Electrophysiological Marker of a Potential Excitatory/Inhibitory Imbalance in Children with Autism Spectrum Disorder

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ABSTRACT

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Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder characterized by impairments in social interaction and the presence of stereotypic behaviors or restricted interests. To explore possible consequences of an excitatory/inhibitory (E/I) imbalance on the visual system in ASD, we investigated spatial suppression in 16 children with ASD and 16 neurotypical comparison children from 6 - 12 years of age using a visual motion processing task during high-density electroencephalography (EEG) recording in order to derive the N1 event related potential (ERP). Consistent with prior behavioral research, neurotypical participants displayed spatial suppression in conditions of large, high-contrast sinusoidal gratings as indexed by delayed N1 response latency. As predicted, children with ASD displayed weakened surround suppression, i.e. shorter N1 response latency to large, high-contrast sinusoidal gratings. However, this study also unexpectedly revealed that children with ASD showed longer N1 latencies in response to small, high-contrast sinusoidal gratings as compared to neurotypical control children. Although there were no statistically significant differences between children with ASD and NT children for N1 peak amplitude, there was a strong negative correlation between N1 amplitude represented in absolute values for large, high-contrast sinusoidal gratings and hyper-responsiveness item mean scores on the Sensory Experiences Questionnaire for children with ASD, but not for NT children. As predicted, no significant differences were found within or between groups in the low-contrast experiment. Our results are indicative of weakened spatial suppression and deficits in contrast gain in children with ASD, suggestive of an underlying E/I imbalance in ASD.
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1. INTRODUCTION

Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by impairments in social interaction, communication, and the presence of stereotypic behaviors or restricted interests (DSM-V; American Psychiatric Association, 2013). Based on their 2010 surveillance study, the Centers for Disease Control and Prevention (CDC) estimate that 1 in 68 children has autism, more than a 30% increase in prevalence since the 2008 surveillance study (Centers for Disease Control and Prevention, 2014). ASD occurs across all racial, ethnic, and socioeconomic groups (Centers for Disease Control and Prevention, 2014). The increase in ASD diagnoses is likely due to heightened awareness, more effective diagnosis and classification, and increased incidence rates. ASD is typically diagnosed between 2 and 3 years of age, with a disproportionate male-to-female ratio of 5:1 (Centers for Disease Control and Prevention, 2014). Presently, the factors leading to ASD are unknown, with no specific biological, genetic, or environmental markers known to be common to all disorders that encompass the spectrum.

Despite the apparent rise in prevalence over the last decade and the associated public health significance, the underlying pathophysiology of ASD is not well understood, nor are there many evidence-based treatment options. Because of the implications and social effects of the behavioral and social difficulties experienced by children with ASD, it is important that research is directed at enhancing our understanding of the etiology and mechanisms that give rise to these symptoms. One of the diagnostic criteria set forth by the Diagnostic and Statistical Manual of Mental Disorders (DSM-V; American Psychiatric Association, 2013) includes the presence of hyper- or hypo-reactivity to sensory input(s), or unusual interests in sensory input(s). This may include a heightened or attenuated response to temperature, auditory input, texture, smell, visual input, or moving objects.
Recent findings suggest that abnormalities in sensory processing in ASD may be due to aberrations in glutamate (Glu) and \( \gamma \)-aminobutyric acid (GABA) neurotransmission, a proposal known as the excitatory/inhibitory (E/I) theory of ASD (Hussman, 2001; Evers & Hollander, 2008). Visual processing deficits are perhaps the most widely researched sensory issue in ASD, with abnormalities in multiple domains having been documented. Visual processing deficits in ASD include (but are not limited to): deficits in low-level visual processing; abnormal perceptual integration; holistic processing deficits; reduced sensitivity to biological motion; enhanced perception of fine details; impairments in object-boundary detection; and deficits in facial processing (for a review, see Simmons et al., 2009). Since a balanced interaction between inhibitory GABA and excitatory Glu is imperative for the regulation of emotion, learning, cognitive processes, and memory, this theory posits that an imbalance between insufficient GABA and abundant glutamate synaptic transmission results in hyperexcitation (excitotoxicity), resulting in sensory abnormalities in individuals with ASD (Hussman, 2001; Evers & Hollander, 2008). The current study seeks to further examine the E/I theory of ASD by investigating an electrophysiological marker of spatial suppression during a visual task, probing GABAergic neural inhibition through the implementation of event-related potential (ERP) methodology. The results of this study may contribute to the understanding of pathophysiology of sensory issues in ASD, as well as provide potential groundwork for evidence-based treatments specific to GABA and/or Glu dysregulation.

This dissertation is organized as follows: Chapter 2 provides an introduction to \( \gamma \)-Aminobutyric acid and glutamate neurotransmission and the E/I theory of autism spectrum disorder. It also reviews relevant evidence from post-mortem studies, magnetic resonance spectroscopy studies, and blood plasma studies pertinent to comprehending a potential imbalance
between excitation and inhibition in ASD. Chapter 3 outlines experimental questions and expected outcomes of this study. Chapter 4 discusses the event-related potential (ERP) technique that was implemented in this study, along with study methods. Chapter 5 describes the equipment and electroencephalography (EEG) recording parameters, along with a detailed study protocol. Chapter 6 discusses data processing procedures and data analysis. Chapter 7 contains the results and discussion of study findings. Finally, Chapter 8 presents study limitations, and Chapter 9 discusses conclusions and future directions.
2. BACKGROUND

Multiple theoretical frameworks have been developed in the attempt to elucidate the etiology of ASD, but there is still no single theoretical approach that has been widely accepted by the scientific community, and no theory that can account for all the symptoms experienced by those with an ASD. Due to the heterogeneous nature of ASD, it is unlikely that any single theory, environmental factor, biological marker, genetic marker, or any unitary phenomenon will account for the pathophysiology of ASD in its entirety. The E/I theory of autism spectrum disorder posits that sensory abnormalities, a core feature of ASD, are due to aberrations in glutamate (Glu) and γ-aminobutyric acid (GABA) neurotransmission, leading to excitotoxicity (Hussman, 2001; Evers & Hollander, 2008). Excitotoxicity is a neurotoxic process resulting in apoptosis and/or damage to neurons due to overstimulation via glutamate or similar excitotoxic substances, discussed further below. Sensory symptoms are a core feature of autism, meaning they are universal in nature, unrelated to other core symptomatology, and not a manifestation of some other co-morbid disorder (Evers & Hollander, 2008). Therefore, the study of sensory features in autism is exigent to uncover the etiology of hypo- or hyper-reactivity to sensory inputs in ASD. This research line may provide meaningful information regarding the pathophysiology of sensory issues in ASD, provide potential groundwork for evidence-based treatments specific to insufficient GABA neurotransmission, and be implemented as a clinical outcome measure during clinical research studies of investigational medications that act on the GABAergic or glutamatergic pathways.
2.1 \( \gamma \)-Aminobutyric acid and Glutamate Neurotransmission

\( \gamma \)-Aminobutyric acid and glutamate are both prevalent neurotransmitters within the human central nervous system. There are four main criteria for classification as a neurotransmitter: (1) presynaptic localization, (2) release by physiological stimuli, (3) identical action with naturally occurring transmission, and (4) an existing mechanism for rapid termination of transmitter action (Fonnum, 1984).

2.1.1 Introduction to \( \gamma \)-Aminobutyric acid (GABA)

\( \gamma \)-Aminobutyric acid (C\(_4\)H\(_9\)NO\(_2\)), also known as GABA, is the most critical and widespread inhibitory neurotransmitter in the adult human central nervous system (CNS), with GABA neurotransmission occurring at 25 – 40% of all synapses (Li & Xu, 2008). GABA acts as an inhibitory neurotransmitter by inhibiting Ca\(^{2+}\) influx into the neuron, thereby weakening the excitability of the neuron (Li & Xu, 2008). GABA is a functional amino acid (non-protein) primarily biosynthesized from L-glutamic acid, whose reaction is catalyzed by glutamic acid decarboxylase (GAD). GAD has two main isoforms, GAD\(_{65}\) and GAD\(_{67}\), and requires pyridoxal-phosphate for action (Watanabe, Maemura, Kanbara, Tamayama, & Hayasaki, 2002; Binder, 2009). L-glutamate is synthesized from \( \alpha \)-ketoglutaric acid, a product of glucose metabolism, since glutamate and glutamine cannot cross the blood-brain barrier (BBB). \( \alpha \)-ketoglutaric acid, generated by the tricarboxylic acid (TCA) cycle (see Figure 1), is supplied through active transport to the CNS (Watanabe et al., 2002).
When a neuron is hyperpolarized, GABA is released from the axon terminal to the synapse and binds to the post-synaptic membrane (Li & Xu, 2008). The release of synaptic GABA is dependent on vesicular inhibitory amino acid transport, which loads GABA into synaptic vesicles (Coghlan et al., 2012). After the synaptic release of GABA, its actions are terminated primarily by glia through active reuptake, or reuptake into the pre-synaptic terminals via Na\textsuperscript{+}/Cl\textsuperscript{−} (sodium/chloride) dependent GABA transporters known as GAT1, GAT2, and GAT3 (Madsen, Larsson, & Schousboe, 2008). GABA transaminase (GABA-T) is responsible for degrading unbound GABA (Li & Xu, 2008). In areas of the brain that have high concentrations of GABA, the GABA shunt pathway is implicated in both GABA production and conservation rather than the TCA cycle. In brief, the GABA shunt pathway involves a closed loop to mediate
catabolism of GABA and channel L-glutamate to the TCA cycle, bypassing the first two steps of the TCA cycle (Watanabe et al., 2002).

**2.1.1.1 γ-Aminobutyric acid (GABA) receptors.** There are two classes of GABA receptors, known as GABA_A and GABA_B.¹ GABA_A receptors are ligand gated (ionotropic) receptors² that mediate the fast inhibitory action of GABA, while GABA_B receptors are G protein-coupled (metabotropic) receptors³ that mediate the long term inhibitory activity of GABA (Bowery, Hill, & Hudson, 1983; Bormann, 1988). GABA_A and GABA_B receptors are pharmacologically, electrophysiologically, and biochemically distinct from one another (Olsen & DeLorey, 1999).

**2.1.1.2 GABA_A receptors.** GABA_A receptors are the most common receptors in GABAergic neurotransmission, consisting of four subunits, and one subfamily – GABA_A-ρ (Olsen & Sieghart, 2008), each with a unique expression in the brain (Laurie, Seeburg, & Wisden, 1992). To date, the majority of studies specify that six α subunit isoforms (GABRA1 – GABRA6), three β subunit isoforms (GABRB1 – GABRB3), three γ subunit isoforms (GABRG1 – GABRG3), and one δ subunit isoform (GABRD) have been identified in the human brain (Johnson et al., 1992; Wingrove et al., 1992; McLean, Fard, & Russek, 1995), with the most abundant combination being the α₁ β₂ γ₂ subunit (Bormann, 2000; Sarto-Jackson & Sieghart, 2008). Due to the multitude of potential permutations of subunits (and subclasses),

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¹ Since GABA_C’s structure, function, and sequence are so closely related to GABA_A, GABA_C has been deemed a subfamily of GABA_A by the International Union of Basic and Clinical Pharmacology, now known as GABA_A-ρ (Olsen & Sieghart, 2008).

² A ligand-gated ion channel is a group of proteins that allow for ions such as K⁺, Na⁺, Ca²⁺, or Cl⁻ to pass through the membrane via the binding of a ligand, i.e. a chemical messenger, in this case a neurotransmitter.

³ A G protein-coupled receptor is a group of proteins that activate signal transduction pathways and cellular responses primarily via the cAMP signal pathway or the phosphatidylinositol signal pathway.
there is a wide variety of receptor diversity resulting in receptors with highly specific properties (Coghlan et al., 2012).

GABA<sub>A</sub> receptors, which may have five or more binding sites, allow the flow of chloride ions (Cl<sup>−</sup>) into the neuron by opening the Cl<sup>−</sup> channel when bound to GABA, thus hyperpolarizing the neuronal membrane (Bowery, Hill, & Hudson, 1983; Watanabe et al., 2002). Membrane hyperpolarization results in neuronal inhibition due to the increase in firing threshold (Olsen & DeLorey, 1999). By this action, GABA<sub>A</sub> makes the neuron less reactive to excitatory neurotransmitters (Bormann, 1988; Hevers & Lüddens, 1998). However, in some cases GABA<sub>A</sub> receptors can mediate GABA as an excitatory neurotransmitter if the potential of chloride ions inside the neuron is greater than in the extracellular environment, pushing Cl<sup>−</sup> ions out of the neuron (Sieghart, 1995). In this case, depolarization of the membrane potential occurs (Sieghart, 1995). GABA<sub>A</sub> receptors are imperative for regulation of neuronal excitability and signal transduction. Additionally, GABA<sub>A</sub> receptors contain binding sites for a variety of clinically relevant drugs such as the positive allosteric modulators benzodiazepines, barbiturates, neuroactive steroids, and anesthetics, in addition to the non-competitive channel blockers known as picrotoxins (Sarto-Jackson & Sieghart, 2008). Each of these drugs acts on GABA<sub>A</sub> differently. For example, benzodiazepines enhance the inhibitory action of GABA via the GABA<sub>A</sub> <em>α</em> subunit receptor, which is clinically relevant for its anti-anxiety, anti-epileptic, sleep-inducing, and muscle relaxant properties (Page, Michael, Sutter, Walker, & Hoffman, 2002), while barbiturates bind to the GABA<sub>A</sub> <em>β</em> subunit receptor, acting as a central nervous system depressant resulting in sedation or anesthesia (Sarto-Jackson & Sieghart, 2008). Additionally, other drugs such as ethanol and penicillin have been shown to have an effect on GABA<sub>A</sub> receptors (Harris, Mihic, Dildy-Mayfield, & Machu, 1995).
2.1.1.3 \textit{GABA}_B receptors. GABA\textsubscript{B} receptors have lower concentrations in the CNS than GABA\textsubscript{A} receptors, and bind with very few pharmacological agents; therefore, less information is known about their structure and function (Li & Xu, 2008). Presently, two GABA\textsubscript{B} receptor subunits have been identified: GABA\textsubscript{B} R1 and GABA\textsubscript{B} R2 (Isomoto et al., 1998). GABA\textsubscript{B} receptors are coupled indirectly to potassium ion (K\textsuperscript{+}) channels, allowing for decreases in calcium ion (Ca\textsuperscript{2+}) conductance and inhibition of cAMP production mediated by G-proteins (Olsen and DeLorey, 1999). Although GABA\textsubscript{B} receptors can mediate pre- and post-synaptic inhibition via a decrease in Ca\textsuperscript{2+} influx, they are always inhibitory (Hevers & Lüddens, 1998; Olsen & DeLorey, 1999). Presently researchers do not fully comprehend GABA\textsubscript{B} receptor capabilities in the human brain, but it is known that GABA\textsubscript{B} receptors are crucial for long-term inhibition of synaptic transmission via regulating the release of glutamate, noradreneline, dopamine, substance-p, and other critical neurotransmitters (Bowery, 1989). In the human brain GABA\textsubscript{B} receptor distribution is less widespread than GABA\textsubscript{A} receptor distribution, and due to this narrow distribution there are only a few clinically relevant drugs known to act on GABA\textsubscript{B} receptors; one such is Baclofen, a GABA analogue, which is an agonist that can be used as a muscle relaxant (Mezler, Müller, & Raming, 2001).

2.1.1.4 \textit{GABA and neurodevelopment}. Although in the healthy adult brain GABA is the primary inhibitory neurotransmitter, during neurodevelopment GABA can also act as an excitatory neurotransmitter (Sibilla & Ballerini, 2009). Research in this domain has shown that in early murine CNS development, GABA acts as an excitatory neurotransmitter by raising the intracellular Ca\textsuperscript{2+} levels through GABA\textsubscript{A} receptors (Li & Xu, 2008). In rodent models, GABA\textsubscript{A} \( \alpha_1 \) receptors have been shown to play a critical role in defining the critical period for neuroplasticity of the visual cortex (Fagiolini et al., 2004).
2.1.2 Introduction to Glutamic acid (Glu)

Glutamic acid (C$_5$H$_9$NO$_4$), also known as Glu, is the most crucial excitatory neurotransmitter in the human CNS, with widespread Glu concentrations evenly distributed throughout the brain (Engelsen, 1986; Broman, Hassel, Rinvik, & Ottersen, 2000). Neuronal Glu is biosynthesized from glucose, a Glu precursor that enters the brain via the blood brain barrier (BBB), whose transport is mediated via GLUT1. Approximately 85% of serum glucose that crosses the BBB is metabolized by astrocytes via glycolysis into lactate, which can then occupy the extracellular fluid to later be taken up by neurons (Tsacopoulos & Magistretti, 1996). Lactate is converted to pyruvate to form citrate via acetyl-coA. Citrate is then converted to $\alpha$-ketoglutarate, which then forms Glu (see Figure 1, TCA cycle). Since all neurons contain Glu as a by-product of energy metabolism, Glu is widely dispersed in the vesicles of glutamatergic terminals, in GABAergic neurons since Glu serves as a precursor to GABA, in cells for metabolic processes, and in the glia serving as a precursor to glutamine (Engelsen, 1986; Broman, Hassel, Rinvik, & Ottersen, 2000). Therefore, a neuron can only be defined as glutamatergic if there is Glu in the synaptic vesicles, which can be determined via immunoprecipitation. Lund-Karlsen and Fonnum (1978) found that 20 to 30% of Glu in the brain is synthesized and utilized for glutamatergic neurotransmission through vesicular uptake. Although neuronal Glu is primarily formed from glucose, transmitter Glu is primarily formed from glutamine. Approximately 60% of $\alpha$-ketoglutarate formed via astrocytes is converted to Glu, and eventually to glutamine (known as the astrocytic TCA cycle) or to malate, which is then converted to pyruvate (Hassel, 2001). However, since the astrocytic TCA cycle results in a loss of $\alpha$-ketoglutarate, thereby impairing ATP production, neurons must additionally carboxylize pyruvate so they are not completely dependent on glutamine as a precursor for Glu.
neurotransmission (Broman, Hassel, Rinvik, & Ottersen, 2000). During Glu neurotransmission, the Glu synthesized in nerve terminals enters the synaptic vesicles through vesicular glutamate transporter 1 (VGLUT1) mediated by an electrochemical gradient via an ATP dependent proton pump stimulated by chloride (Thompson et al., 2005). After synaptic release of Glu, its actions are terminated primarily by astrocytes and amidated to glutamine or metabolized via the TCA cycle, meaning that approximately 50 – 80% of brain glutamine is formed from neurotransmitter Glu (Broman, Hassel, Rinvik, & Ottersen, 2000).

2.1.2.1 Glutamate receptors

There are two classes of Glu receptors, known as ionotropic and metabotropic receptors (Gereau & Swanson, 2008). Ionotropic glutamate receptors consist of ligand-gated ion channels that permit the flow of Na$^{+}$, K$^{+}$, and occasionally Ca$^{2+}$ in direct response to glutamate binding (Gereau & Swanson, 2008). During binding, ion flow results in an excitatory post-synaptic current, which is depolarizing, resulting in an action potential in the post-synaptic neuron (Gereau & Swanson, 2008). Metabotropic glutamate receptors consist of seven transmembrane-domain proteins that couple to intracellular signaling pathways through GTP-binding proteins (Riedel, Platt, & Micheau, 2002). These classes of receptors are pharmacologically, electrophysiologically, and biochemically distinct from one another.

