Auditory Brainstem Development in Autism: From Childhood Hypo-Responsivity to Adult Hyper-Reactivity

Ala Seif [1-5], Renee Guerville [1-3], Mohammad Rajab [1,2], Cassandra Marceau-Linhares [3,4], Kristina Schaaf [3,4], Samantha E. Schulz[3,4], Susanne Schmid [1,2,5], Ryan A. Stevenson [1-4]*

[1] *Program in Neuroscience, University of Western Ontario*
[2] *Western Institute of Neuroscience, University of Western Ontario 3*
[3] *Centre for Brain and Mind, University of Western Ontario*
[4] *Department of Psychology, University of Western Ontario*
[5] *Department of Anatomy and Cell Biology, Schulich School of Medicine and Dentistry, University of Western Ontario*

*Corresponding author: aseif5@uwo.ca

Abstract

Background: Autism Spectrum Disorder (ASD) is characterized by sensory disruptions, including auditory processing differences, which can significantly impact social, emotional, and cognitive development. This study investigates auditory brainstem development in Autistic children and adults using auditory brainstem responses (ABRs) and acoustic startle responses (ASRs), two key measures of auditory processing. We hypothesize that early hypo-responsivity in children, measured with ABRs, may lead to compensatory neural adaptations, resulting in hyper-reactivity in adulthood, measured by ASRs.

Methods: The study included 40 Autistic children, 57 non-Autistic children, 20 Autistic adults, and 21 non-Autistic adults. Participants underwent peripheral hearing screening, ABR testing at slow and fast click-rates, and ASR measurements. ABR wave and ASR latencies and amplitudes were analyzed. Statistical analyses included mixed-model ANOVAs and Spearman's correlations to examine group differences and associations with age.

Results: Autistic children exhibited increased ABR wave latencies and reduced amplitudes, indicating slower neurotransmission and reduced neural responsivity in the ascending auditory pathway. In contrast, Autistic adults showed normalized ABR latencies but increased ASR magnitude, suggesting hyper-reactivity to auditory stimuli. Age-related correlations revealed that ABR latencies increased with age in non-Autistic participants, while ASR magnitude was negatively correlated with age in non-Autistic participants. The associations were significantly different between groups.

Conclusion: The findings support the hypothesis that Autistic children experience auditory brainstem hypo-responsivity, which may normalize in adulthood but lead to maladaptive hyper-reactivity. These results highlight the role of early auditory disruptions in shaping long-term sensory processing and reactivity in Autism, emphasizing the need for further research into the neural mechanisms underlying these differences.

Keywords: Sensory reactivity, startle response, auditory brainstem response, autism

Introduction

Autism is a neurodevelopmental condition characterised by challenges in social interaction, communication, and the presence of restricted and/or repetitive behaviors and interests. Sensory disruptions, such as hyper- and hypo- sensitivities, were only included in the most recent version of the diagnostic criteria under the umbrella of restricted and repetitive behaviors[1]. Sensory disruptions are well documented in clinical[2] and experimental measures[2]. Difficulties in sensory processing can cascade to affect social communication and interactions[3,4], education[5], and everyday activities[6]. Moreover, they are associated with higher rates of social-emotional dysregulation[7], anxiety[7], and other mental health difficulties[8]. Sensory disruptions are often categorized into three subcategories, hyper-responsiveness, hypo-responsiveness, and sensory seeking[8]. They are reported in multiple modalities including olfactory[9], tactile[10], auditory[11], visual[12], as well as in the interplay of those modalities[13–16].

Auditory processing plays a crucial role in cognitive development[17], impacting how individuals interpret, understand, and respond to sound stimuli in their environment. Disruptions in auditory processing during early development and critical periods is especially impactful[18]. These periods close after structural consolidation, reducing future plasticity as the brain reaches adulthood. Although critical periods provide an exceptional time window for learning and consolidation, they also represent a period of great vulnerability for the developing brain[19]. This study focused on the auditory sensory disruptions at the level of the brainstem across development. There are multiple studies reporting alterations in the ascending auditory brainstems in Autistic individuals, including slower neurotransmission[20] and atypical cell structures[21]. In addition, there is evidence from animal models of Autism indicating reduced responsivity of the ascending auditory pathway[22].

