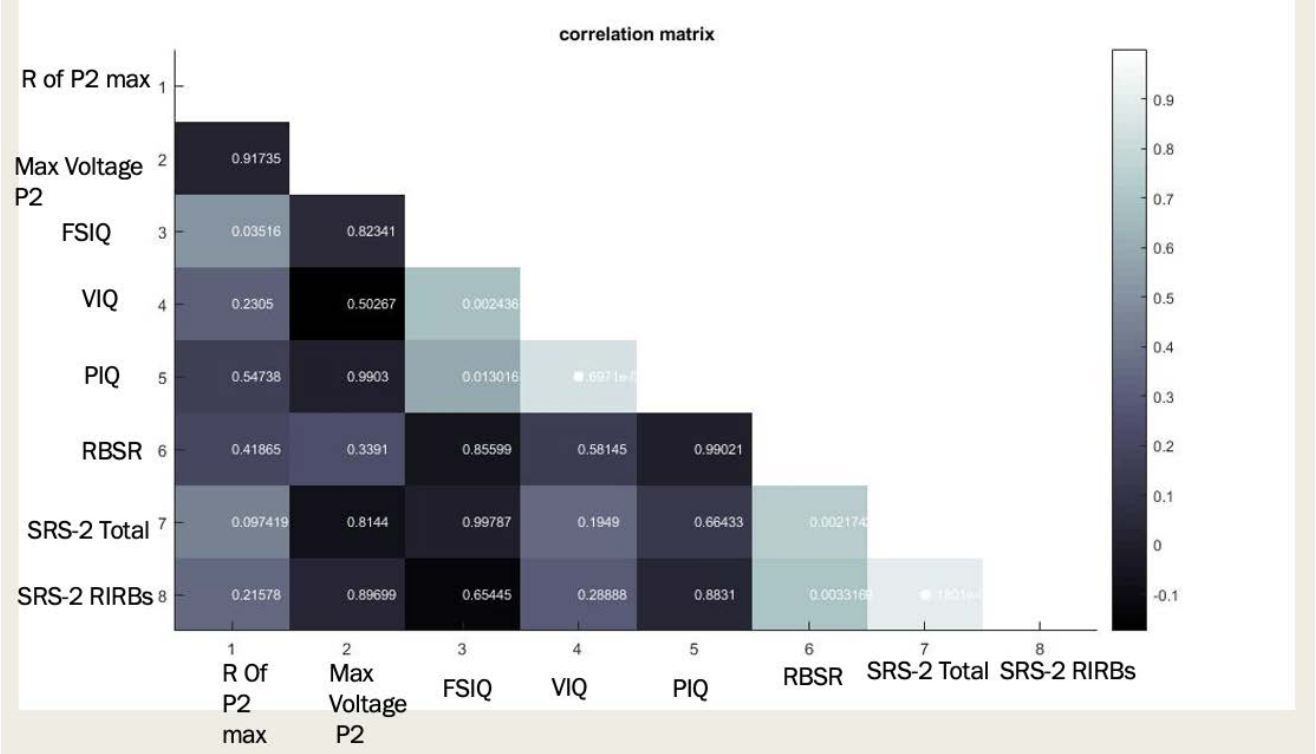


Figure 8: Correlation Matrix relating neuronal data from the entrainment study to various cognitive tests' results for TD experimental group. R values are pictured in the boxes of the matrix. Significance was measured with p value ≤0.05. White dot indicates values that survived a correction for multiple comparisons.

The same code was followed in creating a matrix for the ASD study group and figure 9 was produced. There are two important distinctions between the TD and ASD matrices. First, item three in the ASD matrix are ADOS scores which were not included for the TD sample since they did not have ADOS administered. Second, there is an additional item of consideration, the vineland test. This was excluded from the TD group since only four TD participants had the vineland administered to them. In direct comparison, there were 11 out of 17 ASD participants with vinland scores so this was a valuable measurement to include. Items 1 and 2 are the same as they were for TD and items 4-9 correspond to items 3-8 for TD respectively. Here too there are correlations with a p score below 0.05, however they did not end up with the white dot since they

did not survive the correction for multiple comparisons. Those that did survive include items 4 and 5 which translate to FSIQ and VIQ, items 5 and 6 which translate to FSIQ and PIQ, and items 8 and 9 which translate to SRS total and Restrictive interests and Repetitive Behaviors, just as with the TD group . This again, makes sense that the subscores within a test would correlate well with the overall exam. Here too, the EEG data does not correlate with any of the clinical measurements. In fact, there is one correlation between neuronal and cognitive scores that had a p-score that was less than 0.05 and that is the correlation between the vineland and the maximum voltage of the P2 peak, but this data set did not survive all of the statistical analyses performed. Unfortunately, again, I did not find any statistically significant correlation between the neuronal data and the cognitive test scores.