**Ionotropic Receptors**

2.1.2.2 **AMPA Receptors:** The amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor is an ionotropic glutamate receptor that mediates fast neurotransmission in the CNS. AMPA receptors are distributed evenly throughout the nervous system as one of the most common receptor types, although some studies have reported regional increases in the hippocampus (CA1 & CA2) and pyramidal cell layer (Ozawa, Kamiya, & Tsuzuki, 1998).
AMPAs exist in all neuronal types as well as in glial cells (Ashby, Daw, & Isaac, 2008). AMPA receptors consist of four subunits known as GluR1, GluR2, GluR3, and GluR4. GluR2 is the most common monomer to form a dimer with other subunits. Due to this, GluR2 is essential and its absence can drastically change AMPA signaling, altering neurotransmission (Derkach, Oh, Guire, & Soderling, 2007). AMPA receptors are crucial for neurotransmission at post-synaptic membranes, and play a critical role in long-term potentiation, which is imperative for synaptic plasticity (Cook & Bliss, 2006). AMPA receptors are additionally associated with facilitating cognitive functions such as memory and learning (Derkach, Oh, Guire, & Soderling, 2007). Notably, barbiturates act as antagonists on AMPA Glu receptors while kainate acts as a potent agonist (Riedel, Platt, & Micheau, 2003).

2.1.2.3 **Kainate Receptors:** Kainate receptors are ionotropic glutamate receptors that are implicated in excitatory neurotransmission pre-synaptically and inhibitory neurotransmission post-synaptically (Ozawa, Kamiya, & Tsuzuki, 1998). Similar to AMPA receptors, kainate receptors can be found throughout the entire CNS, but with increased expression in the CA3 region of the hippocampus and granular layer of the cerebellum (Ozawa, Kamiya, & Tsuzuki, 1998). There are five subunits of kainate receptors: GluR5, GluR6, GluR7, KA1, and KA2. Activation of GluR5 is associated with seizures, which are known to occur at a greater incidence rate in autism (Fritsch, Reis, Gasior, Kaminski, & Rogawski, 2014). Also akin to AMPA receptors, kainate receptors form a ligand-gated ion channel permeable to K⁺ and sodium (Na⁺) ions (Huettner, 2003).

2.1.2.4 **NMDA Receptors:** The N-methyl-D-aspartate (NMDA) receptor is an ionotropic glutamate receptor that is primarily implicated in neuroplasticity, learning, and memory (Broman, Hassel, Rinvik, & Ottersen, 2000). Unlike AMPA and Kainate receptors, NMDA receptors
require both glutamate and glycine agonists for activation (Petralia & Wenthold, 1992), and are both voltage-dependent as well as ligand-gated (Kleckner & Dingledine, 1988). NMDA receptors have two subunits, GluN1 and GluN2, each with many isoforms (Petralia & Wenthold, 2008). When an NMDA receptor is activated, an ion channel opens and calcium rushes into the cell, which in turn acts as a second messenger affecting synaptic plasticity (Petralia & Wenthold, 2008). Compared to AMPA receptors, NMDA receptors have a sluggish excitatory postsynaptic potential (EPSP) since AMPA receptor depolarization is altered after Glu neurotransmission (Petralia & Wenthold, 2008). Aside from glutamate and glycine, notably aspartate, cycloserine, and D-serine act as agonists at NMDA receptors while some drugs with potential addictive qualities such as ketamine, methoxetamine, phencyclidine, ethanol, and nitrous oxide act as antagonists (Petralia & Wenthold, 2008).

**Metabotropic Receptors**

2.1.2.5 mGluR Receptors: Metabotropic glutamate receptors (mGluRs) are glutamate receptors that function through secondary messenger systems, such as signal transduction or G-proteins, rather than an ion channel pore. mGluRs are implicated in synaptic transmission and excitability in the human nervous system, and are imperative for learning, memory, and pain perception (Saugstad & Ingram, 2008). mGluRs are not as evenly distributed as ionotropic receptors and are primarily found in pre- and post-synaptic neurons of the cerebellum, hippocampus, peripheral tissue, and cerebral cortex (Chu & Hablitz, 2000). mGluRs are class-C G-protein coupled receptors that consist of eight transmembrane domains in three distinct families (Hampson, Rose, & Antflick, 2008). Group I consists of mGluR1 and mGluR2, Group II consists of mGluR2 and mGluR3, and Group III consists of mGluR4, mGluR5, mGluR6,
mGluR7, and mGluR8 (Swanson et al., 2005). Group I mGluRs are principally activated by quisqualic acid, an excitatory amino acid, while the main role of Group II and Group III is to prevent the formation of cAMP via activation of a G-protein (Hampson, Rose, & Antflick, 2008).

2.2 The Excitatory/Inhibitory Theory of Autism Spectrum Disorder

Although the etiology of ASD remains largely undetermined, a large body of research suggests that sensory abnormalities in ASD may be due to aberrations in glutamate (Glu) and γ-aminobutyric acid (GABA) neurotransmission; this proposal is known as the E/I theory of autism spectrum disorder. Since a balanced interaction between inhibitory GABA and excitatory Glu is imperative for the regulation of emotion, learning, cognitive processes, and memory, this theory posits that an imbalance between insufficient GABA signaling and abundant Glu signaling results in hyperexcitation (excitotoxicity), which in turn leads to the sensory processing issues in individuals with autism.

The term hyperexcitation, or excitotoxicity, was first described by Olney (1969) in a paper that discussed brain lesions and other neurological symptoms resulting from administering monosodium glutamate to mice. Excitotoxicity is a neurotoxic process resulting in apoptosis and/or damage to neurons due to overstimulation via Glu or similar excitotoxic substances, primarily to NMDA or AMPA receptors (Manev, Favaron, Guidotti, & Costa, 1989). This process generally occurs when glutamate receptors are overactivated by glutamatergic storm (Manev, Favaron, Guidotti, & Costa, 1989), leading to a disproportionate influx of Ca\(^{2+}\) into the cell (Evers & Hollander, 2008). An unwarranted influx of Ca\(^{2+}\) can activate enzymes that damage cellular structures, cellular membranes, cytoskeletal structure and critical genetic material, as well as impairing ATP production necessary for neurotransmission (Evers & Hollander, 2008). Excess Ca\(^{2+}\) can additionally contribute to oxidative stress, a disturbance in the intricate balance
between the production of free radicals and a system’s ability to repair the resulting damage (Betterridge, 2000). Group I mGluRs increase NMDA receptor activity and risk of excitotoxicity, while Group II and Group III receptors decrease NMDA receptor activity and risk of excitotoxicity (Hampson, Rose, & Antflick, 2008).

2.2.1 Excitotoxicity and Autism Spectrum Disorder. Excitotoxicity is implicated in a number of disorders such as Parkinson’s disease, Huntington’s disease, Alzheimer’s disease, amyotrophic lateral sclerosis, and autism spectrum disorder. However, the precise mechanisms by which hyperexcitation is implicated in ASD are not fully understood. Hussman (2001) proposed that the dysfunctions observed in ASD may be related to insufficient GABAergic inhibition, leading to excess stimulation of Glu neurons (glutamatergic storm) and a loss of sensory gating. GABAergic neurotransmission can be suppressed by GABA antagonists and receptor impairment (Hussman, 2001). A lack of inhibitory regulation via GABAergic signaling can result in neurotoxic hyperexcitation, damaging pyramidal and multipolar neurons (Hussman, 2001). Hussman’s hypothesis regarding GABA/Glu dysregulation in ASD is based primarily on two concepts: (1) GABA receptor pathologies have been cited to play a significant role in ASD, and, (2) Blocking GABAergic signaling via NMDA antagonists generates a pathology that appears similar to ASD (Hussman, 2001). Hussman even went so far as to state that suppressed GABA signaling is a common feature of the autistic brain (Hussman, 2001).

Rubenstein and Merzenich (2003) also theorized that some forms of ASD are caused by high levels of excitation from overactive Glu neurotransmission, or a disproportionately weak level of inhibition from GABA neurotransmission. The authors proposed that the imbalance between GABA and Glu neurotransmission that mediates sensory, mnemonic, social, and emotional systems may be due to a combination of genetic and/or environmental factors, but
additionally suggested that local circuitry and cellular components may be impacted. For example, minicolumns, vertical columns of functionally related Glu and GABA neurons in cortical layers, have been found to be smaller, have atypical structure, and are more abundant in individuals with autism (Rubenstein & Merzenich, 2003). The authors also suggest that hyperexcitation due to GABA/Glu imbalances increases the chance of developing epilepsy, which occurs in a high proportion (approximately 30%) of those with an ASD (Viscidi, 2013).

Belmonte et al. (2004) posited that the anatomical and functional differences in autism leading to altered neural processing/neural connectivity might potentially involve low signal-to-noise in neural assemblies. This may involve disinhibition of the cerebellar nuclei and overexcitation of the cerebral cortex and/or thalamus (Belmonte et al., 2004). Building upon this theory, Polleux and Lauder (2004) also suggest that an imbalance between GABA and Glu during the development and activation of cortical networks can result in functional deficits as well as a lack of synchronization in neuronal signaling. Individuals with autism often show disruptions in raw EEG recordings. Neuronal discharges measured via the gamma frequency band (20 – 80 Hz, with 40 Hz thought to be typical) are thought to reflect synchronized neuronal activity at both the cortical and sub-cortical levels (Gold, 1999). This activity is presumed to play a critical role in sensory perception and attention, and is dependent on GABA$_A$ receptor inhibition amongst interneurons (Polleux & Lauder, 2004). Since preliminary support for this notion, numerous studies have demonstrated gamma band abnormalities in ASD (Baruth et al., 2010; Gandal et al., 2010; Maxwell et al., 2013; Orekhova et al., 2008; Richard, Lajiness-O’Neill, & Bowyer, 2013; Rojas & Wilson, 2014; Sun et al., 2012). Polleux and Lauder (2004) suggest that this disruption between excitation and inhibition could result in drastic consequences for the networks that underlie sensory perception and attention.
2.2.1.1 Evidence from post-mortem studies. Blatt et al. (2001) were the first to find an abnormality in the human GABA system in post-mortem tissue from adults with ASD. In a ligand binding study (radioimmunoassay) they demonstrated that H-muscimol labeled GABA$_A$ receptors and H-flunitrazepam labeled benzodiazepine binding sites were reduced in high binding regions of the hippocampus, which could account for decreased neural inhibition (Blatt et al., 2001). A follow-up study set out to determine whether the reduction of GABA$_A$ receptors and reduced benzodiazepine binding sites were due to decreased binding site quantities or due to an altered affinity to bind to the ligand, and found a trend towards decreased binding site numbers in hippocampal tissue (Guptill et al., 2007). Blatt and Fatemi (2011) reported that GAD65 and GAD67 were decreased in the cerebellum, which may be due to decreases in Purkinje cells, while Yip, Soghomonian and Blatt (2007; 2008; 2009) observed decreased levels of GAD67 mRNA in Purkinje cells, increased GAD67 mRNA cerebellar interneurons, and decreased GAD65 mRNA in the dentate nucleus. Blatt and Fatemi also reported GABA receptor density decreases in the cerebellum and some cortical regions, while GABA$_A$ and GABA$_B$ protein expression were significantly reduced in the cerebellum, BA9, and BA40 (Blatt & Fatemi, 2011). Individuals with ASD also showed reduced quantities of benzodiazepine binding in the hippocampus and anterior cingulate cortex (ACC) compared to controls (Blatt & Fatemi, 2011). Lawrence, Kemper, Bauman, and Blatt (2010) found increased densities of GABA interneurons in postmortem brain tissue of adults with ASD in the dentate gyrus, CA1, and CA3 regions of the hippocampus. Reduced quantities of GABA$_A$ receptors and benzodiazepine binding sites in the superficial fusiform gyrus and posterior cingulate cortex have also been demonstrated, in addition to reduced benzodiazepine binding sites in the deep layers of the fusiform gyrus (Oblak, Gibbs, & Blatt, 2011).
A further postmortem study used Western blotting opposed to ligand binding assays to investigate GABA<sub>A</sub> receptor protein levels in postmortem tissue from individuals with ASD. This study found downregulation of GABA<sub>A</sub> subunits GABA<sub>A</sub> α 1, GABA<sub>A</sub> α 2, GABA<sub>A</sub> α 3, and GABA<sub>A</sub> β 3 in the cerebellum (Fatemi, Reutiman, Folsom, & Thuras, 2009a) as well as downregulated GABA<sub>B</sub> subunits GABA<sub>B</sub>1 and GABA<sub>B</sub>2 in the cerebellum (Fatemi, Folsom, Reutiman, & Thuras, 2009b). Dysregulation of GABA<sub>B</sub>1 and GABA<sub>B</sub>2 is associated with seizure activity, which is a common issue in individuals with ASD (Fatemi et al., 2009b). An additional study observed significantly increased levels of mGluR5 in children with ASD, with no differences between the ASD group and controls in GABA<sub>A</sub> β 3 levels (Fatemi, 2011). Fewer studies have set out to examine post-mortem brain tissue for expression of glutamate in ASD. One postmortem Glu study found that excitatory amino acid transporter 1 and GluR1 had upregulated mRNA levels and there was decreased AMPA Glu receptor density in the cerebellum (Purcell, Jeon, Zimmerman, Blue, & Pevsner, 2001). A further study demonstrated that individuals with ASD had reduced expression of GLS1 (kidney-type glutaminase) in the anterior cingulate cortex (Shimmura et al., 2013), but GLS1 and GLS2 (liver-type glutaminase) are not thought to play a significant role in synaptic transmission.

In a review of twenty-three post-mortem studies of cellular abnormalities in ASD, Dickinson, Jones and Milne (2016) found that sixteen studies pointed to decreased neural inhibition, three studies pointed to decreased excitation, and four studies found no group differences. Although further research needs to be conducted in this domain, such post-mortem evidence advances the notion that there is dysregulation of the brain’s E/I system in ASD, affecting cellular circuitry, sensory functioning, and ultimately behavior.
2.2.1.2 Evidence from magnetic resonance spectroscopy studies. Magnetic Resonance Spectroscopy (MRS) is a non-invasive analytical method used to complement Magnetic Resonance Imaging (MRI) in the characterization of tissue or identification of metabolic changes. Through MRS, researchers can determine relative concentrations of key neuronal metabolites, such as Glu and/or GABA. Although target metabolites such as N-acetylaspartate, creatine, and choline have been studied in ASD since the late 1990’s, Glu and GABA levels were not examined using MRS until relatively recently (Otsuka, Harada, Mori, Hisaoka, & Nishitani, 1999). Page et al. (2006) used 1T MRS to evaluate Glu and glutamine levels in twenty-five adults in ASD and neurotypical (NT) controls. Adults with ASD were found to have elevated concentrations of Glu and glutamine in the amygdala and hippocampus, but not in the parietal region (Page et al., 2006).

In a more recent study, Harada (2011) set out to determine the levels of GABA and Glu in the frontal lobes and lenticular nuclei of twelve children with ASD and ten controls using 3T MRS. The results indicated that GABA was significantly reduced in the frontal lobes of ASD patients compared to NT controls. Moreover, the GABA/Glu ratio was significantly reduced in the frontal lobes of individuals with ASD compared to neurotypical controls, suggestive of GABA/Glu dysregulation (Harada, 2011). In another MRS study, concentrations of key metabolites were determined for fourteen high-functioning individuals with ASD and fourteen IQ-matched NT controls using 3T MRS (Bernardi et al., 2011). Results indicated that the ASD group demonstrated reduced Glu concentrations in the right anterior cingulate cortex, but no significant differences were found in the thalamus, temporoparietal junction, or intraparietal sulcus (Bernardi et al., 2011). Horder et al. (2013) investigated Glu and glutamine levels using 3T MRS in the basal ganglia (caudate and anterior putamen), dorsolateral prefrontal cortex, and
parietal cortex as a control region in twenty-eight adults with ASD and fourteen matched controls. The ASD group had reduced concentrations of Glu and glutamine in the basal ganglia, which was correlated with degree of social impairment (Horder et al., 2013). However, no significant differences were found in the dorsolateral prefrontal cortex or parietal lobe (Horder et al., 2013). In a review of 38 MRS studies of varying brain regions in individuals with ASD published between 2006 – 2015 (Dickinson, Jones, & Milne, 2016), 11 studies found increased Glx or glutamate suggestive of increased excitation, 7 studies found decreased Glx and/or glutamate suggestive of decreased excitation, 3 studies found decreased GABA suggestive of decreased inhibition, and 17 studies found no group differences. Taken in aggregate, all these findings reflect the heterogeneity of ASD and the difficulty in measuring GABA, Glu, and relevant precursors in clinical studies.

2.2.1.3 Evidence from blood plasma studies. Currently only a few studies have set out to investigate blood plasma levels of GABA, Glu, or their precursors in children or adults with ASD, which have produced mixed results. One of the first studies to investigate plasma amino acid levels in autism via high-pressure liquid chromatography (HPLC) demonstrated that glutamic acid and aspartic acid were elevated in 14 children with ASD as compared to normed control values (169 +/- 142 uM and 22.1 +/- 13 uM) (Moreno-Fuenmayor, Borjas, Arieta, Valera, & Socorro-Candanoza, 1996). Glutamine levels were significantly lower in ASD than control values (241 +/- 166 uM; p < 0.01) (Moreno-Fuenmayor et al., 1996). Dhossche et al. (2002) measured blood plasma GABA levels via gas chromatography/mass spectrometry in 9 children with Autistic Disorder and 9 children with attention deficit hyperactivity disorder (ADHD), aged from 5 to 15 years. Findings showed that the ASD group had elevated GABA levels compared to the ADHD group (Dhossche et al., 2002). This study also demonstrated that plasma GABA
levels decreased with increasing age, and were not affected by the use of psychotropic medications (Dhossche et al., 2002). Aldred et al. (2003) measured blood plasma amino acid levels in 12 individuals with ASD (aged 4-29 years), 11 individuals with Asperger’s syndrome (aged 7-28 years), 32 parents (aged 24-58 years), and 13 siblings (aged 9-30 years). Comparison subjects were neurotypical individuals 4-16 years of age. Fluorescence spectroscopy analyses revealed that patients with autism or Asperger’s, their parents, and their siblings all had elevated levels of glutamic acid (Aldred et al., 2003). Shinohe et al. (2006) measured serum levels of amino acids related to glutamatergic neurotransmission (glutamate, glutamine, glycine, D-serine, and L-serine) in 18 males with ASD and 19 neurotypical comparison subjects via HPLC. Serum Glu levels in the participants with ASD were significantly elevated compared to comparison subjects (p < 0.001), while other amino acid levels did not significantly differ (Shinohe et al., 2006). In a more recent study by Shimmura et al. (2011), blood plasma levels from 23 male children with high-functioning autism (HFA) (aged 8-17 years) and 22 neurotypical comparison children (aged 9-17 years) were analyzed for twenty-five amino acids, including GABA, glutamate, and glutamine via HPLC. The HFA group had elevated levels of plasma glutamate, but lower levels of plasma glutamine compared to the comparison group, and no significant differences were found for GABA (Shimmura et al., 2011). Russo (2013) assessed plasma GABA concentrations in 48 children with ASD and 29 comparison children (mean age 8 years) via ELISA. Children with ASD had significant increased levels of GABA (p = 0.002) as compared to NT control children (Russo, 2013). Increased GABA levels were correlated with hyperactivity (p = 0.0007), hypersensitivity to light (p = 0.02), and hypersensitivity to tactile stimuli (p = 0.01) (Russo, 2013).
2.3 Sensory Abnormalities in Autism Spectrum Disorder

Sensory abnormalities in ASD were featured in the original description of autism by Kanner in 1943. One of the diagnostic criteria set forth in the Diagnostic and Statistical Manual of Mental Disorders (DSM-V: American Psychiatric Association, 2013) includes the presence of hyper- or hypo-reactivity to sensory input(s), or unusual interests to sensory input(s). This may include a heightened or attenuated response to temperature, auditory input, texture, smell, visual input, or moving objects. Visual processing deficits are perhaps the most cited sensory related issue in ASD, with abnormalities in multiple domains (see review by Simmons et al., 2009). Prior research suggests that these abnormalities in sensory processing in ASD may be due to aberrations in glutamate (Glu) and γ-aminobutyric acid (GABA) neurotransmission.