The functionality of the auditory brainstem pathway is commonly measured through the auditory brainstem response (ABR) a well-characterized auditory evoked potential of the brainstem. The ABR output is a waveform with five major wave peaks that have known bio generators. Wave I is linked to the distal portion of the auditory nerve with an absolute latency of 1.5 msec[23]. While wave II is associated with the proximal portion of the auditory nerve and cochlear nucleus with an absolute latency of 2.5 msec[23]. Wave III is generated by the cochlear nucleus and superior olivary complex with an absolute latency of 3.5 msec[23]. Wave IV is associated with the superior olivary complex and ascending auditory fibers of the lateral lemniscus with an absolute latency of 4.5 msec[23]. Wave V corresponds to the lateral lemniscus and inferior colliculus with an absolute latency of 5.5 msec[23] (Figure 1). The latency of the wave is indictive to the speed

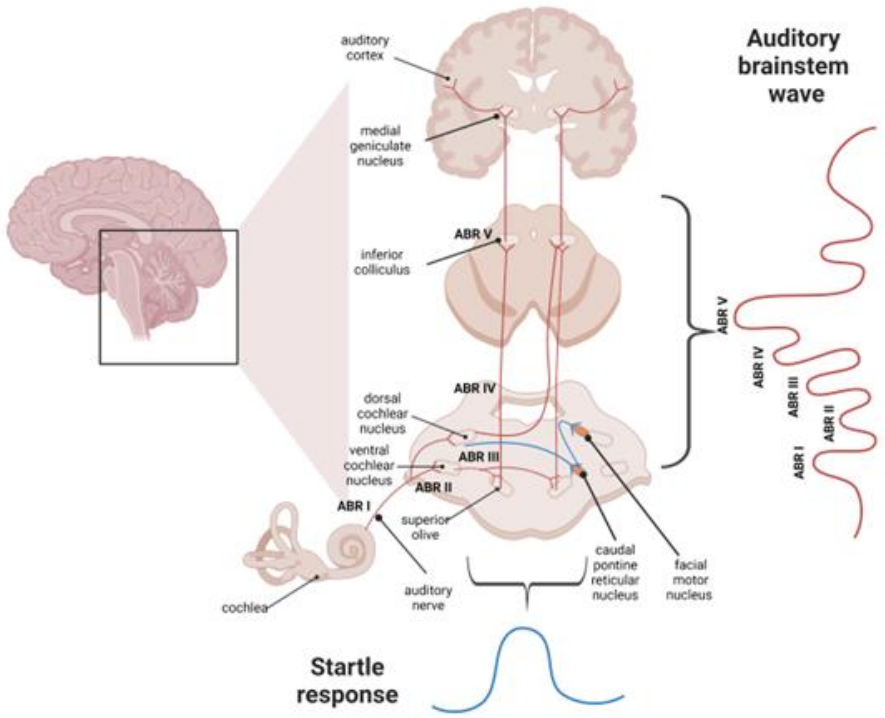


Figure 1: The pathways involved with the acoustic startle reflex and the auditory brainstem response.

of neurotransmission while the amplitude of the waves is indictive of neural responsivity[23].

ABRs are measured as a common newborn hearing test to provide information about brainstem maturation. Given this, is has been possible to detect differences in ABRs in infants later diagnosed with Autism[20]. The ABR reports in Autism, however, have been inconsistent, with some reporting no group differences and others reporting delayed and/or muted ABR waves for Autistic individuals[20]. The inconsistency could be owed to the heterogeneity of Autism, the variation in the ABR parameters, or the participant ages in the studies. Studies that looked at ABRs in younger samples such as newborns[24], toddlers[25], or school-aged[26] children report increased ABR peak latencies. However adult study results are less consistent[20]. This could be due to a delay in the development of the ascending auditory pathway.