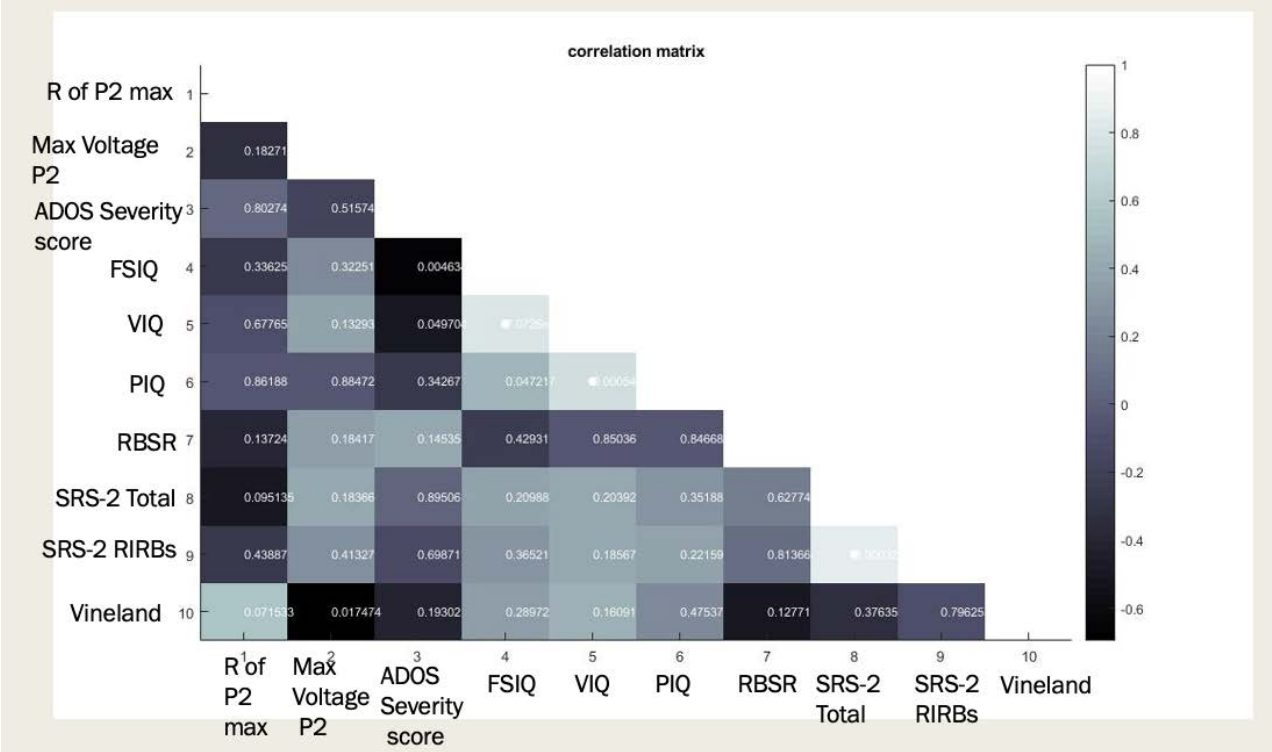


Figure 9: Correlation Matrix relating neuronal data from the entrainment study to various cognitive tests' results for ASD experimental group. R values are pictured in the boxes of the matrix. Significance was measured with p value ≤0.05. White dot indicates values that survived a correction for multiple comparisons.

Discussion

The goal of this study was to determine whether individuals with ASD habituate differently to sensory stimuli when compared to TD participants. Based on the neuronal data for the first 60, there was some evidence to say that our hypothesis was correct, however, this was not the case across all the trials. Even when analyzing the first sixty trials, the significance was minimal. Using this neuronal data and the cognitive test scores a correlation matrix was utilized to determine whether there is a correlation between the cognitive test scores and EEG results. For the ASD experimental group, there is one correlation between neuronal and cognitive scores that had a p-score that was less than 0.05 and that is the correlation between the vineland and the maximum voltage of the P2 peak (Figure 9). It is evident by the negative r value, indicated by the dark blue color in that area, that there is an inverse relationship between the two scores, meaning the higher the vineland score the lower the voltage of the p2 peak. This makes sense since a higher vineland score indicates an individual who is more independent, and the lower voltage indicates habituation. Although this data point did not pass all statistical tests, there is a trend indicating that individuals with ASD who are able to achieve greater levels of independence habituate to sensory stimuli more effectively than those with lower levels of self-sufficiency. There may be many contributing factors as to why the significance level found in terms of difference in habituation was so slight. One possibility is that the stimuli presented to the participating individuals were distinct and often unpredictable. The isochronous condition was

the only condition analyzed, however, since the isochronous condition was one of six conditions presented, this may have interfered with the participants brain response to the highly predictable stimulus. If only predictable stimuli were presented to participants, the results may have been more pronounced. Moreover, the isochronous condition was not always presented in the beginning of the trial blocks, so the resulting data may not be as clean as it should be, as the participants' responses may be skewed from previously observed conditions. Lastly, this analysis is only based on sixty trials. For more significant results, we would modify the entrainment to have a more predictable stimulus and many more trials.

The goal in finding a correlation between the neuronal data and the EEG data was to assert the idea that the degree of habituation impairment may be useful as one of many factors that will help participants with ASD gain the proper services they need. Additionally, the collection of neuronal data measuring the response to sensory stimuli may aid in the early diagnosis of ASD, but that is using the assumption that reduced habituation is present in all cases of autism. Additionally, reduced rates in habituation for ASD may confirm the theory that people with ASD have difficulty making temporal predictions. Since predictability is very connected to habituation, this is also something to consider when discovering findings of this nature. Lastly, reduction in habituation can explain both the hyposensitivity and hypersensitivity found in many cases of ASD. For a NT developing individual certain stimuli lose their appeal as they are repeated again and again due to habituation. This is not the case for an ASD individual. Since they do not habituate to the same extent, they are hyper focused on this stimulus for lengthy periods of time. This is hypersensitivity. The focus on this specific stimulus, however, makes it difficult for the individual to disengage and attend to a different stimulus presenting as hyposensitivity to the other stimuli around them. All of these theories need further studies to

confirm them, but these are groundbreaking findings for the diagnosis and increasingly specialized treatment of individuals with ASD.

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