As a diagnostic criterion and one of the core features of ASD, sensory features are unique in the sense that they are not a manifestation of another co-morbid occurring disorder (American Psychiatric Association, 2013). Abnormalities in at least one domain of sensory processing have been shown to occur in up to 95% of surveyed children with ASD, based on parent questionnaires (Baranek et al., 2006; Baker et al., 2007; Tomcheck & Dunn, 2007) and more than 96% of children with ASD report hyper- or hypo-sensitivity to sensory inputs (Marco, Hinkley, Hill, & Nagarajan, 2011). However, presently there are very few standardized methods available for assessing sensory profiles in children with ASD, even though sensory abnormalities are classified as a diagnostic feature. Three of the most common assessments for diagnosing ASD in children only assess limited information regarding sensory features. The Autism Diagnostic Interview – Revised (ADI-R) is broken down into three domains: Language and Communication; Reciprocal Social Interests; and Restrictive, Repetitive, and Stereotypic Behaviors (Rutter, Lecouteur, & Lord, 2003). There are narrow questions about unusual sensory
interests under the Restrictive, Repetitive, and Stereotypic Behaviors section, but these questions do not provide a full sensory features panel (Rutter, Lecouteur, & Lord, 2003). The Autism Diagnostic Observation Schedule, Second Edition (ADOS-2) specifically assesses sensory issues, but does not provide information about whether a child is hyper- or hypo-responsive to sensory inputs (Lord et al., 2012). Another common assessment for diagnosing ASD, the Childhood Autism Rating Scale-2 (CARS-2), has a single question regarding taste, smell, touch response, and use on Item 9 (Schopler, Reichler, & Renner, 1980; Schopler, Bourgondien, Wellman, & Love, 2011). Assessments specific to sensory issues in ASD are less often used, but may provide a more comprehensive analysis of hypo- or hyper-responsiveness. This may include assessments such as the Sensory Profile, Second Edition (SP-2), a self-administered or parent questionnaire to assess response to sensory events in daily life (Tomcheck & Dunn, 2007). A second practical assessment, The Sensory Experiences Questionnaire (SEQ), aids to characterize sensory features and differentiate sensory patterns in children with ASD using a Likert scale for self-administration or caregiver questions (Baranek, David, Poe, Stone, & Watson, 2006).

In a study by Leekam, Nieto, Libby, Wing, and Gould (2007), patterns of sensory processing in children with ASD were assessed via the Diagnostic Interview for Social Communication Disorders (DISCO), which assesses abnormalities in audition, vision, touch, smell, taste, other oral, kinesthetic sensation, and pain (Wing, Leekam, Libby, Gould & Larcombe, 2002). In a group of thirty-three children with ASD, results revealed that over 90% of the children had sensory issues in more than one domain (Leekman et al., 2007). Tomcheck and Dunn (2007) assessed 281 children with ASD and neurotypical age-matched comparison children using the Short Sensory Profile (SSP), a shorter version of the SP-2 (Dunn, 2014). This study found that sensory issues were present in 95% of the children with ASD. A meta-analysis
of fourteen studies that assessed sensory modulation symptoms in ASD found heightened sensory modulation symptoms across ages and severity of ASD, with 80% of 6 – 9 year olds being symptomatic (Ben-Sasson et al., 2009). Kern (2006) found significant group differences between 104 persons with ASD aged from 3 to 56 years and neurotypical age matched comparison participants in visual, touch, and oral sensory processing, assessed via the SP (Kern, 2006). Sensory symptoms are correlated with severity of social impairment in ASD, but they appear to lessen with age (Kern, 2007).

2.3.1 Visual sensory processing in autism spectrum disorder. Individuals with ASD have been shown to use visual information insufficiently and to display atypical visual behaviors in numerous domains. Visual processing abnormalities are one of the most cited sensory issues in ASD despite the majority of studies reporting a lack of optometric abnormalities and normal visual acuity, aside from a high prevalence of strabismus (Beaudot, 2009; Kaplan, Rimland, & Edelson, 1999; Kaplan, 2006; Simmons et al., 2009).

Numerous studies have demonstrated that individuals with ASD display atypical visual processing expressed through both strengths and weaknesses as compared to neurotypical controls in numerous domains, including but not limited to deficits in low-level visual processing, insusceptibility to visual illusions, abnormal perceptual integration, holistic-configural processing deficits, abnormalities in motion perception, deficits in processing biological motion, enhanced perception of fine details, differences in visual search and detection, impairments in object-boundary detection, abnormalities in gaze detection, and deficits in facial processing (see review by Simmons et al., 2009). Individuals with ASD also use visual input inadequately, as evidenced by eye tracking data, and additionally have issues maintaining visual attention (Beaudot, 2009). Some studies suggest that children and adults with ASD have difficulties
processing visual information due to deficits in central and peripheral vision, leading to poor integration (Beaudot, 2009). Moreover, research in visual neuroscience suggests that the majority of receptive fields in the visual system are inhibitory, meaning that neuronal inhibition plays a critical role in cortical neurons and sensory responses (Haider et al., 2013). These studies provide support for the idea that there is a deficit in processing unimodal sensory input in ASD, which in turn affects higher cortical abilities such as socialization (Marco, Hinkley, Hill, & Nagarajan, 2011).

2.4 Psychophysical Evidence for an Excitatory/Inhibitory Imbalance in Autism Spectrum Disorder

Although hyper- or hypo-reactivity to sensory input or an unusual interest in sensory aspects of the environment is part of DSM-V diagnostic criterion for ASD, our comprehension of sensory processing abnormalities in autism is limited. A few studies have addressed the psychophysical or neural mechanisms that potentially underlie hyper- or hypo-reactivity to sensory input, the majority of which have focused on defining visual perceptual thresholds in autism through behavioral measures (Marco, Hinkley, Hill, & Nagarajan, 2011). We have previously discussed evidence for an E/I imbalance in ASD from post-mortem, magnetic resonance spectroscopy, and blood plasma studies. The focus of the following section will be to outline research on providing a marker for measuring the potential E/I imbalance in individuals with autism using psychophysical tools and/or brain imaging methodology.

2.4.1 Binocular rivalry. Currently, four studies have investigated a potential E/I imbalance in ASD by measuring binocular rivalry, a psychophysical phenomenon that occurs in the dichoptic presentation of two different images, whereby individuals generally perceive each image separately for a few moments and/or mixed perception of both images (Blake, Randolf,
and Nikos, 2002; Dickinson et al., 2016). Rivalry suppresses the ventral pathway for visual processing, but the interplay between excitation and inhibition is thought to account for visual awareness of both images (Said, Egan, Minshew, Behrmann, & Heeger et al., 2013). Robertson, Ratai and Kanwisher (2016) used magnetic resonance spectroscopy to demonstrate a tight linkage between binocular rivalry dynamics and GABA/glutamate levels in the visual cortex of neurotypical individuals. In a study of two distinct aspects of binocular rivalry, mixed visual perception and traveling waves, Said et al. (2013) found no significant differences between adults with ASD and neurotypical adults. Other studies have also demonstrated significant differences between neurotypical individuals and those with ASD in various aspects of binocular rivalry, suggestive of an E/I imbalance (Robertson, Kravitz, Freyberg, Baron-Cohen, & Baker, 2013; Freyberg, Robertson, & Baron-Cohen, 2015; Robertson, Ratai, & Kanwisher, 2016). In each of these studies, individuals with ASD displayed slower binocular rivalry characterized by longer transitional states between percepts (Robertson et al., 2013; Freyberg, Robertson, & Baron-Cohen, 2015; Robertson, Ratai, & Kanwisher, 2016).

2.4.2 Spatial suppression and contrast gain. Spatial suppression, also known as surround suppression, is a well-studied non-linear visual-sensory effect first described by Hubel and Wiesel (1965). It is defined as the suppressed neuronal activity to a given stimulus outside of a neuron’s classic receptive field in the surround, or non-classical receptive field, since neurons in the primary visual cortex are size-selective (Hubel & Wiesel, 1965). Stimulation of the center of a classic receptive field by an optimal stimulus will generate an evoked action potential. Experiments in macaques have revealed that the contrast-response function saturates when viewing drifting sinusoidal gratings, also known as Gabor patches, centered on a neuron’s receptive field, which are designed to reflect the receptive field properties of cells in the early
visual cortex (Bonneh & Sagi, 1998; Khoe, Freeman, Woldorff, & Mangun, 2004). In other words, the neuronal response rate increases until a preferred size is reached, and then once the classic receptive field and surround are stimulated, the magnitude of response is attenuated (Tailby, Solomon, Peirce, & Metha, 2007). Modulation of the response is either excitatory or inhibitory depending on the contrast and size of the stimulus (Cavanaugh, Bair, & Movshon, 2002; Karas & McKendrick, 2011). Electrophysiological studies have demonstrated that neural responses in the primary visual cortex can be suppressed in as many as 90% of neurons by a given stimulus outside of the classic receptive field (Cavanaugh, Bair, & Movshon, 2002). Spatial suppression observed in V1 is likely reflective of modulatory mechanisms operating at varying levels of the visual system such as in retinal ganglion cells, the lateral geniculate nucleus, the cortex, and cortical receptive fields (Solomon, Peirve, & Lennie, 2004; Tailby, Solomon, Peirce, & Metha, 2007). Cortical processing is not considered to be entirely hierarchical since feedforward connections have corresponding reverse feedback connections, which modulate responses to stimuli within a receptive field (Lamme, Supér, & Spekreijse, 1998). Both feedforward and feedback connections are primarily excitatory and are thought to play a role in attention and visual awareness (Lamme, Supér, & Spekreijse, 1998).

The magnitude of the suppressive response is considered to be contrast-dependent, with the greatest degree of suppression occurring in conditions of high-contrast and reduced or absent suppression occurring in conditions of low-contrast (Geisler & Albrecht, 1992; Tang, Sang, & Liu, 2016). The lack of suppression in conditions of low contrast can be explained by the phenomenon of contrast gain control, a concept first described by Ohzawa and Freeman (1985), who measured contrast-response functions of neurons in the striate cortices of cats while adapting them to varying contrast levels of sinusoidal gratings. This study demonstrated that
contrast gain control is likely a cortical function and that the majority of neurons in visual cortex increase their firing rates with increasing contrast levels (Ohzawa & Freeman, 1985). However, more recently gain control has been studied in the lateral geniculate nucleus (LGN) and primary visual cortex. Contrast gain control is functionally advantageous since it can aid in optimal detection of slight differences in local contrast levels (Ohzawa & Freeman, 1985; Boynton, 2005). Gain control allows for sensory systems to adapt responses dependent on the context of the stimuli and can be influenced by neural excitation, neural inhibition, and feedback connections (Butler, Silverstein, & Dakin, 2008). Although visual pathways principally rely on glutamate as their primary transmitter, NDMA plays a critical role in gain control by amplifying the effect of spatial suppression. Therefore, an NMDA deficit could result in reduced spatial suppression (Butler, Silverstein, & Dakin, 2008).

Animal studies have largely informed our understanding of the visual system. While brain organization varies across species, there are significant similarities in cell types and circuits making it possible to draw inferences about the human visual system (Krubitzer, 1995; Sereno, 1998). Spatial suppression has recently been studied in various clinical contexts. For example, weakened spatial suppression has been demonstrated through paradoxical increases in motion discrimination thresholds in large, high-contrast stimuli for aging populations (Tadin & Blake, 2005), individuals with depression (Golomb et al., 2009), individuals who suffer from migraine headaches (Battista, Badcock, & McKendrick, 2011), and individuals with schizophrenia (Tadin et al., 2006). It has been theorized that weakened spatial suppression reflects abnormalities in the GABAergic system (Blin, Mestre, Paut, Vercher, & Audebert, 1993; Giersch & Lorenceau, 1999; Betts, Taylor, Sekuler, & Bennett, 2005). GABA’s role in the visual pathways, specifically visual perception and motion integration, has been well defined in primate and pharmacological
intervention studies (Blin et al., 1993; Giersch & Lorenceau, 1999; Betts et al., 2005). More recent studies have shown that psychophysical motion processing tasks reflect GABAergic lateral inhibition amongst cortical neurons in visual cortex in humans (Tadin et al., 2006; Golomb et al., 2009; Foss-Feig et al., 2013; Horder et al., 2014).

Figure 2. Classic Receptive Fields and Spatial Suppression: Visual stimulation of the classic receptive field and the surround produces a response smaller in magnitude than stimulation of the classic receptive field alone. Stimulation of the surround alone produces no response. Reproduced from Alledel (2012), permitted under the Creative Commons Attribution 3.0 Unported License.

2.4.3 Spatial suppression in autism spectrum disorder. To date, only three studies have set out to investigate GABA/Glu imbalances in ASD through the implementation of psychophysical motion processing tasks. In a behavioral study by Foss-Feigg et al. (2013), researchers investigated the E/I imbalance theory of ASD in a paradigm requiring twenty participants with ASD and twenty-six neurotypical individuals between the ages of 8 and 17 years to view drifting sinusoidal gratings moving either left or right. There were three conditions of sinusoidal gratings, small, medium, and large, in the high contrast experiment; and separately, three conditions of sinusoidal gratings, small, medium, and large, in the low contrast experiment.
Results revealed that there was an enhancement of direction of motion perception in ASD in conditions of high contrast compared to NT controls across all stimulus sizes (Foss-Feigg et al., 2013). However, this study did not reveal any differences specifically in spatial suppression. In a second behavioral study by Horder et al. (2014), researchers implemented a similar task with a group of twelve adult males with ASD and eighteen age and IQ matched controls. This study revealed a higher sensory threshold for large, high-contrast stimuli compared to small, low-contrast stimuli in both groups, but in the ASD group the degree of the paradoxical effect was significantly reduced. In other words, they found that individuals with ASD showed a selective enhancement of motion perception in the large, high-contrast condition. In a recent behavioral study, Sysoeva et al. (2017) aimed to replicate Foss-Feig’s findings and to explore contrast gain control in children and adolescents with ASD. Spatial suppression and contrast gain control were examined in 40 males with ASD and 44 neurotypical comparison participants from 6-15 years of age. Stimuli were similar to those used by Foss-Feig et al. (2013), but Sysoeva et al. (2017) found no significant differences in threshold discrimination in large, high-contrast stimuli. Rather, they found that children/adolescents with ASD demonstrated significantly delayed processing of small, stimuli in both the high- and low-contrast conditions as compared to the neurotypical control group. Spatial suppression and contrast gain indices were created by calculating the residual values of discrimination thresholds for large stimuli after removing the effect of small stimuli via a regression analysis. The spatial suppression index was shown to be atypically reduced in children/adolescents with ASD, while contrast gain was abnormally enhanced. The spatial suppression and contrast gain indices were highly correlated in neurotypical participants, but this correlation was absent in children/adolescents with ASD.
2.4.4 Electrophysiological marker of spatial suppression. Few studies have established an electrophysiological marker of spatial suppression in human beings. Vanegas, Blangero, and Kelly (2015) investigated steady-state visual evoked potentials (SSVEP) by flickering foreground stimuli within full-screen static surround patterns to examine whether suppressive response patterns exhibit orientation specificity, location effects, or effects of temporal adaptation. Vanegas et al. demonstrated that the foreground response was significantly suppressed in the presence of a visual surround, and that this suppression was enhanced during trials with matching orientation as compared to orthogonally oriented stimuli. Additionally, suppression was stronger at peripheral as compared to foveal locations. Depending on the relative contrast of the surround, the foreground signal either fell or grew over time, which is consistent with adaptation to suppressive drive (Vanegas, Blangero, & Kelly, 2015).

2.4.5 Visual evoked potentials in autism spectrum disorder. To our knowledge, only a few studies have implemented visual evoked potentials (VEPs) in order to examine the E/I imbalance theory of ASD. VEPs are a non-invasive technique implemented to assess excitation and inhibition from post-synaptic potentials on apical dendrites (Zemon, Gordon, & Welch, 1988; Siper et al., 2016). In a steady-state VEP study, Takare, Sablich, White, and Sweeney (2016) examined visual evoked responses to vertical circular gratings oscillating at 3.76 Hz at varying contrasts ranging from 5% to 90% in 13 individuals with ASD and 12 NT controls. Spectral power of responses at each level of contrast was estimated using discrete Fourier transforms in order to create an index of magnitude for neural responses. In individuals with ASD, neurocortical activation increased with increasing contrast levels; on this basis, the authors proposed that cortical hyperexcitability is a fundamental characteristic of ASD. Siper et al. (2016) recorded transient visual evoked potentials (tVEPs) from 37 children with ASD, 23
unaffected siblings, and 36 neurotypical children from 2-12 years of age with non-verbal IQs ranging from 42-140. Stimuli consisted of a contrast-reversing 32 x 32 checkerboard pattern displayed for either 60 seconds or 3 seconds, and EEG was recorded using was recorded using Oz as the electrophysiological channel (Siper et al., 2016). The contrast-reversing checkerboard stimulus produced a negative VEP response at approximately 75 milliseconds (ms) post-stimulus presentation, which is thought to reflect depolarization and glutamatergic post-synaptic activity in the primary visual cortex. Children with ASD demonstrated smaller P60-N75 and N75-P100 amplitudes compared to neurotypical children while unaffected siblings demonstrated intermediate responses relative to the ASD and neurotypical groups (Siper et al., 2016). The authors suggested that smaller P60-N75 amplitude in the ASD group reflects weaker excitatory input to the cortex, which reduces the inhibitory component of the VEP (Siper et al., 2016). Therefore, P60-N75 amplitude can be considered a measure of relative strength of inhibition to excitation (Siper et al., 2016). By contrast, pharmacological studies of VEPs have demonstrated that a topical GABA_A receptor antagonist results in greater VEP negative amplitude and attenuation or elimination of positive amplitude; while a topical GABA agonist results in smaller or attenuated VEP negative amplitude and enhanced positive amplitude (Purpura, 1959; Zemon, Kaplan, & Ratliff, 1980; Siper et al., 2016).