There is also evidence supporting increased behavioral responsivity to sound associated with Autism, including brainstem-mediated reflexive responses. These responses are commonly measured by the acoustic startle response (ASR), which is an evolutionary conserved protective response to sudden auditory stimuli[27,28]. The primary startle pathway includes spiral ganglion cells innervating the cochlea and synapsing on secondary auditory neurons in the cochlear nucleus which innervate the ventrolateral caudal pontine reticular nucleus (PnC)[29]. PnC giant neurons project to the spinal cord where they ipsilaterally innervate motor neurons[29] (Figure 1). The startle response is a measure of acoustic reactivity and is typically quantified in humans using electromyography (EMG) of the eyeblink, in which electrodes measure orbicularis oculi muscle activity. Autistic individuals are reported to have increased startle reactivity. According to the literature, Autistic individuals have been reported to express acoustic hyper-reactivity[3] and

hyposensitivity to complex stimuli in the ascending auditory pathway[30], measured by the auditory brainstem response. It is unclear if these two seemingly contradicting constructs are explained by the heterogeneity of Autism.

This study focused on the auditory brainstem development by investigating the auditory brainstem response and the acoustic startle response in a sample of Autistic children and Autistic adults. According to previous report, we expect that Autistic children will have hypo-responsivity to sounds quantified by muted and delayed ABR wave peaks compared to non-Autistic children, while Autistic adults will have normalized ABR. We hypothesize that this hypo-responsivity in Autistic children will lead to the development of an adaptive compensatory gain of auditory signals downstream of the ABR. As the hypo-responsivity of the ABR gets resolved with age, the adaptive compensatory gain would become maladaptive. Therefore, we predict that the Autistic adults will have increased behavioral reactivity measured by startle response compared to non-Autistic adults. Finally, we hypothesize that ABR parameters will negatively correlate with age while behavioural reactivity will positively correlate with age for the Autistic participants.

Methodology

Participants

This study includes 40 Autistic children (10 ± 2.9 years, 85% males, FSIQ-2 composite score 93.6 ± 20.8) and 57 non-Autistic children (9 ± 2.9 years, 51% males, FSIQ-2 composite score 109.7 ± 13.6). It also included 20 Autistic adults (28 ± 6.5 years, 47% males, FSIQ-2 composite score 112.4 ± 14.3) and 21 non-Autistic adults (23 ± 5.1years, 31% males, FSIQ-2 composite score 113.6 ± 15.9). 10 additional participants were recruited but excluded for reasons outlined below. All participants had normal hearing (confirmed in the study, details below) and absence of neurological disorders, history of seizures, or past head injury. Participants' cognitive ability was assessed by the Wechsler Abbreviated Scales of Intelligence, second edition[31] or Kaufman Brief Intelligence Test, Second Edition[32]. A subset of the adult non-Autistic sample were undergraduate university students, and they did not complete a cognitive assessment as it was assumed to be IQ >70. The study was a fully passive study therefore allowing us to include three Autistic participants that could not sit through the IQ test due to communication difficulties. Participants in the Autism group had a clinical diagnosis of Autism by a professional including medical doctors such as family physicians, pediatricians and psychiatrists, psychologists and psychological associates, or nurse practitioners. The diagnosis was confirmed by an Autism Diagnostic Observation Schedule, Second Edition[33] conducted by a research-reliable administer, or a clinically-reliable administer supervised by a research-reliable administer. Participants in the non-Autism group had no family history of Autism in first-degree relatives. An additional two participants were excluded due to ADOS-2 not confirming the Autism diagnosis. Final groups did not differ on the hearing screening. Participants aged eighteen or more or the parents of younger participants gave informed assent, and children gave assent.

Periphery Hearing Screening

A periphery hearing screening was conducted before being included in the study. The screening consists of a visual otoscope inspection, an audiometric assessment (Midimate 602), and a tympanometry test (MT10 by interacoustics). The evaluation criteria are (1) no perforation in the eardrum, (2) pass an audiometer screen for frequencies (250 Hz, 500 Hz, 1000 Hz, 2000 Hz, 4000 Hz, 8000 Hz) < 25 dB, and (3) have a detected tympanogram peak with compliance(ml) = 0.30-1.5, pressure (dapa)= -/+ 100 and ear canal volume (ECV)= 0.5-1.35. All assessments were completed for both ears and the participants were included if they passed two out of the three assessments for every ear.