2.5 Electrophysiological Outcome Measures in Clinical Trials.

Although there are currently no FDA approved medications to treat the core symptoms of ASD, there are more than 50 pharmaceutical trials for individuals with ASD presently listed as open and recruiting on the U.S. National Institutes of Health clinical trials registry (clinicaltrials.gov). Selecting appropriate outcome measures in clinical trials of
neurodevelopmental disorders defined by behavior is challenging since measures often rely on parent report, self-report, or clinical observations. Inadequate outcome measures may be unable to capture changes from study specific interventions or distort results. A handful of clinical trials have used electrophysiological outcome measures in clinical trials enrolling children with ASD.

Dawson et al. (2013) examined EEG spectral power and event-related potential (ERP) latency to faces vs. objects in young children with ASD who were randomized to receive either the Early Start Denver Model (ESDM) behavioral intervention or community intervention. The ESDM group demonstrated greater improvements in IQ, language, adaptive skills, and pro-social behaviors than the community intervention group. This study was the first to demonstrate that responses to intervention were associated with changes in EEG spectral power and ERP responses (Dawson et al., 2013). Specifically, this study found that the ESDM group and neurotypical children showed shorter ERP latency and increased cortical activation when viewing faces, while the community intervention group demonstrated shorter ERP latency and increased cortical activation to objects (Dawson et al., 2013). In a trial of 20 children with regressive ASD from 3-5 years of age treated with corticosteroid therapy and 24 children with ASD who received no steroid treatment, Duffy et al. (2014) examined frequency modulated evoked responses (FMAER), language, and behavior. The steroid-treated group demonstrated a significant increase in FMAER spectral response, which was associated with improvements on language assessments, compared to the non-steroid-treated group (Duffy et al., 2014). In addition to these studies, the Autism Biomarkers Consortium for Clinical Trials (ABC-CT) is currently working to validate objective tools, including EEG biomarkers, in clinical trials of behavioral and pharmaceutical interventions.
3. RESEARCH QUESTIONS AND HYPOTHESES

The purpose of this research is twofold. The first aim is to determine whether the N1 event-related potential (ERP) component can be used as a marker of spatial suppression in conditions of high-contrast stimuli and contrast gain in conditions of low-contrast stimuli, as discrimination threshold has been used as a measure of spatial suppression in behavioral studies. In neurotypical individuals, delayed processing of large, high-contrast stimuli as compared to small, high-contrast stimuli would suggest that this ERP component could be used as an electrophysiological marker of spatial suppression in this paradigm. The absence of differences in N1 peak amplitude and latency in the same population when processing differences between large and small low-contrast stimuli would lend additional support to the validation of our experimental paradigm, since spatial suppression is most prevalent in conditions of high-contrast.

The second aim of this study is to determine whether children with ASD demonstrate a lack of spatial suppression when perceiving and processing large, high-contrast stimuli as compared to neurotypical children. Based on previous studies that lend support to the E/I theory of ASD, we expect that children with ASD will not exhibit spatial suppression in conditions of large, high-contrast stimuli, and therefore will display a selective enhancement of motion processing in conditions of large, high-contrast stimuli. If children with ASD exhibit decreased spatial suppression in conditions of large, high-contrast stimuli, then this paradigm may have potential for use as a clinical outcome measure in clinical trials to evaluate the effectiveness of drugs that act on GABAergic neurotransmission.
3.1 Research Question 1

Can the N1 event-related potential (ERP) probe visual perceptual hyperexcitability, that can be associated with GABA/Glu imbalances in children with autism spectrum disorder?

_Hypothesis 1:_ Latency and amplitude of the visual N1 response relates to spatial suppression capabilities, and is therefore impacted by insufficient GABA and abundant glutamate in children with autism spectrum disorder.

Predictions: Although direct measurement of GABA/Glu in children is not possible for the current study, this hypothesis allows us to predict that N1 latency in response to large, high-contrast visual stimuli in ASD children will be shorter, and N1 amplitude will be larger, compared to neurotypical controls, demonstrative of a lack of spatial suppression, which by hypothesis reflects a GABA/Glu imbalance. We also predict shorter latency of the N1 ERP to large, high-contrast visual stimuli as compared to small, high-contrast stimuli in individuals with ASD; and longer latency of the N1 ERP to large, high-contrast visual stimuli as compared to small, high-contrast stimuli in neurotypical individuals.

3.2 Research Question 2

Is there a relationship between the responsiveness pattern of sensory symptoms in autism spectrum disorder and the degree of visual perceptual hyperexcitability? _Hypothesis 2:_ Hyper-responsiveness sensory symptoms correlate with the severity of imbalance between insufficient GABA and abundant glutamate in children with autism spectrum disorder, and hence with the extent to which the latency of the N1 response is impacted in spatial suppression.

Predictions: In the ASD group, we predict children with hyper-responsiveness patterns to have shorter N1 latencies than those with hypo-responsiveness patterns.
4. RESEARCH DESIGN AND METHODS

EEG is a non-invasive neuroimaging technique for recording electrical activity generated by post-synaptic potentials (PSPs) in cortical pyramidal cells due to their orientation in relation to the cortex ( Luck & Kappenman, 2012). PSPs are the result of fluctuations in membrane potential from negative to positive caused by the rapid opening and closing of voltage-gated ion channels in the postsynaptic terminal ( Luck & Kappenman, 2012). PSPs can be excitatory postsynaptic potentials (EPSPs) or inhibitory postsynaptic potentials (IPSPs). EPSPs depolarize the postsynaptic membrane potential from a resting state of approximately -70 microvolts (µV) to -55µV, which makes the postsynaptic neuron more likely to produce an action potential (Meldrum, 2000). IPSPs hyperpolarize the postsynaptic membrane potential, which makes the postsynaptic neuron less likely to produce an action potential (Chaves & Marty, 2003). When a group of spatially aligned neurons are simultaneously active, the summed PSPs can be measured via electrodes placed on the surface of the scalp in predetermined locations in relation to a reference electrode ( Luck & Kappenman, 2012). EEG has high temporal resolution and accurate spatial resolution, through source localization, when voltage deflections are sampled over superior and inferior surfaces (Song et al., 2015). From electroencephalography (EEG) recordings, we can derive event-related potentials (ERPs), which are averaged voltage fluctuations from the raw EEG recording that are time-locked to a specific stimulus ( Luck & Kappenman, 2012). ERPs can be used to answer a variety of inquiries in cognitive neuroscience and related fields. An ERP waveform is the representation of positive or negative voltage deflections that vary in amplitude and polarity over time and reflect a sensory, cognitive, or motor process elicited by a specific stimulus ( Luck & Kappenman, 2012). In order to produce an ERP waveform from raw EEG data, multiple single-trial waveforms are averaged together for
each specific stimulus type at each electrode site, or within a pre-determined montage of
electrodes (Handy, 2005). Through the averaging of the electrical signal recorded in relation to a
time-locked event, a waveform representing the averaged brain activity associated with each
stimulus type can be produced (Handy, 2005).

Visual evoked potentials (VEPs) are voltage deflections recorded as a result of cortical
and subcortical activation of the visual pathway (Celesia & Brigell, 1999; Pratt, 2012). The
visual system is highly sensitive to changes in luminance and changes in visual patterns;
therefore, stimuli implemented to evoke VEPs are generally luminance- or pattern-defined (Pratt,
2012). The visual N100 (N1) ERP is a large, negative visual evoked potential (VEP) that peaks
approximately 100 to 250 ms after stimulus presentation in neurotypical adults, and is enhanced
with spatial attention (Johannes, Munte, Heinze, & Mangun, 1995; Vogel & Luck, 2000). The
N1 occurs over occipital, parietal, and/or central electrode sites (Mangun & Hillyard, 1991) and
has been observed as early as 24 weeks’ gestational age (Taylor & McCulloch, 1992; Coch &
Gullick, 2012). The N1 has been observed to appear earliest at occipital electrode sites prior to
moving towards fronto-central sites (Hennighausen, Remschmidt, & Warnke, 1994). The N1
ERP is elicited by primarily parvocellular and mixed parvo- and magnocellular stimuli attributed
to extrastriate activity (Schechter et al., 2005; Pratt, 2012). The magnocellular pathway is elicited
by low-contrast and low spatial frequency stimuli, whereas high-contrast visual stimuli activate
both the magno- and parvocellular inputs to the cortex (Pratt, 2012). The N1 has many
subcomponents, all of which appear to be influenced by attention (Hillyard & Anllo-Vento,
1998). N1 amplitude is generally larger in response to attended stimuli than unattended (Pratt,
2012).
4.1 Design

This study followed a 2 X 2 mixed factorial design. The factors were group (NT vs. ASD) and size (Small vs. Large) within contrast categories. The data was analyzed within and between groups to determine differences in N1 latency and amplitude. Each condition (Small/High-Contrast, Large/High-Contrast, Small/Low-Contrast, and Large/Low-Contrast Vertical Gray Scale Sinusoidal Gratings) was a within-subjects factor, and group (NT, ASD) was the between-subjects factor. Comparisons between luminance levels (high contrast vs. low contrast) were not conducted because spatial suppression is contrast dependent, with the greatest degree of suppression occurring in conditions of high contrast, and reduced or absent suppression occurring in conditions of low-contrast (Geisler & Albrecht, 1992; Tang, Sang, & Liu, 2016); therefore, comparisons between luminance-defined stimuli would not be meaningful.

<table>
<thead>
<tr>
<th>Neurotypical</th>
<th>Autism Spectrum Disorder</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small/High Contrast</td>
<td>Small/High Contrast</td>
</tr>
<tr>
<td>Large/High Contrast</td>
<td>Large/High Contrast</td>
</tr>
</tbody>
</table>

Table 1. High-Contrast Planned Comparisons: 2 X 2 Mixed Design Within- (Vertical) and Between- (Horizontal) Group Comparisons for High-Contrast Stimuli

<table>
<thead>
<tr>
<th>Neurotypical</th>
<th>Autism Spectrum Disorder</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small/Low Contrast</td>
<td>Small/Low Contrast</td>
</tr>
<tr>
<td>Large/Low Contrast</td>
<td>Large/Low Contrast</td>
</tr>
</tbody>
</table>

Table 2. Low-Contrast Planned Comparisons: 2 X 2 Mixed Design Within- (Vertical) and Between- (Horizontal) Group Comparisons for Low-Contrast Stimuli
4.2 Screening Assessments and Behavioral Evaluations

All participants were administered the following evaluations: Visual acuity screening; the Pelli-Robson Contrast Sensitivity Chart; Stanford-Binet Intelligence Scales, Fifth Edition (SB5) Abbreviated IQ; Autism Diagnostic Observation Schedule, Second Edition (ADOS-2); and the Childhood Autism Rating Scale, Second Edition (CARS-2).

All parents of participants completed the following questionnaires: Sensory Experiences Questionnaire; Autism Center of Excellence (ACE) Family Medical History Questionnaire; and a Demographic Questionnaire. Each of these measures is described further below.

4.2.1 Visual acuity screening. The visual acuity screening consisted of reading the Snellen Eye Chart (Snellen, 1862) from 20 feet away. If a participant was unable to identify letters, the Kindergarten Test Chart was implemented. Both the Snellen Eye Chart and the Kindergarten Test Chart have a series of letters or objects that become progressively smaller from the top row to the bottom row. If the individual being tested can read the row labeled “20/20,” this individual is deemed to have 100% visual efficiency. Only children with at least 20/40 vision were included in this experiment. Additional inclusion and exclusion criteria are described in the participant section starting on page 50.

4.2.2 The Pelli-Robson Contrast Sensitivity Chart. The Pelli-Robson Contrast Sensitivity Chart (Pelli & Robson, 1988) can be implemented for clinical determination of an individual’s contrast sensitivity function. Contrast sensitivity function is the ability to perceive fluctuations in luminance across varying spatial frequencies. This chart consists of letter sequences organized into three-letter groups, with contrast decreasing from one group to the next. The observer must stand 40 inches away from the chart in a room with an approximate illumination of 85 cd/m². A score above 1.5 is considered within normal range, while a score
below 1.5 suggests that a contrast sensitivity impairment may be present. Children who scored below a 1.5 were excluded from this study.

4.2.3 Stanford-Binet Intelligence Scales, Fifth Edition (SB5) Abbreviated Battery IQ. The Stanford-Binet Abbreviated Battery IQ (ABIQ) is an estimated general ability index useful for screening purposes in research studies. The score is obtained by administering two routing subtests of the Stanford-Binet Intelligence Scales, Fifth Edition (Roid, 2003). The combination of a fluid reasoning measure (Object Series/Matrices) and a knowledge measure (Vocabulary) is generally recognized as a good estimate of global ability. The ABIQ results in a standard score, with a mean of 100 and a standard deviation of 15. Scores between 85 and 115 are in the average range. Neurotypical children (comparison group) with an ABIQ less than 85 and children with ASD with an ABIQ less than 70 were excluded from this study.

4.2.4 Autism Diagnostic Observation Schedule, Second Edition (ADOS-2). The Autism Diagnostic Observation Schedule, Second Edition (ADOS-2) (Lord, Rutter, DiLavore, Risi, Gotham, & Bishop, 2012), allows clinicians/researchers to accurately diagnose autism spectrum disorder and assess severity across age, language ability, and developmental level through a semi-structured behavioral observation. The ADOS-2 is divided into five modules, administered dependent upon chronological age and expressive language ability (toddlers and pre-verbal children through to adolescents and adults with fluent, complex speech). Each module assesses the following domains: language and communication; reciprocal social interaction; play and imagination; stereotyped behaviors and restricted interests; and other autism-specific behaviors (Lord et al., 2012).

The ADOS-2 is viewed as the “gold standard” for assessing ASD, in both clinical practice and research settings, with the highest sensitivity and specificity of available ASD
assessments (Falkmer, Anderson, Falkmer, & Horlin, 2013). The ADOS-2 was standardized on a sample of 1,574 children, in addition to a replication sample of 1,282 children (Lord et al., 2012). Internal consistency is high (.87 - .92) for Modules 1, 2, and 3 in the Social Affect domain, and moderate for the Restricted and Repetitive Behaviors domain (.51 - .66) (McCrimmon & Rostad, 2014). For Module 4, internal consistency is also high for the Communication (.75) and Social Interaction (.85) domains, but poor for the Restricted and Repetitive Behavior domain (.47) (McCrimmon & Rostad, 2014). Test-retest reliability for Modules 1, 2, and 3 ranges from .68 - .92, but has not been calculated for Module 4 (McCrimmon & Rostad, 2014). Sensitivity of the ADOS-2 ranges from 60 to 90%, while specificity ranges from 75 to 100% (McCrimmon & Rostad, 2014). The appropriate ADOS-2 module was selected for each individual participant based on language ability and age. For each module, ADOS-2 codes are converted to algorithm scores, which results in a Social Affect (SA) total and a Restricted and Repetitive Behavior (RBR) total based on individually coded items for the following: Language and Communication, Reciprocal Social Interaction, Imagination, Stereotyped Behaviors and Restricted Interests, and Other Abnormal Behaviors. SA and RBR totals are combined to create an Overall Total. The Overall Total is then compared to cut-off scores for autism and autism spectrum disorder for each module in order to determine if an individual meets instrument criteria for a diagnosis of autism or autism spectrum disorder (Tables 3–6, below). Lastly, an ADOS-2 comparison score is also obtained from the Overall Total to determine severity. Only children and adolescents who met instrument criteria for a diagnosis of autism or autism spectrum disorder on the ADOS-2 were included in the experimental group. Tables 3 through 6, below, summarize the cut-off scores for each ADOS module.
### Table 3. Cut-off Scores for ADOS-2 Classification by Module: Module 1 (Pre-Verbal/Single Words)

<table>
<thead>
<tr>
<th></th>
<th>Few to no words</th>
<th>Some words</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autism</td>
<td>16</td>
<td>12</td>
</tr>
<tr>
<td>Autism Spectrum Disorder</td>
<td>11</td>
<td>9</td>
</tr>
</tbody>
</table>

### Table 4. Cut-off Scores for ADOS-2 Classification by Module: Module 2 (Phrase Speech)

<table>
<thead>
<tr>
<th></th>
<th>Younger than 5 years</th>
<th>Aged 5 years or older</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autism</td>
<td>10</td>
<td>9</td>
</tr>
<tr>
<td>Autism Spectrum Disorder</td>
<td>7</td>
<td>8</td>
</tr>
</tbody>
</table>

### Table 5. Cut-off Scores for ADOS-2 Classification by Module: Module 3 (Fluent Speech: Child/Adolescent)

<table>
<thead>
<tr>
<th></th>
<th>Cut-off scores</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autism</td>
<td>9</td>
</tr>
<tr>
<td>Autism Spectrum Disorder</td>
<td>7</td>
</tr>
</tbody>
</table>

### Table 6. Cut-off scores for ADOS-2 Classification by Module: Module 4 (Fluent Speech: Adolescent/Adult)

<table>
<thead>
<tr>
<th></th>
<th>Communication</th>
<th>Social Interaction</th>
<th>Communication &amp; Social Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autism</td>
<td>3</td>
<td>6</td>
<td>10</td>
</tr>
<tr>
<td>Autism Spectrum Disorder</td>
<td>2</td>
<td>4</td>
<td>7</td>
</tr>
</tbody>
</table>

### 4.2.5 Childhood Autism Rating Scale, Second Edition (CARS-2). The Childhood Autism Rating Scale, Second Edition (CARS-2) (Schopler, Van Bourgondien, Wellman, & Love, 2010), aims to identify children who have ASD, and to determine severity through direct observation and a parent/guardian interview. The CARS-2 is administered in one of two versions, for either standard (ST) or high-functioning (HF) individuals. Each version has 15 category...
ratings, to be completed by the clinician/researcher, which assess primarily social understanding and interaction, sensory features, verbal and non-verbal communication, restricted or repetitive interests, and general cognition. The high-functioning (CARS-2 HF) version was developed for the Second Edition to assess individuals 6 years of age or older with an IQ greater than 80, who exhibit fluent communication (Schopler, Bourgondien, Wellman, & Love, 2011). The CARS-2ST and CARS-2HF were in total evaluated on a sample of 3,500 children and adults (Schopler et al., 2011), and demonstrated a robust internal consistency (CARS-2ST = .93; CARS-2HF = .96). Validity of the CARS-2ST and HF versions was moderate to high, ranging from .42 to .77 (Schopler et al., 2011). Neurotypical comparison children with total raw scores above 27.5 on the CARS-2 were excluded from the study.

4.2.6 Sensory Experiences Questionnaire, Second Edition (SEQ 2.1). The Sensory Experiences Questionnaire, Version 2.1 (SEQ: Baranek, 1999a & b; Baranek et al., 2006), is a caregiver questionnaire that evaluates sensory responsiveness patterns in children with ASD and other neurodevelopmental disorders. The SEQ total score is an aggregate off 33 quantitative items that provide an overall picture of a child’s level of sensory processing difficulties. A total “mean score” can be derived via dividing the sum of the scores on the 33 items by the number of items. The SEQ 2.1 aids in the identification of hypo-responsiveness, hyper-responsiveness, and sensory seeking behaviors within social and non-social contexts (Baranek, 1999b). The SEQ is reliable in identifying hyper-responsiveness (.73) and hypo-responsiveness (.75) in young children with ASD. This questionnaire was used to categorize study participants as hyper- or hypo-responsive to sensory stimuli. The sensory experiences questionnaire was not used to determine eligibility for this study, but rather to provide information about sensory sensitivities that will help to address Research Question 2.
4.2.7 Autism Center of Excellence (ACE) Family Medical History Questionnaire.
The National Database for Autism Research (NDAR) has created a family medical history questionnaire to be completed by each study participant’s primary caregiver, which consists of a list of neurodevelopmental disorders, sensory disorders, and mental health-related disorders. The respondent is requested to indicate the age, sex, and last grade completed for the participant’s biological mother, father, and all biological siblings, and also to provide information as to whether any of the participant’s biological relatives have ever had a diagnosis of any of the listed conditions. Children in the experimental group who reported a history of Fragile-X Syndrome, epilepsy, or traumatic brain injury (TBI) were excluded. Control participants were excluded if they had a history of attention-deficit/hyperactivity disorder (ADD/ADHD), developmental delays, a genetic neurodevelopmental disorder, TBI, history of epilepsy or seizures, intellectual disability (IQ less than 75), language or learning disorders, Rett Syndrome, Pragmatic Communication Disorder, schizophrenia, or a visual impairment that could not be corrected with glasses.

4.2.8 Demographic questionnaire. Basic demographic information about the participant and their family was collected on a study-specific form: participant and parent/guardian race, ethnicity, parent/guardian education level, parent/guardian occupation, annual household income, household structure, participant’s language ability, participant’s current school program, and participant’s medication history. Participants were excluded if they had taken psychiatric medications within the last six months (see Appendix 11.4).
4.3 Experimental Equipment

4.3.1 Computers and software. The stimulus presentation computer was a Mac mini, Intel Core 2 Duo (8 GB memory), NVIDIA GeForce 320M Graphic card. Continuous EEG data were recorded using EGI’s Netstation Version 4.3 data acquisition software (Tucker, 1993; Electrical Geodesics Inc., Eugene, OR) at 500hz (samples every 2ms) run on a MacBook Pro running Snow Leopard 10.6.8. Stimuli were presented via Psykinematix, psychophysical presentation software for investigating visual impairment due to neurological dysfunction (Beaudot, 2009). Psykinematix allows for designing visual psychophysics experiments through an intuitive graphical user interface that can sync with NetStation (Beaudot, 2009).

4.3.2 Display. Visual stimuli were presented using a 24" NEC MultiSync PA241W LCD display. This display has a native resolution of 1920 x 1200 and refresh rate of 60Hz.

4.3.3 Display calibration. In order to calibrate the 24" NEC MultiSync LCD monitor, a Minolta LS used display calibration settings recommended by Psykinematix (KyberVision Japan LLC, 2016). A MD100 photometer and a Spyder Elite 4 were used to confirm luminance (light) levels of the monitor. The display calibration were performed every 30 days, as recommended by Psykinematix, and consisted of calibrating for geometry, gamma, and color. The geometry calibration is to assure spatial properties such as stimulus size and spatial frequency are displayed accurately. The gamma calibration corrects for intrinsic non-linearities in the monitor’s display luminance to minimize luminance artifacts. Although the experimental stimuli were grayscale, a color calibration was performed by measuring the spectral emission of the display. The display calibration instructions can be found in Appendix 10.1.

4.3.4 Response box. A Cedrus Response Box RB-730 was used to record button press responses to the appearance of an emoticon during stimulus presentation.
4.3.5 Timing tests. A Cedrus Stim Tracker was used to conduct timing tests and provide offset values for data preprocessing. Timing tests were run on the same day as each participant’s EEG session in order to enter the precise offset.

4.3.6 EEG run rooms. Recording chambers were sound attenuated and lit with Nemalux LED lighting (XCANLEDAC- C1D2) fixtures with dimmable power supply and minimal electrical noise. Specific luminance measurements are described further below.

4.4 EEG Experiment

4.4.1 Spatial suppression N1 paradigm. The visuo-spatial suppression task consisted of viewing vertical grayscale sinusoidal gratings drifting either right or left at a consistent speed. The stimuli implemented were similar to those used in behavioral studies by Golomb et al., 2009; Foss-Feig, Taudin, & Schauder, 2013; Horder et al., 2014.

Stimuli were programmed using Psykinematix (Beaudot, 2009) and consisted of one-cycle/degree drifting vertical monochrome gratings surrounded by two-dimensional Gaussian envelopes. Stimulus size (large vs. small) is either 5.0° or 0.7° and stimulus contrast (bright vs. faint) is either 92% or 2.8%. Contrast was defined as \((L_x - L_{ave})/L_{ave}\), where \(L_x\) represents the contrast of a single pixel and \(L_{ave}\) represents the average luminance value of the NEC LCD display (Golomb et al., 2009; Foss-Feig, Taudin, & Schauder, 2013). The stimuli were presented on a uniform gray background so that luminance was consistent across all conditions at 40cd/m², with only contrast and size varying. There were four conditions of stimuli:

*Condition 1:* Small/High-Contrast Vertical Grayscale Sinusoidal Grating

*Condition 2:* Large/High-Contrast Vertical Grayscale Sinusoidal Grating
**Condition 3:** Small/Low-Contrast Vertical Grayscale Sinusoidal Grating

**Condition 4:** Large/Low-Contrast Vertical Grayscale Sinusoidal Grating

The four conditions were divided into separate blocks, each consisting of 250 trials. Block order was counterbalanced across participants. Each individual trial began with a blank screen appearing for 150 ms. A grayscale vertical sinusoidal grating of either high (92%) or low (2.8%) contrast and small (0.7°) or large (5.0°) size then appeared in the center of the screen. The vertical sinusoidal gratings drifted either left or right (at 50% chance) at a rate of 2°/s, for 200ms. To maintain attention, participants were instructed to press “1” on the response box when an emoticon appeared as an attentional target (10% of trials per block). Interstimulus interval was randomized, ranging from 600 to 1000ms. There were four blocks, each consisting of 250 trials, making the total experiment run time approximately 20 minutes, plus additional time for impedance checking and to allow for varying button-press response rates.

Figure 3: Schematic of the Visuo-Spatial suppression ERP Task
4.5 Participants

All study procedures were approved by the Teachers College, Columbia University Institutional Review Board. Administrations of the Stanford-Binet Intelligence Scales, Fifth Edition (SB5) Abbreviated IQ and the Autism Diagnostic Observation Schedule, Second Edition (ADOS-2) were also approved by the Weill Cornell Medical College (WCMC) Institutional Review Board for screening purposes that took place at the Center for Autism and the Developing Brain at NewYork-Presbyterian’s Westchester Division. Informed consent was obtained from a parent or legal guardian of all participating children. Verbal assent was acquired from all children cognitively capable of providing assent before study participation. Participants were assured that they could stop participating at any time during the duration of the study without penalty. Participants were given a $25 gift card from Amazon or Toys “R” Us as a thank-you for participating. Compensation was paid for by a Teachers College, Columbia University Dean’s Grant that was awarded for this study in 2013.

Participants were recruited from the tri-state area through advertising at Teachers College, Columbia University and nearby institutions with programs for children with ASD, support groups for parents of children on the autism spectrum, word of mouth, and social media postings (e.g., Facebook, online Teachers College, Columbia University message board). Participants from the experimental group were also recruited through the Center for Autism and the Developing Brain (CADB), a collaborative program between NewYork-Presbyterian, Weill Cornell Medical College, and Columbia University College of Physicians and Surgeons. Current patients who indicated they were interested in being contacted about research studies, prior patients who had consented to be contacted for future research studies, and patients on the waiting list for the CADB clinic were contacted using the recruitment brochure.
For the experimental group, ASD classification was based on DSM-5 diagnostic criteria for autism spectrum disorder and confirmed using the Autism Diagnostic Observation Schedule, Second Edition (ADOS-2) by a research reliable rater (Lord et al., 2012). In order to meet instrumental criteria for a diagnosis of autism or autism spectrum disorder, an individual was required to have an overall total score greater than the cut-off score for autism or autism spectrum disorder for a specific module, as described in Tables 3-6, above. Comparison participants were medically healthy and had no current or prior psychiatric diagnoses.

Exclusion criteria included: Fragile-X Syndrome, Down syndrome, traumatic brain injury (TBI), a history of epilepsy or seizures, intellectual disability (IQ less than 75), a contrast-sensitivity impairment, or a visual impairment unable to be corrected with glasses. Comparison participants were excluded from the current study if they had a prior DSM-IV or DSM-V diagnosis, a history of developmental delays, or a first-degree relative with ASD. Comparison children were also excluded if they scored in the range of ASD on the CARS-2.

The following participants were recruited for this study: 40 children from 6 to 12 years of age, 21 with a diagnosis of autism spectrum disorder, and 19 neurotypical (NT) comparison children. All participants reported no history of neurological disorder, and had normal or corrected-to-normal vision. Additionally, all participants had normal contrast sensitivity function (as indicated by the Pelli-Robson Contrast Sensitivity Chart). All participants reported no use of psychiatric medications within the last six months.

4.6 Sample Size and Power Calculations

In any given research study, the sample size must be sufficient to exhibit an experimental effect and adequately represent the population in which results will be generalized (Picton et al.,
Since ASD is a heterogeneous disorder, a sufficient number of individuals must be enrolled in order to determine whether a given experimental effect is autism-specific. However, power analyses to calculate the minimum necessary sample size in ERP studies are particularly difficult, and research tools available to fMRI researchers to generate power curves are not yet available for ERP researchers (Mumford, 2012). Estimation of power requires researchers to know the effect size (expected percent signal change between conditions) and an estimation of the variability in signal change. These factors are generally not well defined in neuroimaging studies. In ERP studies, signal-to-noise ratios are typically low due to repeated presentation of given stimuli during each individual condition.

This study consisted of approximately five to seven minutes of EEG recording during four separate conditions, for a total of 20 minutes of recording time, depending on response time for the attentional component (button press in response to emoticons). The raw data collected consisted of continuous digital recordings at a rate of 500 samples per second of voltage deflections at 128 electrodes on each participant’s scalp. During this ERP experiment 210,000 data points for each of the 128 electrodes for each condition for each participant were collected (500 samples/second x 60 seconds/minute x 7 minutes/condition). Within the time-series data, there were two sources of variability of interest: within-subject time course variability (fluctuations from one time point to another), and within-subject experimental variability (variation in the effectiveness of each experimental condition in producing a percentage signal change). Sample sizes and numbers of trials per condition were therefore established using available guidelines for ERP researchers (e.g., Handy, 2005; Luck, 2005; Luck & Kappenman, 2012) and relying on prior studies of the same population and/or ERP component.
This ERP study probed the N1 ERP component. The visual N1 ERP is a large, negative visual evoked potential (VEP) that peaks approximately 100 to 250 ms after stimulus presentation in neurotypical adults (Johannes, Munte, Heinze, & Mangun, 1995; Vogel & Luck, 2000). To our knowledge, this study was the first to implement the presented experimental paradigm to examine N1 ERP latency and amplitude fluctuations in neurotypical children as well as those with ASD. A minimum of 40 to 150 trials are typically implemented when targeting small- to medium-sized ERP components to provide a sufficient signal-to-noise ratio (Handy, 2005). This study had 225 experimental trials and 25 attentional trials per condition, which provided a sufficient balance between the number of trials presented and the total length of runtime for research with a vulnerable population. Behavioral studies of spatial suppression in individuals with autism, major depressive disorder, and schizophrenia that have implemented similar visual stimuli have enrolled an average of 14 experimental participants and 15 control participants (Foss-Feig et al., 2013; Golomb et al., 2009; Tadin et al., 2006). An EEG study of spatial suppression in neurotypical adults that measured steady-state visual evoked potentials enrolled 21 participants (Vanegas, Blangero, & Kelly, 2015), while a similar study enrolled 13 young adults with autism and 12 neurotypical young adults (Takarae Sablich, White, & Sweeney, 2016). Visual N1 studies of individuals with autism have enrolled an average of 13 experimental and 12 control participants. The current study enrolled 40 participants, with usable data obtained from 32 participants, which was consistent with existing literature in this field.
5. DATA ACQUISITION

5.1 Electroencephalography Electrode Nets

All acquisition of EEG data took place at the Neurocognition of Language Lab, in the Department of Biobehavioral Studies at Teachers College, Columbia University. The Neurocognition of Language Lab uses Electrical Geodesics, Inc. (EGI) 128-electrode high-density, HydroCel Geodesic Sensor nets (Electrical Geodesics; Tucker, 1993), which consist of carbon fiber silver chloride electrodes embedded in small, soft sponges woven into a geodesic array. This system allows for fast and precise application of large numbers of electrodes in high-density arrays without scalp abrasion or electrode gel. The circumference of each participant’s head was measured to ensure the correctly sized sensor net was selected. The net was then soaked in a potassium-chloride solution for five minutes; the solution consisted of one liter of purified water, two teaspoons of potassium chloride, and three ccs of Johnson & Johnson baby shampoo to break down oils on the scalp. Prior to placing the net, the vertex (center of the head) was marked to ensure accurate net placement. The electrode net was then connected to a high impedance amplifier (EGI NetAmps 200 Series) manufactured by Electrical Geodesics.

5.2 Electroencephalography Recording and Experimental Procedures

For the EEG portion of the experiment, the participant was seated in an electrically shielded and sound attenuated testing chamber containing the computer monitor that delivered task instructions and stimuli. Thirty minutes prior to each recording session, monitors were turned on to allow luminance levels to stabilize to 120 cd/m². The ambient room lighting was
held constant across experimental blocks at low light conditions of 15.1 volts (mesopic vision\(^4\)).

To minimize the effects of environmental electrical noise, participants were seated in a wooden chair 46” away from the monitor and provided with a footrest if necessary. The average room temperature and humidity was recorded.

In order to precisely correlate recorded electrical activity to specific locations on each participant’s scalp, we placed EGI’s 128 Geodesic Sensor Nets (Tucker, 1993) on each participant’s head following measurement of head circumference and marking of the vertex. As previously mentioned, the electrodes were embedded in sponges, which were soaked in a potassium-chloride (KCl) solution to minimize impedances. The EGI NA200 amplifier was checked and calibrated prior to plugging in the net. Continuous EEG data were recorded using EGI’s Netstation (v4.5.6) data acquisition software with a sampling rate of 500 Hz, or a sample taken every two ms. Prior to digitization to prevent antialiasing of the signal, raw EEG data were filtered using an analog low-pass filter determined based on the Nyquist frequency of the sampling rate. After the sensor net was applied, individual sensors were adjusted so that they were in good contact with the scalp. Impedances were measured by feeding a 400-microvolt electrical field through each electrode, which was then read back by the acquisition system to calculate electrode impedance values. Electrodes that had impedances greater than 40kΩ were re-positioned and rehydrated with potassium chloride as needed. Since this experiment had multiple blocks, electrode impedance was reassessed after each block and electrodes were further rehydrated with potassium-chloride solution as needed. The data recording was monitored in real

\(^4\) Mesopic light levels range from luminance values of 0.001 to 3 cd m\(^{-2}\). Mesopic vision is a combination of photopic vision (well-lit conditions) and scotopic vision (low-light conditions) (Stockman & Sharpe, 2006).
time, and bad channels and artifacts were noted and marked so that they could be addressed offline during analysis.

5.3 Experimental Protocol

1. The parent or guardian of each child participant was presented with a consent form and asked to read it carefully. Risks were explained fully and any questions were answered before the participant’s parent signed the form. The parent and child were reminded that they could stop participating at any time. The parent was given a $25 gift card at this time for his or her child’s participation.

2. The researcher read a social story with each child in order to familiarize him/her with the lab and the tasks that s/he would carry out during the visit. If cognitively capable, the child assented to the study.

3. The child and parent were shown around the lab and familiarized with the equipment and experimental tasks. Questions were encouraged and answered throughout the laboratory introduction. A written or picture schedule was provided for each child based on his/her developmental ability in order to alleviate anxiety in this unfamiliar situation.

4. The child completed the visual acuity screening and the Pelli-Robson Contrast Sensitivity Chart.

5. The parent/guardian(s) was provided with the following questionnaires that were completed while their child was completing assessments with the research staff: Sensory Experiences Questionnaire, Childhood Autism Rating Scale, Second Edition Parent Questionnaire, Autism Center of Excellence (ACE) Family Medical History Questionnaire, and a
demographic questionnaire. Parent/guardian(s) were encouraged to ask questions about the questionnaires. A research assistant was available to answer these questions.


7. The head circumference of each child was measured and the net size corresponding with that measurement was determined. The researcher then measured the vertex of the head according to lab protocol in order to ensure proper placement of net electrodes. During this time, the child was engaged in activities designed to decrease anxiety and ensure participant comfort with the situation; for example, measuring the lab teddy bear’s head and putting a net on him; or watching a video on the lab iPad.

8. Using the written or picture schedule as a guide, the child was introduced to the experimental tasks and equipment.

9. The child was fitted with the appropriate 128-channel HydroCel Geodesic Sensor Net (HCGSN) (Net Amps 200, Electric Geodesics Inc., Eugene, OR). Electrodes were referred to the vertex marking made previously on the child’s scalp.

10. The child was seated in a chair in a sound attenuated chamber within the Neurocognition of Language Lab. A video camera provided the researcher with visual information about the child during the experiment. The child was reminded to signal at any time during the experiment if he or she did not wish to continue. Parents were able to sit in the room during the EEG portion of the study at the child’s request. The amplifier was checked and calibrated, the net was connected, and impedances (loss of signal between scalp and sensor) were measured. In order to improve impedances, the electrodes were adjusted as necessary, so that they were in good contact with the participant’s scalp.
11. If cognitively capable of understanding, the child completed an artifact reduction demonstration, which consisted of watching the effects on live EEG recordings of various movements, including blinking, moving his/her head, and clenching his/her jaw while growling like a tiger. This demonstration was used to help the child understand why he or she was being asked to remain as still as possible while completing the experiment. The research team then told the child that he/she was going to see some lines either moving right or left on the screen for about seven minutes. Participants were instructed to press the response box each time they saw an emoticon. The experiment was discontinued if the child became distressed during the recording.

12. Following the experiment, the net was removed. Parents and participants were debriefed and had the opportunity to ask any further questions.
6. DATA PROCESSING AND ANALYSES

6.1 Data Pre- and Post-Processing

Raw EEG data were pre-processed using NetStation, version 4.5.6 (Electrical Geodesics Inc), following standard protocols for ERP analysis (outlined by Handy, 2005; Luck, 2005; Picton et al., 2007; Handy, 2014). Raw data were digitally filtered offline using a using a 30Hz lowpass and a 0.1Hz highpass filter (Parks, Beck, & Kramer, 2013; Luck, 2014; Gannon, Knapp, Adams, Long, & Parks, 2016). Data were subjected to automatic artifact rejection protocols for the remove of movement and physiological artifacts. To remove bad channels (defined as >150µV), blink artifacts (defined as >140µV), and eye saccades (defined as >55µV) we used an in-house NetStation script. Segments were marked as bad and automatically excluded from analyses if more than 10% of channels were outside the bad channel, blink, or eye saccade thresholds. Channels with fluctuations over 100µV were replaced and interpolated using spherical spline interpolation based on recorded data from surrounding sensors. Therefore, only trials free of artifacts from participant movements were included in ERP averages for each condition. Data were re-referenced to an average reference, which was calculated by subtracting the mean of all electrodes from each individual channel (DeBoer, Scott, & Nelson, 2005). Re-referencing to an average reference is most effective in high-density systems consisting of more than 64 electrodes (DeBoer, Scott, & Nelson, 2005).

The ERP waveform was segmented into 600 ms epochs, including 200 ms pre-stimulus (baseline) and 400 ms post-stimulus. Individual segments were averaged together for each condition in order to identify time-locked event-related responses associated with the onset of the sinusoidal gratings. Segments were baseline corrected using the 200 ms before the start of the epoch, when no stimuli were being presented, to provide an average of brain activity unrelated to
stimulus processing. Data were then exported from NetStation into individual text files for each condition, for each participant.

6.2 Data Analysis Protocol

Post-processed data files were read into R version 3.1.2 (1.65 Mavericks build). A data frame was produced and a montage was applied to the data in order to examine responses recorded by fronto-central electrodes (see Figure 4 below). Prior research has shown that the N1 occurs at central electrode sites, especially when modulated by visual attention (e.g., Mangun & Hillyard, 1991; Hennighausen, Remschmidt, & Warnke, 1994). Using in-house R scripts, we determined peak latency and amplitude for the N1 ERP for each individual participant during each of the four conditions. A peak latency analysis requires determination of the maximum amplitude (in this case, the maximum negative deflection) within a pre-determined time window. Peak latency and amplitude were visually examined and defined as the time and magnitude of the greatest negative deflection between 85 and 220 ms after stimulus presentation. This study followed a 2 X 2 mixed factorial design, which is described in Section 4.1. The assumption of normality was tested prior to conducting within- and between-group analyses, using Levene’s statistic and the Shapiro-Wilk test of normality. A one-way analysis of variance (ANOVA) was conducted for the high- and low-contrast experiments separately to test for between-group effects (Size: Small (0.7°), Large (5.0°) x Group: Autism spectrum disorder, Neurotypical). In order to determine within-group differences, paired-samples t-tests were conducted for the high- and low-contrast experiments separately (Small x Large, Autism spectrum disorder; Small x Large, Neurotypical). For data presentation, individual files were grand-averaged together by group
(ASD vs. NT) for each individual condition. Grand average ERP waveforms were created in R by averaging conditions across participants.

Figure 4: N1 Fronto-central Montage of Electrodes
7. RESULTS

7.1 Participants.

Forty children from 6 to 12 years of age, 21 with a diagnosis of autism spectrum disorder (ASD) and 19 neurotypical (NT) comparison children, were recruited to participate. Eight participants were excluded from the present study due to: unwillingness to wear the EEG net (n = 2), participant movement resulting in too few usable trials per condition (n = 4), abbreviated IQ < 75 (n = 1), and current use of psychiatric medication (n = 1). After exclusions, 32 participants were included in data analysis, 16 with a diagnosis of ASD, and 16 NT comparison children. Although the ASD group (mean age = 8.04 years, SD = 1.78) was younger on average than the NT group (mean age = 9.44 years, SD = 2.29), there was no statistically significant difference in chronological age between the two groups (t (30) = -1.92, p = .06). Further demographic information is provided in Table 7 and Figure 5, below.

<table>
<thead>
<tr>
<th></th>
<th>Autism (n=16)</th>
<th>Neurotypical (n=16)</th>
<th>Total (n=32)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>11</td>
<td>9</td>
<td>20</td>
</tr>
<tr>
<td>Female</td>
<td>5</td>
<td>7</td>
<td>12</td>
</tr>
</tbody>
</table>
## Table 7. Demographic Information

Demographic information for participants with autism spectrum disorder, neurotypical comparison participants, and combined demographic information.

<table>
<thead>
<tr>
<th>Age in years: Mean (SD)</th>
<th>Autism (n=16)</th>
<th>Neurotypical (n=16)</th>
<th>Total (n=32)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.04 (1.78)</td>
<td>9.44 (2.29)</td>
<td>8.94 (2.14)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Race</th>
<th>Autism (n=16)</th>
<th>Neurotypical (n=16)</th>
<th>Total (n=32)</th>
</tr>
</thead>
<tbody>
<tr>
<td>African-American</td>
<td>4</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>Asian-American</td>
<td>1</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>Caucasian</td>
<td>6</td>
<td>9</td>
<td>15</td>
</tr>
<tr>
<td>Hispanic/Latino</td>
<td>3</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Native American</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Native American/African-American</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Ethnicity</th>
<th>Autism (n=16)</th>
<th>Neurotypical (n=16)</th>
<th>Total (n=32)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hispanic or Latino</td>
<td>7</td>
<td>2</td>
<td>9</td>
</tr>
<tr>
<td>Not Hispanic or Latino</td>
<td>9</td>
<td>14</td>
<td>23</td>
</tr>
</tbody>
</table>

*46% of the sample population represented U.S. racial or ethnic minority groups.*
7.2. Assessment Results

7.2.1 Visual acuity screening. All participants had normal or corrected-to-normal visual acuity on the Snellen Eye Chart.

7.2.2 The Pelli-Robson Contrast Sensitivity Chart. All participants scored within the normal range (>1.50) on the Pelli-Robson Contrast Sensitivity Chart. There was no significant difference in contrast sensitivity function on the Pelli-Robson Contrast Sensitivity Chart between children with ASD ($M = 1.83$, $SD = .11$) and neurotypical comparison children ($M = 1.80$, $SD = .14$); $t (30) = .80$, $p = .42$).

7.2.3 Stanford-Binet Intelligence Scales, Fifth Edition (SB5), Abbreviated IQ. All participants had average or above average abbreviated IQ on the Stanford-Binet Intelligence Scales, Fifth Edition (SB5). Abbreviated IQ ranged from 94 to 130 for children with ASD and from 85 to 155 for the neurotypical comparison group. There was no significant difference in abbreviated IQ on the SB5 between children with ASD ($M = 105$, $SD = 10.05$) and neurotypical
children ($M = 110.56, SD = 17.86$); $t (30) = -1.02, p = .31)$. Figure 6 depicts the distribution of ABIQ for children with ASD and neurotypical comparison children.

![Figure 6](image)

Figure 6. Abbreviated IQ Distribution: Histograms depicting the distribution of abbreviated IQ in children with autism spectrum disorder (left) and neurotypical comparison participants (right).

### 7.2.4 Autism Diagnostic Observation Schedule, Second Edition (ADOS-2)

ADOS-2 scores for the experimental group confirmed that all participants were on the autism spectrum. ADOS-2 calibrated severity scores ranged from 6 to 10 ($M = 7.56, SD = 1.36$). Half of the participants in the experimental group ($n = 8$) were categorized as having a high level of autism-spectrum related symptoms, and half ($n = 8$) were categorized as having a moderate level of autism-spectrum related symptoms.
<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>Standard Deviation</th>
<th>Standard Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADOS-2 Overall Total</td>
<td>13.75</td>
<td>3.39</td>
<td>0.84</td>
</tr>
<tr>
<td>ADOS-2 Social Affect Total</td>
<td>10.12</td>
<td>2.47</td>
<td>0.61</td>
</tr>
<tr>
<td>ADOS-2 Restricted Repetitive Behavioral Total</td>
<td>3.62</td>
<td>1.31</td>
<td>0.32</td>
</tr>
<tr>
<td>ADOS-2 Calibrated Severity Score</td>
<td>7.56</td>
<td>1.36</td>
<td>0.34</td>
</tr>
</tbody>
</table>

Table 8. ADOS-2 Scores: Mean score, standard deviation, and standard error for the ADOS-2 overall total, social affect domain, restricted repetitive behavior domain, and calibrated severity scores for children in the experimental group. All experimental participants met instrument criteria for a diagnosis of autism or autism spectrum disorder.


7.2.6 Sensory Experiences Questionnaire, Second Edition (SEQ 2.1). In children with ASD, SEQ mean scores ranged from 1.49 to 3.09 ($M = 2.45$, $SD = .40$) with $n = 6$ participants classified as in the “typical range,” $n = 5$ participants classified as in the “at risk” range, and $n = 5$ participants classified as in the “deficient” range. Mean hypo-responsiveness scores ranged from 1.33 to 3.00 ($M = 2.07$, $SD = .52$), and mean hyper-responsiveness scores ranged from 1.78 to 3.35 ($M = 2.57$, $SD = .40$) in the experimental group. Based on Table 9, below, $n = 6$ participants were classified as hyper-responsive, $n = 1$ participant was classified as hypo-
responsive, n = 8 participants were classified as being both hyper- and hypo-responsive, and n = 1 participant was classified as having no pattern of responsiveness to sensory stimuli from the experimental group. Among the neurotypical comparison participants, mean scores range from 1.00 to 2.87, with (M = 1.53, SD = .44), with n = 15 participants classified as in the “typical range” and n = 1 participant classified as being in the “deficient” range. Mean hypo-responsiveness scores ranged from 1.00 to 2.83 (M = 1.40, SD = .49), and hyper-responsiveness scores ranged from 1.00 to 2.78 (M = 1.55, SD = .45) in the neurotypical comparison group. One participant from the neurotypical comparison group was classified as being hyper- and hypo-responsive to sensory stimuli, and the remaining n = 15 participants from the comparison group showed no specific pattern of responsiveness. These findings are summarized in Table 9 and Figure 7, below.

<table>
<thead>
<tr>
<th></th>
<th>Reference Norms for Typical Children</th>
<th>Cut-off for Typical Range Scores between 0 and -1 SD</th>
<th>Cut-off for At Risk Range Scores between -1 and -2 SD</th>
<th>Cut-off for Deficient Range Scores &gt; 2 SD</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>TOTAL RAW SCORE</strong></td>
<td>62.49 (11.50)</td>
<td>33 - 74</td>
<td>75 – 86</td>
<td>87 - 165</td>
</tr>
<tr>
<td><strong>Hypo-responsive</strong></td>
<td>8.68 (1.91)</td>
<td>6 - 10</td>
<td>11 – 12</td>
<td>13 - 70</td>
</tr>
<tr>
<td><strong>Hyper-responsive</strong></td>
<td>24.78 (8.68)</td>
<td>14 - 29</td>
<td>30 – 34</td>
<td>35 - 70</td>
</tr>
</tbody>
</table>

Table 9. Sensory Experiences Questionnaire, Version 2.1 Criterion Cut Points.
Figure 7. Sensory Experiences Questionnaire Distribution: *Histograms depicting the distribution of sensory experiences questionnaire total mean scores, hypo-responsiveness mean scores, and hyper-responsiveness mean scores in children with ASD.*

### 7.3 EEG Results

Following data pre- and post-processing (described in section 6.1), number of useable trials was documented for each participant. A two-way ANOVA was conducted to examine the effect of group (ASD, NT) and condition (Small, Large) on number of usable trials in the high-contrast experiment. Although children with ASD had fewer usable trials per condition (Small, High Contrast $M = 96.87$, SD = 51.93; Large, High Contrast $M = 98.50$, SD = 51.77) than NT comparison children (Small, High Contrast $M = 125.68$, SD = 54.68; Large, High Contrast $M = 125.62$, SD = 57.43), this difference was not statistically significant ($F(1, 60) = .01, p = .90$). A two-way ANOVA was also conducted to examine the effect of group (ASD, NT) and condition (Small, Large) on number of usable trials in the low-contrast experiment. Although children with ASD had fewer usable trials per condition (Small, Low-Contrast $M = 97.50$, SD = 55.10; Large, Low-Contrast $M = 98.07$, SD = 54.28) than NT comparison children (Small, Low-Contrast $M = 148.07$, SD = 41.23; Large, Low-Contrast $M = 142.78$, SD = 52.31), this difference was not statistically significant ($F(1, 52) = .16, p = .68$). The threshold for inclusion based on useable trials per condition was set at 40 usable trials per condition.
7.3.1 High-contrast experiment.

7.3.1.1 Within-group peak latency analyses. Paired samples t-tests were conducted to evaluate the significance of N1 peak latency differences in processing small, high-contrast sinusoidal gratings versus large, high-contrast sinusoidal gratings for children with ASD and neurotypical comparison children, separately. Latencies were normally distributed for both small, high-contrast and large, high-contrast conditions in children with ASD, as assessed by Shapiro-Wilk’s test (Small, High-Contrast: \( p = .20 \), Large, High-Contrast: \( p = .66 \)). As predicted, in the high-contrast experiment participants with ASD demonstrated enhanced processing of large, high-contrast sinusoidal gratings (N1 mean peak latency = 121.06ms, SD = 19.81) compared to small, high-contrast sinusoidal gratings (N1 mean peak latency = 148.50ms, SD = 28.95) as indexed by significantly shorter N1 ERP latencies to large, high-contrast stimuli \( (t (15) = 3.25, p = .005, d = .81) \). Latencies were also normally distributed for both small, high-contrast and large, high-contrast conditions in neurotypical comparison children, as assessed by Shapiro-Wilk’s test (Small, High-Contrast: \( p = .37 \), Large, High-Contrast: \( p = .47 \)). Neurotypical comparison participants demonstrated delayed processing of large, high-contrast sinusoidal gratings (N1 mean peak latency =139.38ms, SD = 26.38) compared to small, high-contrast sinusoidal gratings (N1 mean peak latency = 125.00ms, SD = 13.54) as indexed by significantly longer N1 ERP latencies to large, high-contrast stimuli \( (t (15) = -2.12, p = .05, d = .53) \).
7.3.1.2 Between-group peak latency analysis. A one-way analysis of variance (ANOVA) was conducted to evaluate the significance of N1 latency differences in processing large, high-contrast sinusoidal gratings between children with ASD and neurotypical comparison children. Latencies were normally distributed for both children with ASD and neurotypical children in the large, high-contrast condition as assessed by Shapiro-Wilk’s test (ASD: \( p = .66 \), NT \( p = .47 \)). As predicted, in the high-contrast experiment, participants with ASD demonstrated more rapid N1 responses to large, high-contrast sinusoidal gratings (N1 mean peak latency = 121.06ms, SD = 19.81) compared to the NT group (N1 mean peak latency =139.38ms, SD = 26.38), (F (1, 30) = 4.92, \( p = .03 \), \( \eta^2 = 0.14 \)). A one-way analysis of variance (ANOVA) was also conducted to evaluate the significance of N1 latency differences in processing small, high-contrast sinusoidal gratings between children with ASD and neurotypical comparison children. Although latencies were normally distributed for both children with ASD and neurotypical children in the small, high-contrast condition as assessed by Shapiro-Wilk’s test (ASD: \( p = .20 \), NT \( p = .37 \)), the assumption of homogeneity of variances was violated, as assessed by Levene’s test for equality.
of variances \((p = .01)\). Therefore, a Mann-Whitney U test was run to determine differences in processing small, high-contrast sinusoidal gratings between children with ASD and neurotypical comparison children. The distribution of latencies for children with ASD and neurotypical comparison children were not similar, as assessed by visual inspection (Figure 9). N1 latency for children with ASD (N1 mean peak latency = 148.50ms, SD = 28.95, mean rank = 20.88) was statistically significantly longer than for neurotypical children (N1 mean peak latency = 125.00ms, SD = 13.54, mean rank = 12.13), \((U = 58, z = -2.64, p = .007)\). Although children with autism had a younger mean chronological age than neurotypical comparison participants (ASD mean age = 8.04 years, SD = 1.78; NT mean age = 9.44 years, SD = 2.29), a standard multiple regression established that chronological age does not predict N1 latencies to small, high-contrast or large, high-contrast sinusoidal gratings in children with ASD; \(F (2,13) = 1.52, p = .25\), adj. \(R^2 = .06\) or neurotypical children; \(F (2,13) = 4.42, p = .41\), adj. \(R^2 = -.009\).

Figure 9. Small, High-Contrast Latency Distributions: Distribution of N1 peak latencies for children with ASD and neurotypical comparison children for the small, high-contrast condition.

7.3.1.3 Between-group peak amplitude analysis. A one-way analysis of variance (ANOVA) was conducted to evaluate the significance of N1 peak amplitude differences in

1.52, \(p = .25\), adj. \(R^2 = .06\) or neurotypical children; \(F (2,13) = 4.42, p = .41\), adj. \(R^2 = -.009\).
processing large, high-contrast sinusoidal gratings between children with ASD and neurotypical comparison children. Although amplitudes were normally distributed for both children with ASD and neurotypical children in the large, high-contrast condition as assessed by Shapiro-Wilk’s test (ASD: $p = .92$, NT $p = .81$), the assumption of homogeneity of variances was violated, as assessed by Levene’s test for equality of variances ($p = .03$). Therefore, a Mann-Whitney U test was run to determine differences in processing large, high-contrast sinusoidal gratings between children with ASD and neurotypical comparison children. The distributions of amplitudes for children with ASD and neurotypical comparison children were not similar, as assessed by visual inspection (Figure 10). Although N1 amplitude for children with ASD (N1 peak amplitude = -4.04, SD = 2.14, mean rank = 15.28) was greater in magnitude than NT comparison children (N1 peak amplitude = -3.48, SD = 1.31, mean rank = 17.72), this difference was not statistically significant ($U = 108.50$, $z = - .73$, $p = .46$).

The same analysis (one-way ANOVA) was conducted to evaluate the significance of N1 peak amplitude differences in processing small, high-contrast sinusoidal gratings between children with ASD and neurotypical comparison children. Latencies were normally distributed for both children with ASD and neurotypical children in the small, high-contrast condition, as assessed by Shapiro-Wilk’s test (ASD: $p = .27$, NT $p = .24$). Although N1 amplitude for children with ASD (N1 peak amplitude = -2.96, SD = 2.01) was smaller in magnitude than for NT comparison children (N1 peak amplitude = -3.36, SD = 1.44), this difference was not statistically significant ($F (1, 30) = .39$, $p = .53$, $\eta^2 = 0.01$). These comparisons are depicted in Figures 10 and 11, below.
Figure 10. Large, High-Contrast Amplitude Distributions: *Distribution of amplitudes for children with ASD and neurotypical comparison children for the large, high-contrast condition.*

Figure 11. Within-Group Low-Contrast ERP Plots: *Children with autism spectrum disorder exhibited enhanced processing of large, high-contrast sinusoidal gratings as compared to neurotypical comparison children (left). Children with autism spectrum disorder exhibited delayed N1 responses to small, high-contrast sinusoidal gratings as compared to neurotypical comparison children (right).*

7.3.1.4 Peak latency: correlations with cognitive and behavioral measures. A Pearson’s product-moment correlation revealed no significant correlations between N1 latency for small, high-contrast sinusoidal gratings in children with ASD or NT children and the following measures: abbreviated IQ (ASD: \( p = .89 \), NT: \( p = .53 \)), total item mean scores on the SEQ (ASD:
p = .85, NT: p = .28), hypo-responsiveness item mean scores on the SEQ (ASD: p = .71, NT: p = .36), hyper-responsiveness item mean scores on the SEQ (ASD: p = .64, NT: p = .55), or ADOS-2 Calibrated Severity Scores (ASD: p = .48). Similarly, no significant correlations were found between N1 latency for large, high-contrast sinusoidal gratings in children with ASD or NT children and abbreviated IQ (ASD: p = .35; NT: p = .33), total item mean scores on the SEQ (ASD: p = .62, NT: p = .32), hypo-responsiveness item mean scores on the SEQ (ASD: p = .62, NT: p = .43), hyper-responsiveness item mean scores on the SEQ (ASD: p = .41, NT: p = .52), or ADOS-2 Calibrated Severity Scores (p = .88).

7.3.1.5 Peak amplitude: correlations with cognitive and behavioral measures. Pearson’s product-moment correlations again revealed no significant correlations between N1 amplitude for small, high-contrast sinusoidal gratings in children with ASD or NT children and the following measures: abbreviated IQ (ASD: p = .99, NT: p = .49), total item mean scores on the SEQ (ASD: p = .25, NT: p = .43), hypo-responsiveness item mean scores on the SEQ (ASD: p = .98, NT: p = .30), hyper-responsiveness item mean scores on the SEQ (ASD: p = .10, NT: p = .52), or ADOS-2 Calibrated Severity Scores (ASD: p = .27). A strong negative correlation was found between N1 amplitude for large, high-contrast sinusoidal gratings and hyper-responsiveness item mean scores on the SEQ for children with ASD (r = .61, p = .01), but not for NT children (p = .52). No other significant correlations were found between N1 amplitude for large, high-contrast sinusoidal gratings and the following measures: abbreviated IQ (ASD: p = .70, NT: p = .97), total item mean scores on the SEQ (ASD: p = .09, NT: p = .69), hypo-responsiveness item mean scores on the SEQ (ASD: p = .81, NT: p = .63), or ADOS-2 Calibrated Severity Scores (ASD: p = .19). These correlations are depicted in Figure 12, below.
Figure 12. Relationship between Large, High-Contrast N1 Response Amplitude and Hyperresponsiveness in Children with autism spectrum disorder: *Scatterplot depicting mean hyperresponsiveness scores on the x-axis and N1 response amplitude to large, high-contrast stimuli in absolute values on the y-axis. There is a strong negative correlation between N1 amplitude for large, high-contrast sinusoidal gratings and hyper-responsiveness item mean scores on the SEQ for children with ASD.*

7.3.2 Low-contrast experiment. Since the threshold for useable trials per condition was set at 40, two participants from the experimental group and two participants from the comparison group were excluded from analysis for low-contrast conditions due to < 40 usable trials per condition. After exclusions, 28 participants were included in data analysis for the low-contrast experiment (ASD: 14; NT: 14). Although the ASD group after exclusions was younger on average than the NT group after exclusions (ASD mean age = 8.32 years, SD = 1.72; NT mean age = 9.47 years, SD = 2.10), there was no significant difference in chronological age between the two groups (t (1, 26) = 2.46, p = .12).

7.3.2.1 Within-group peak latency analyses. Paired samples t-tests were conducted to evaluate the significance of N1 peak latency differences in processing small, low-contrast sinusoidal gratings versus large, low-contrast sinusoidal gratings for children with ASD and
neurotypical comparison children, separately. Latencies were not normally distributed for small, low-contrast and large, low-contrast conditions in children with ASD as assessed by Shapiro-Wilk’s test (Small, Low-Contrast: $p = .004$, Large, Low-Contrast: $p = .008$), but the assumption of homogeneity of variances was not violated, as assessed by Levene’s test for equality of variances ($p = .29$).

For the neurotypical comparison group, latencies were normally distributed for the small, low-contrast condition ($p = .31$), but not for the large, low-contrast condition ($p = .006$). The assumption of homogeneity of variances was not violated, as assessed by Levene’s test for equality of variances ($p = .23$).

In the low-contrast condition, there were no significant N1 latency differences between small stimuli and large stimuli for either children with ASD (small stimuli N1 mean peak latency = 152.85ms, SD = 28.10; large stimuli N1 mean peak latency = 161.71ms, SD = 29.91; $t(13) = -.83$, $p = .41$) or for the NT comparison group (small stimuli N1 mean peak latency = 163ms, SD = 39.86; large stimuli N1 mean peak latency = 157.35, SD = 27.55; $t(13) = .46$, $p = .64$). See Figure 13, below.
7.3.2.2 Between-group peak latency analysis. A one-way ANOVA was conducted to evaluate the significance of N1 latency differences in processing large, low-contrast sinusoidal gratings between children with ASD and neurotypical comparison children. Latencies were not normally distributed for large, low-contrast conditions in children with ASD ($p = .008$) or NT children ($p = .006$), as assessed by Shapiro-Wilk’s test. The assumption of homogeneity of variances was not violated, as assessed by Levene’s test for equality of variances ($p = .75$). In the large, low-contrast condition, there were no significant N1 latency differences between children with ASD (N1 mean peak latency = 161.71ms, SD = 29.91) and NT comparison children (N1 mean peak latency = 157.35, SD = 27.55; $F (1, 26) = .16$, $p = .69$).

The same analysis was applied to evaluate N1 peak latency differences between groups for the small, low-contrast condition. Latencies were not normally distributed for small, low-contrast conditions in children with ASD ($p = .004$), but were normally distributed for NT children ($p = .31$), as assessed by Shapiro-Wilk’s test. The assumption of homogeneity of variances was not violated, as assessed by Levene’s test for equality of variances ($p = .25$). In the small, low-contrast condition, there were also no significant N1 latency differences between children with ASD (N1 mean peak latency = 152.85ms, SD = 28.10) and NT comparison children (N1 mean peak latency = 163ms, SD = 39.86; $F (1, 26) = 1.00$, $p = .32$).

7.3.2.3. Between-group peak amplitude analysis. A one-way ANOVA was conducted to evaluate the significance of N1 amplitude differences in processing large, low-contrast sinusoidal gratings between children with ASD and neurotypical comparison children. Amplitudes were normally distributed for the large, low-contrast condition in children with ASD ($p = .20$) and NT
children ($p = .34$), as assessed by Shapiro-Wilk’s test. The assumption of homogeneity of variances was not violated, as assessed by Levene’s test for equality of variances ($p = .33$). In the large, low-contrast condition, there were no significant N1 amplitude differences between children with ASD (N1 mean peak amplitude = -1.87, SD = .84) and neurotypical comparison children (N1 mean peak amplitude = -1.59, SD = .63; $F (1, 26) = .97, p = .33$).

Amplitudes were also normally distributed for the small, low-contrast condition in children with ASD ($p = .29$) and NT children ($p = .43$), as assessed by Shapiro-Wilk’s test. The assumption of homogeneity of variances was not violated, as assessed by Levene’s test for equality of variances ($p = .93$). In the small, low-contrast condition, there were no significant N1 amplitude differences between children with ASD (N1 mean peak amplitude = -1.28, SD = .83) and neurotypical comparison children (N1 mean peak amplitude = -1.61, SD = .76; $F (1, 26) = 1.20, p = .28$). See figure 14 below.

Figure 14: Between Group Low-Contrast ERP Plots: Children with autism spectrum disorder exhibited no processing differences as compared to neurotypical comparison children in the large, low-contrast condition (left). Children with autism spectrum disorder exhibited no processing differences as compared to neurotypical comparison children in the small, low-contrast condition (right).
7.3.3 Results Summary. In brief, our main experimental findings are as follows:

(1) Neurotypical participants displayed longer N1 response latencies to large, high-contrast stimuli as compared to small, high-contrast stimuli with no significant latency differences in the low-contrast experiment.

(2) Participants with ASD displayed shorter N1 response latencies to large, high-contrast stimuli as compared to small, high-contrast stimuli with no significant latency differences in the low-contrast experiment.

(3) Participants with ASD displayed shorter N1 response latencies compared to neurotypical participants in the large, high-contrast condition and longer latencies compared to neurotypical participants in the small, high contrast condition. No between group latency differences were observed in the low-contrast condition.

(4) Although there were no significant between group peak N1 response amplitude differences in the high- or low-contrast experiment, there was a strong negative correlation between N1 amplitude (represented in absolute values) between large, high-contrast stimuli and hyper-responsiveness scores on the Sensory Experiences Questionnaire for children with ASD, but not for neurotypical children.
8. DISCUSSION

In the present study, neurotypical participants displayed longer latencies to large, high-contrast stimuli as compared to small, high-contrast stimuli and no differences between small and large stimuli in conditions of low-contrast. Delayed motion processing in the large, high-contrast condition in neurotypical participants supports N1 ERP latency as a reliable measure of spatial suppression and is consistent with behavioral findings of increased discrimination thresholds in conditions of large, high-contrast stimuli. A lack of significant differences in the low-contrast condition is also consistent with surround suppression being absent in conditions of low-contrast stimuli since contrast gain is primarily operating at this level. Therefore, we can consider a lack of significant differences in the low-contrast condition as a control to examine contrast-dependent effects. High-functioning, medication-free children with ASD displayed weakened surround suppression, i.e. shorter latency of N1 responses to large, high-contrast stimuli as compared to small, high-contrast stimuli. When examining differences in N1 latencies between groups, as predicted, children with ASD demonstrated shorter latencies in response to large, high-contrast stimuli compared to neurotypical children.

The finding of shorter N1 latencies in conditions of large, high-contrast stimuli for children with ASD could lend support to the E/I imbalance theory of ASD, since weakened neural inhibition or increased neural excitation would result in a diminished spatial suppression effect resulting in enhanced processing of large, high-contrast stimuli (Wallisch & Bornstein, 2013). However, this study also unexpectedly revealed that children with ASD showed electrophysiological evidence of delayed processing in response to small, high-contrast stimuli as compared to neurotypical control children, as indexed by longer N1 latencies. This finding is partially consistent with the results of a recently published behavioral study by Sysoeva et al.
(2017), who found that children/adolescents with ASD demonstrated significantly delayed discrimination thresholds to small, stimuli in both high- and low-contrast conditions as compared to a neurotypical control group.

Pharmacological studies of visual evoked potentials (VEPs) have demonstrated that a topical GABA receptor antagonist results in greater VEP negative amplitude and attenuation or elimination of positive amplitude while a topical GABA agonist resulted in smaller or attenuated VEP negative amplitude and enhanced positive amplitude (Purpura, 1959; Zemon, Kaplan, & Ratliff, 1980; Siper et al., 2016). Based on these results, we would expect greater N1 amplitude to be associated with increased excitation, and smaller N1 amplitude to be associated with increased inhibition. The magnitude of the N1 response was greater for children with ASD in the large, high-contrast condition compared to neurotypical children, which aligned with our prediction. However, findings also indicated that N1 amplitudes for children with ASD were smaller in response to small, high-contrast stimuli, compared to neurotypical children. This unexpected finding could be suggestive of alterations in contrast gain for the ASD group. Since gain control is influenced by neural excitation, neural inhibition, and feedback connections, alterations in an E/I imbalance could lead to differences in gain control between individuals with ASD and neurotypical controls. Although amplitude differences within- and between groups were not statistically significant, there was a strong negative correlation between N1 amplitude (represented in absolute values) between large, high-contrast stimuli and hyper-responsiveness scores on the Sensory Experiences Questionnaire for children with ASD. In other words, children with the highest levels of hyper-responsiveness had the smallest N1 peak amplitudes. Normal contrast sensitivity function is also dependent on intact contrast gain control; therefore, it is possible that hyper-responsiveness to sensory stimuli could reflect deficits in contrast gain.
control (Rosenberg, Patterson, & Angelaki, 2015). As predicted, there were no significant within or between group differences in the low-contrast experiment, for either latency or amplitude.

As previously discussed, cortical processing is not considered to be entirely hierarchical since feedforward connections have corresponding reverse feedback connections, which modulate responses to stimuli within a receptive field (e.g., Lamme, Supér, & Spekreijse, 1998). Spatial attention is considered to be mediated by feedback interactions by the parietal cortex or feedforward connections via the thalamus. Both feedforward and feedback connections are primarily excitatory and are thought to play a role in attention and visual awareness (Lamme, Supér, & Spekreijse, 1998). Bullier et al. (1996) inactivated V2 with a GABA injection in non-human primates while recording from V1 and found a reduction in the strength of response to stimuli within the V1 receptive field. However, when presenting to the surround of the receptive field, they unexpectedly found a strong response in the V1 cells (Bullier, Hupé, James, & Girard, 1996). V1, V2, and V3 are dependent on feedback from the middle temporal visual area (MT) and inactivation of MT results in a reduction in surround suppression (Nowak, James, & Bullier, 1997). Therefore, it is possible that individuals with autism have functional differences in these projections, which in turn may lead to altered spatial suppression and contrast gain.

8.1 Study Limitations and Delimitations

To our knowledge, this study represents the first to examine spatial suppression in children with ASD using ERP methodology. Similar to behavioral studies, we found that children with ASD demonstrated weakened spatial suppression and aberrations in contrast gain control in conditions of high-contrast visual stimuli. This finding is reflected in the ERP data, which revealed that children with ASD had shorter N1 latencies in conditions of large, high-
contrast stimuli and longer latencies in response to small, high-contrast stimuli, which may be reflective of disproportionate levels of inhibition and/or abundant levels of excitation.

This study has a number of limitations that we plan to take into consideration for future studies. First and foremost, ASD is a heterogeneous neurodevelopmental disorder; therefore, if an E/I imbalance is present in this population, it is likely that it is only present in a sub-group of the population. However, an E/I imbalance may be present in medicated children (who were excluded from the present study), and/or may be present to differing extents in children with ASD who have different sensory processing profiles. The present study was unable to control for such within-group differences comprehensively, but the target population was children with a diagnosis of ASD who are not currently on any psychiatric medications.

Although our task was similar to that used by previous researchers (Golomb et al., 2009; Foss-Feig, Taudin, & Schauder, 2013; Sysoeva et al., 2017), we did not ask participants to respond indicating the perceived direction of motion during the ERP task. Rather, we told participants that they would see lines either moving left or moving right, and we maintained visual attention by instructing participants to respond to emoticons presented on 10% of trials. We utilized ERP latency as a measure of motion discrimination threshold instead of relying on behavioral responses since the majority of children from the experimental group who participated in this study would be unable to provide such responses. According to the normalization model of attention, attention increases response gain to large stimuli when the spatial uncertainty is small, and to small stimuli when the spatial uncertainty is large (Reynolds & Heeger, 2009; Herrmann, Montaser-Kouhsari, Carrasco, & Heeger, 2010). Since visual attention has been thought to affect behavioral performance on similar tasks, future prospective studies should collect behavioral motion discrimination thresholds and electrophysiological data.
in a cohort of older, more capable children. N1 responses in the present study were recorded from fronto-central electrode sites, with no N1 responses occurring in occipital electrodes, which further supports the interpretation that visual attention played an important role in the ERP responses reported here.

Although spatial suppression is known to be a non-linear sensory effect, we were unable to make this observation with only two stimulus sizes. In the present study, we presented stimuli that were small (0.7°) and large (5.0°) and found shorter N1 response latencies to large, high-contrast stimuli and longer latencies to small, high-contrast stimuli in children with ASD as compared to controls. If we had presented a medium-sized stimulus in the high- and low-contrast experiment, we would have been able to make further speculations about contrast gain control in autism. Since latencies decreased from small to large stimuli in children with ASD and increased from small to large stimuli in neurotypical children, we would have been able to determine linearity, or lack thereof, amongst the three conditions had a medium-size condition been included.

Furthermore, we cannot directly measure GABA and/or Glu concentrations using high-density EEG. In measuring spatial suppression through an adapted behavioral paradigm, we used N1 ERP latency and amplitude as markers of neural inhibition in response to sinusoidal gratings. In future prospective studies we hope to assess the consistency of N1 latencies and amplitudes within each participant at different time points. This dissertation study therefore aims to draw a connection between a biological imbalance (GABA/Glu) and a behavioral manifestation (sensory processing differences), neither of which can be evaluated directly. The N1 latency and amplitude measures offer a window into alterations in neurological function that could underpin sensory processing differences in ASD, and that may be associated a GABA/Glu imbalance, but
direct evidence of the connections between these potentially related measures is not likely to emerge given the state of the art at this time.

8.2 Conclusions and Future Directions

The current study provided insight into cortical activity related to a psychophysical motion-processing task in children with ASD. Since to our knowledge this is the first ERP study to investigate the E/I imbalance theory of ASD in a psychophysical motion-processing task, these findings should be replicated in order to further elucidate spatial suppression and contrast gain differences in individuals with ASD. Although the study findings were not entirely consistent with the study hypotheses, we were able to demonstrate that N1 latency can be utilized as a marker of spatial suppression in neurotypical children, while children with ASD demonstrated weakened spatial suppression resulting in enhanced processing of large, high contrast stimuli. Children with ASD also demonstrated delayed processing of small, high-contrast stimuli, which may reflect differences in contrast gain control. Perhaps our most interesting finding was a strong negative correlation between N1 amplitude in absolute values and hyper-responsiveness scores on the Sensory Experiences Questionnaire, despite no statistically significant between group differences for N1 amplitude in the high- or low-contrast experiments. After further investigation of the present experimental effect, this paradigm may have potential for use as a clinical outcome measure in clinical trials to evaluate the effectiveness of drugs that act on the GABAergic or glutamatergic systems. If the neurotoxic process of hyperexcitation / excitotoxicity contributes to development in even a subset of individuals with ASD, then there are a variety of FDA approved drugs (for the treatment of other disorders) that may offer some utility in this population (Evers & Hollander, 2008), such as NDMA antagonists, GABA_B agonists, or GABA_A agonists. If such pharmacological interventions are found useful for
ameliorating sensory symptoms in ASD, future studies could measure the effectiveness of these interventions using EEG/ERP methodology.
9. REFERENCES


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10. APPENDICES

10.1 Psykinematix Calibration

Calibrating your Experimental Display

This step-by-step tutorial teaches you how to:
1) perform the calibration of your experimental setup using Psykinematix.
2) specify a calibration configuration for a given experiment.

Preparation

First, open the Display Preferences panel to specify the default display and settings you wish to use when creating new experiments.

Click on the Display tab to select the Display Preferences. Select the stimulus display and resolution to be used by default. Any new selection creates a Default calibration configuration that could be used to run an experiment without having to calibrate the setup; however, a warning message will indicate any potential problem in this case. To properly calibrate the selected configuration, switch to the Calibration panel.

Tip: To jump directly to the Calibration panel, click on the small color wheel icon outlined in blue.
To switch to the **Calibration** panel from the main panel, click on the **Calibration** button in the toolbar.

**Calibration Panel**

The calibration of your system is an important step to make sure your experiments can be replicated on other experimental systems:

1) Geometry calibration ensures that stimuli look the same on different displays in terms of their spatial properties. All you need is a ruler!

2) Gamma calibration corrects for the intrinsic non-linearities in the display luminance and in the digital-to-analog conversion of the video card. This is especially important to minimize luminance artifact in contrast-modulated patterns (2nd-order stimuli). You may need a photometer or a colorimeter.

3) Color calibration involves measuring the chromatic coordinates of the phosphors or their spectral emission to determine how much light reaches each type of cone (assuming some human cone fundamentals for the spectral absorption). You may need a colorimeter or a spectrometer (to create a file containing the spectral energy distribution of each phosphor).
The calibration of your experimental setup requires the calibration of three aspects of the stimulus display: its geometry, its Gamma function, and the color properties of its phosphors. You need a ruler to perform the geometry calibration along with a special device to measure the emission properties of your display (photometer, colorimeter, or spectrometer).

As outlined in green, this panel can manage multiple configurations of a display (1) as well as multiple calibrations for a given configuration (2). In this example, the current calibration is named 'Default', and corresponds to the display at index 0 (this generally refers to the main display) for a resolution of 1024 x 768 pixels in 32-bit mode and with a frame rate of 60 Hz. Note that a 'Default' calibration is created each time a new display configuration is set in the Display Preferences.

3) Click on the ' + ' button to create a new calibration, named 'NewCal*' by default, and then rename it to better reflect the display type.

4) Note the additional info at the bottom: the unique display name, the calibration progress in % completion and which aspects of the calibration remain to be performed.

**Step 1: Geometry Calibration**

1) Click on the Geometry tab in the Calibration panel.
2) Set the size in pixels of the square patch used to perform the geometry calibration.
3) Click on Perform Calibration.

1) Click on the Geometry tab in the Calibration panel.
2) Set the size in pixels of the square patch used to perform the geometry calibration.
3) Click on Perform Calibration.
You should see this screen on the stimulus display after clicking on the **Perform Calibration** button: a square shape displayed in the center in the specified color and size.

4) Use the ruler to measure the sides of the square and make sure it's actually square.

5) Press **Esc** (escape key) to close the stimulus screen when the measurement is complete.
6) Select the measurement unit and enter the measured value in the Measured side field.
7) The time of the last calibration is indicated in the bottom left-hand corner.
8) The completion % is updated with a clear indication that the geometry calibration has been performed.

The geometry calibration is now complete! Click on the Next Step button to switch to the next section of the calibration process.

**Step 2: Gamma Correction**

To perform a proper Gamma calibration, a device that functions as a photometer is required. Psykinematix interfaces with the Eye-One Display 2 from GretagMacbeth/X-Rite and the Spyder 3 from DataColor (find a list of supported devices in the Calibration Devices section); however, any photometer that displays its readings on a visual output can be used. Finally, make sure your device is set up to produce luminance readings in cd/m^2.

If you don’t have access to a photometer, you can still manually set the Gamma value for each gun but your display may not be correctly calibrated.

To perform the Gamma calibration, follow the steps below:

1) Click on the Gamma tab in the Calibration panel.
2) The stimulus properties (2), and the measurement parameters (3) appear in this panel. The actual calibration is performed after selecting the measurement mode (4) and clicking on the measurement button (5). The plain curve in the graph shows the Gamma of the selected gun.
2) Set the number of luminance readings, the surround color, and the patch size in pixels.

3) Select the gun you want to calibrate: the red, green and blue guns can be calibrated individually or combined to form the white gun. It is advisable to calibrate all 4 guns so the calibration configuration can be used in either the chromatic or achromatic mode set in the Display settings for each experiment.

4) Specify the source of the measurement; by default, it is set to Gamma and its value can be directly edited in the field just below. Assuming you have access to a photometer that provides the readings you will enter manually, select the Readings option. If you have a supported calibration device connected to the computer, select it and the readings will automatically be sent to Psykinematix as you take the measurements.
5) Select **Gamma Measurement** from the **Perform...** pop-up menu. The Gamma measurement should be performed first, followed by a verification of the linearity of the Gamma correction (**Correction Checking**).

You should see this screen on the stimulus display when selecting one of the **Perform...** actions: a square shape displayed in the center in the specified color and size, as well as its surround.

6) Place the **photometer** head in the center of the white square. The square should be larger than the light sensitive area of the photometer; if it is not, press **Esc**, increase the square size, and re-select **Gamma Measurement**.

7) To start the measurement process, press **Esc** when the photometer head is in place.
8) In manual mode, enter the photometer reading using the keypad after each change in the square's luminance, followed by the Return key to move to the next luminance level. The reading index and entry are displayed in the bottom right-hand corner of the stimulus display. Use the Delete key to modify the entry before it is validated with the Return key.

When using a supported calibration device, the reading is sent directly to the computer and the next luminance level is automatically displayed. In this case, the reading index is displayed in the top left-hand corner of the stimulus display.

Press Esc to close the screen when the luminance measurements are completed (or press Esc for 1 second to abort the measurement process).
When the Gamma measurement for the selected gun is completed, the graph shows the luminance readings as solid squares. The Gamma field shows the Gamma value that best fits these data. The inverse plain curve shows the resulting Gamma correction to be applied. The graph always shows the normalized intensities, so note the minimum and maximum measured luminance levels indicated below the Gamma field. Contrast and luminance expressed in % in your stimuli properties will be relative to this luminance range.

9) The last step of the Gamma calibration is the verification of the linearity of the Gamma correction. Select Correction Checking from the Perform... pop-up menu, and follow the same steps as above to perform the measurement process. When the checking process is completed, the corrected Gamma is added to the graph as empty circles. The closer they are to the diagonal line, the more accurate the correction is. Repeat the Gamma calibration for all the other guns, after which the process is complete! Note the updated completion % and the ability to overlap the Gamma data for different guns by clicking on the L, R, G, B check boxes.
It is highly recommended to regularly perform the Gamma calibration of your display (eg. on a daily or weekly basis) and with an appropriate number of readings (higher the number of readings, better the Gamma calibration will be).

**Step 3: Color Calibration**

For proper color calibration, a device that functions as a colorimeter or spectrometer is required. Psykinematix interfaces with the Eye-One Display 2 colorimeter from GretagMacbeth/X-Rite and the Spyder 3 colorimeter from DataColor; however, any colorimeter that displays its readings on a visual output or any spectrometer with a file output can be used.

If you don't have access to a colorimeter or a spectrometer, you can still set color properties that may be close to your display characteristics, but your display may not be correctly calibrated.

To perform the color calibration, follow the steps below:
1) Click on the **Color** tab in the Calibration panel. The stimulus properties (2), and the measured color properties represented graphically as well as in a table (4) appear in this panel. The actual calibration is performed after selecting the phosphor along with the measurement mode and clicking on the measurement button (3).

2) Set the surround color and the patch size in pixels.

3.1) Select the phosphor you want to calibrate: the **red**, **green** and **blue** phosphors can be individually calibrated or combined to form the **white point**. It is mandatory to calibrate all 4. If you have one of the supported devices, the 4 can automatically be calibrated in one step by selecting **All Phosphors**.
3.2) Select the type of measurement to perform for each phosphor: radiometric or colorimetric properties. For the most accurate calibration, both types of measurement should be carried out.

You need a device with a spectrometer function for radiometric measurements and a colorimeter function for colorimetric measurements.

**Colorimetric Data**

Select the Colorimetric mode.

This mode allows you to specify the source of the colorimetric measurement of your display (1) while the measured chromatic properties are presented in the table (2) using the selected CIE standard (4): the chromatic coordinates (\(xy\) or \(u'v'\)) and maximum luminance (\(L_{\text{max}}\)) for each phosphor which form the Maxwell triangle (or gamut) of the display (3). This chromaticity diagram can be shown either as function of the \(xy\) coordinates (CIE 1931 standard) or the \(u'v'\) coordinates (CIE 1976).
1) This pop-up menu provides several choices. If you own a colorimeter, use one of the top options: Custom... allows you to manually edit the table (2) with readings obtained from a device not supported by Psykinematix while the i1 and Spyder options allows you to automatically obtain the readings from one of the supported colorimeters. Click on the Perform Calibration button to perform the colorimetric measurements for the specified phosphors. All the other choices in this pop-up menu correspond to various standard displays and should only be selected if you do not have access to a colorimeter AND you are familiar with your display characteristics. On selection of one of these, the table (2) is filled with pre-defined colorimetric data for the specified display type and the gamut is updated (3).

**Radiometric Data**

Select the Radiometric mode.
This mode allows you to specify the source of the spectral measurement of your display (1) and the cone fundamentals (2), both shown in the graph (3), in order to generate an LMS to RGB matrix (4).

1) The source of the radiometric measurement is specified through a pop-up menu which offers three choices: to produce a custom LMS to RGB matrix by editing the matrix (4), to select a file containing the spectral power distribution for each phosphor, or to select a phosphor standard that closely matches that of your display.

2) The cone fundamentals specify the spectral absorption for each cone and are used in combination with the spectral emission of the display to produce the LMS to RGB matrix which is automatically updated with the selection.
3) The spectral power distributions of both display emission and cone absorption are represented graphically (continuous lines for phosphor emissions, broken lines for cone absorptions) and updated with any change in the selections.
4) The resulting LMS to RGB matrix is displayed in the table and updated with any change in the selections.

Note that Psykinematix does not support any spectrometer yet, but it can import a file containing the data produced by a spectrometer (usually a text file conforming to a specific format described in the documentation). However, Psykinematix can present an appropriate stimulus patch so the spectrometer is able to perform its measurements for each phosphor. To do this, click on Perform Calibration.

Calibration Summary

A summary panel is available under the Summary tab which provides an overview of the performed calibration steps. It also indicates that calibration steps that have not been performed yet as illustrated in the example below:
A fully completed calibration should result in the following summary panel:

Specify a Calibration Configuration for an Experiment

After finishing an experiment design, you should specify the calibration configuration to be used for each session. To do so, select your experiment in the Designer table (Experiment category with the red psi mini-icon), and click on the blue INFO button to inspect or modify its properties.
In this properties panel:
1) Click on the Display tab to view/edit the experiment Display settings. By default, the display settings (in particular the stimulus display and resolution) are those defined in the Display Preferences.
2) Select the stimulus display (internal or external) and its resolution (width x height x frame rate) to be used by the currently edited experiment.
3) Select the calibration configuration,
Note that each time the display and resolution settings change, the calibration pop-up menu Calibration Name (outlined in blue above) is updated with all the calibration configurations available for these settings.
If none is available, the selection in the pop-up menu remains blank with a small warning icon attached to it. Click on this warning icon to reset the display settings to the ones in the Display Preferences, or close this panel and return to the Display Preferences and Calibration panel to create a configuration for the new display settings (see beginning of this tutorial).

Conclusion

In this tutorial, you learned how to: calibrate the geometry of your display, measure and linearize its Gamma, measure its color properties, and set the calibration configuration used during an experiment.
**10.2 Teachers College, Columbia University IRB Approval**

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**Teachers College IRB Continuing Review Approval Notification**

**To:** Lauren Goodwin  
**From:** Curt Naser, TC IRB Administrator  
**Subject:** IRB Approval: 15-265 Protocol  
**Date:** 03/25/2016

Please be informed that as of the date of this letter, the Institutional Review Board for the Protection of Human Subjects at Teachers College, Columbia University has approved your *continuing* study, entitled "Neural Correlates of GABA:Glu Imbalances in Children with Autism Spectrum Disorder (ASD)" on 03/25/2016." This continuing review was approved by expedited review as no subjects have yet been enrolled and no new risks have been identified.

The approval is effective until 03/24/2017.

The IRB Committee must be contacted if there are any changes to the protocol during this period. **Please note:**  
If you are planning to continue your study, a Continuing Review report must be submitted to either close the protocol or request permission to continue for another year. Please submit your report by 02/24/2017 so that the IRB has time to review and approve your report if you wish to continue your study. The IRB number assigned to your protocol is **15-265**. Feel free to contact the IRB Office (212-678-4105 or IRB@tc.edu) if you have any questions.

Please note that your Consent form bears an official IRB authorization stamp. Copies of this form with the IRB stamp must be used for your research work. Further, all research recruitment materials must include the study’s IRB-approved protocol number. You can retrieve a PDF copy of this approval letter from the Mentor site.

Best wishes for your research work.

Sincerely,
Curt Naser, Ph.D.
TC IRB Administrator

**Attachments:**  
- Consent-Amendment 2.0- 3.7.16.pdf
Teachers College IRB Modification Approval Notification

To: Lauren Goodwin
From: Curt Naser, TC IRB Administrator
Subject: IRB Modification Approval: 15-265 Protocol
Date: 12/03/2016

Please be informed that as of the date of this letter, the Institutional Review Board for the Protection of Human Subjects at Teachers College, Columbia University has approved a modification to your study, entitled "Neural Correlates of GABA/Glu Imbalances in Children with Autism Spectrum Disorder (ASD)" on 12/03/2016. This modification approves a new recruitment flyer and participant brochure.

The approval remains effective until 03/24/2017.

The IRB Committee must be contacted if there are any changes to the protocol during this period. Please note: If you are planning to continue your study, a Continuing Review report must be submitted to either close the protocol or request permission to continue for another year. Please submit your report by 02/24/2017 so that the IRB has time to review and approve your report if you wish to continue your study. The IRB number assigned to your protocol is 15-265. Feel free to contact the IRB Office (212-678-4105 or IRB@tc.edu) if you have any questions.

You can retrieve a PDF copy of this approval letter from the Mentor site.

Best wishes for your research work.

Sincerely,
Curt Naser, Ph.D.
TC IRB Administrator
10.3 Weill Cornell Medical College IRB Approval

April 21, 2016

Jeremy Veenstra-VanderWeele, M.D.

Submission Type: New Response to Issues
Protocol Number: 1511016770
Protocol Title: Neural Correlates of GABA: Glu Imbalances in Children with Autism Spectrum Disorder
Risk Level: Minimal Risk; qualifies for future expedited review under category 9
Pediatric Risk Determination: 45 CFR 46.404
Date of Last Convened Meeting Review: March 15, 2016

Dear Dr. Veenstra-VanderWeele,

The Institutional Review Board has conducted an expedited review of your response to the modifications required letter issued on April 11, 2016 regarding the abovementioned protocol.

- Informed Consent and HIPAA Authorization Form, version date January 20, 2016
- Assent Form for ages 7-11, version date January 21, 2016
- Assent Form for ages 12-17, version date January 21, 2016
- Flyer
- Recruitment Brochure
- Assessments:
  - ADOS-2 Module 3
  - CARS-2
  - CARS-2 Parent Questionnaire
  - Sensory Experiences Questionnaire
  - Stanford Binet

The protocol and its relevant documents stand approved for the following period:

**Approved: April 21, 2016**  **Expires: March 14, 2017**

Please do not hesitate to contact the IRB office staff if you have any questions or need assistance in complying with the terms of this approval.

Sincerely,

Rosemary Kraemer, Ph.D.
Director, Human Research Protections Program

Institutional Review Board Mailing Address: 1300 York Ave Box 19, New York, NY 10065 | T. 646.962.8200 | irb@med.cornell.edu
DEMOGRAPHIC QUESTIONNAIRE

Today’s Date: _____/_____/_____

Child’s Full Name: __________________________________________________

Date of Birth: _____/_____/_____
Sex: _____ Female _____ Male

Race/Ethnicity: (Check all that apply)

_____ Asian
_____ Black or African American
_____ Native American/Alaska Native
_____ Native Hawaiian or Other Pacific Islander
_____ White
_____ More than one race
_____ Other ___________________________________________

(please specify)

Is your child Hispanic or Latino? (circle one) YES NO

CONTACT INFORMATION

Parent/Caregiver A’s Name: ____________________________________________

Parent/Caregiver B’s Name: ____________________________________________

Mailing address: _______________________________________________________

Street

________________________________________________________(____________)

City State Zip Country

Home Phone: ___________________________

Cell Phone: __________________________

Email address: ________________________
CAREGIVER INFORMATION

CAREGIVER A:  
Relationship to participant: ____________________________
Date of Birth: ___/___/____
Race/Ethnicity (If more than one race, check all that apply):

- Asian
- Black or African American
- Native American/Alaska Native
- Native Hawaiian or Other Pacific Islander
- White
- More than one race
- Other ____________________________________________  
  (please specify)

Caregiver A: Hispanic or Latino? (circle one)  YES  NO

CAREGIVER B:  
Relationship to participant: ____________________________
Date of Birth: ___/___/____
Race/Ethnicity (Check all that apply):

- Asian
- Black or African American
- Native American/Alaska Native
- Native Hawaiian or Other Pacific Islander
- White
- More than one race
- Other ____________________________________________  
  (please specify)

Caregiver B: Hispanic or Latino? (circle one)  YES  NO

CAREGIVER INFORMATION Continued

<table>
<thead>
<tr>
<th>Mother</th>
<th>Father</th>
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<tbody>
<tr>
<td>_____</td>
<td>_____</td>
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<tr>
<td>graduate/professional degree</td>
<td>(M.D., Ph.D, M.A. /M.S., M.B.A., etc.)</td>
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<td>_____</td>
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<tr>
<td>baccalaureate degree (four year degree)</td>
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<td>_____</td>
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<td>some college</td>
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<tr>
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<tr>
<td>associate degree (two year degree)</td>
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</table>
high school graduate

GED diploma

Some high school (without diploma)

completed up through ninth grade

completed less than ninth grade

Mother’s occupation:____________________________________________________

Father’s occupation:____________________________________________________

Annual household income:

□ Less than $20,000
□ $21,000 – $35,000
□ $36,000 – $50,000
□ $51,000 - $65,000
□ $66,000 - $80,000
□ $81,000 - $100,000
□ $101,000 - $130,000
□ $131,000 - $160,000
□ Over $161,000

HOUSEHOLD INFORMATION

Your child currently lives with:

_____ both parents

_____ biological father and stepmother

_____ biological mother

_____ group setting

_____ biological father

_____ adoptive parent(s)

_____ biological mother and stepfather

_____ foster parent(s)

Child’s Current Language:

Please estimate your child’s present vocabulary size:

_____ no words

_____ 1 to 5 words

_____ 5 to 25 words

_____ 25 to 100 words

_____ more than 100 words

First words (if applicable):________________________________________________

Education

Does your child currently attend:

_____ Day care

_____ Nursery

_____ Kindergarten

_____ School

Type of program:_________________________________________________________

Grade or kind of class:___________________________________________________
**PARTICIPANT’S MEDICAL HISTORY**

Medications and/or vitamins that the participant is **CURRENTLY** taking (if necessary, please use back side):

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<th>Name</th>
<th>Dosage</th>
<th>Frequency</th>
<th>How long has med been taken?</th>
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Medications and/or vitamins that the participant has taken in the **PAST FOR ONE MONTH OR LONGER** (except for antibiotics)

<table>
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<tr>
<th>Name</th>
<th>Dates taken</th>
<th>Reason</th>
<th>Effectiveness</th>
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