Auditory Brainstem Response

The ABR was measured ABR Integrity™ V500 System. The acoustic stimuli were presented via ER-2 insert earphones. Presentations were made monoaurally and click pulses were repeated until a total of 2000 accepted evoked potential sweeps had been collected and accepted. The acoustic stimuli were presented at a slow click-rate (19.1 clicks/sec) and a fast click-rate (59.1 clicks/sec) at sound pressure level of 80 dB HL. Two trials were recorded for every condition from each ear. The peaks were marked by two trained personnels. The ABR variables were averaged across both trials and ears for every participant.

Acoustic Startle Response

The acoustic startle response was recorded using SR-HLAB™ EMG. All auditory stimuli and background white noise were delivered binaurally to participants through stereophonic headphones. Startle eyeblink electromyographic responses were recorded from the left orbicularis oculi muscle. The eyeblink magnitude of every startle response was defined as the voltage of the peak electromyographic activity within a latency window of 20 to 160 msec following startle-eliciting stimulus onset. The startle onset was defined as the point of positive inflection of the volts before startle peak and the startle latency is the latency of the peak voltage. The peaks were marked automatically by the software. The paradigm consisted of a five-minute acclimation period and consistent 60 dB SPL white noise background. The acoustic reactivity trials consisted of white noise pulse ranging from 65 to 105 dB SPL for 40 msec with 10 dB SPL increments and all trial conditions were repeated 10 times. All trials were presented in a fixed pseudorandom order, separated by intertrial intervals of 10 to 20 s (15 s on average). Startle magnitude was averaged for every condition.

Statistical Analysis

The main objective of the study was to investigate functional and behavioral measures of the auditory brainstem across development in Autism. To compare group differences, we used a four-way, mixed-model ANOVA with between-subjects factors of diagnostic group (Autistic, non-Autistic), sex (females, males), and age (kids, adults) and a within-subjects factors of click rate for ABR (slow, fast) and sound-level intensity for ASR (65, 75, 85, 95, 105 dB SPL). In cases when the parametric assumptions were not met, a rank-based ANOVA was completed. The Mauchly test was used to determine whether the data violated the sphericity assumption. In the case of a violation, the degrees of freedom were corrected using the Greenhouse–Geisser correction. To control the false discovery rate for multiple testing across features, we applied the Benjamini–Hochberg

procedure (FDR q = .05) separately within each effect (Diagnosis, Sex, Age, and their interactions). When an interaction was significant after FDR correction, we conducted simple main effects and pairwise comparisons restricted to that interaction and controlled FDR within each set of follow-up tests. In all experiments, interactions involving diagnostic group or a main effect of diagnostic group were of utmost interest. Spearman's rank correlation was used to assess the relationship between ABR and reactivity features, and Age in both the Autistic and non-Autistic groups. Bootstrapping with 1,000 resamples was applied to estimate 95% confidence intervals (CIs) for the correlation coefficients and the difference in correlations between groups. Analysis was performed with R and graphical display was completed with GraphPad Prism 6.01. R was used for statistical analysis. The study was pre-registered on the Open Science Framework osf.io/hg6b2.

Results

Periphery Hearing Screening

There were 10 participants excluded from the study based on their periphery hearing screening (Tables S1 and S2).

ABR: Absolute and Interpeak Latencies

We analyzed absolute latency of ABR (Figure 2) wave I, negative wave 1, wave II, wave III, wave V and negative wave V and interpeak latencies (IPLs; Figure 4) between waves I and III (I–III IPL) as well as waves III and V (III-V IPL) and overall waveform of wave I and V (I-V IPL; sex X click-rate X diagnostic group X Age group). Overall, the diagnosis group differences are summarized with increased absolute latencies for Autistic children compared to non-Autistic children. The main effects of sex (Table S3), age (Table S4), and ABR click-rate (Table S5) are summarized in the supplementary tables.

ABR: Wave Amplitudes

The amplitudes of waves I, III, and V were analyzed and there was a main effect of group for all waves (Figure 2). There were no interactions with group. In addition, there was no main effect of group on central gain which is the ratio of wave V to wave I. The Autistic participants had reduced neural responsivity across the ascending auditory pathway.

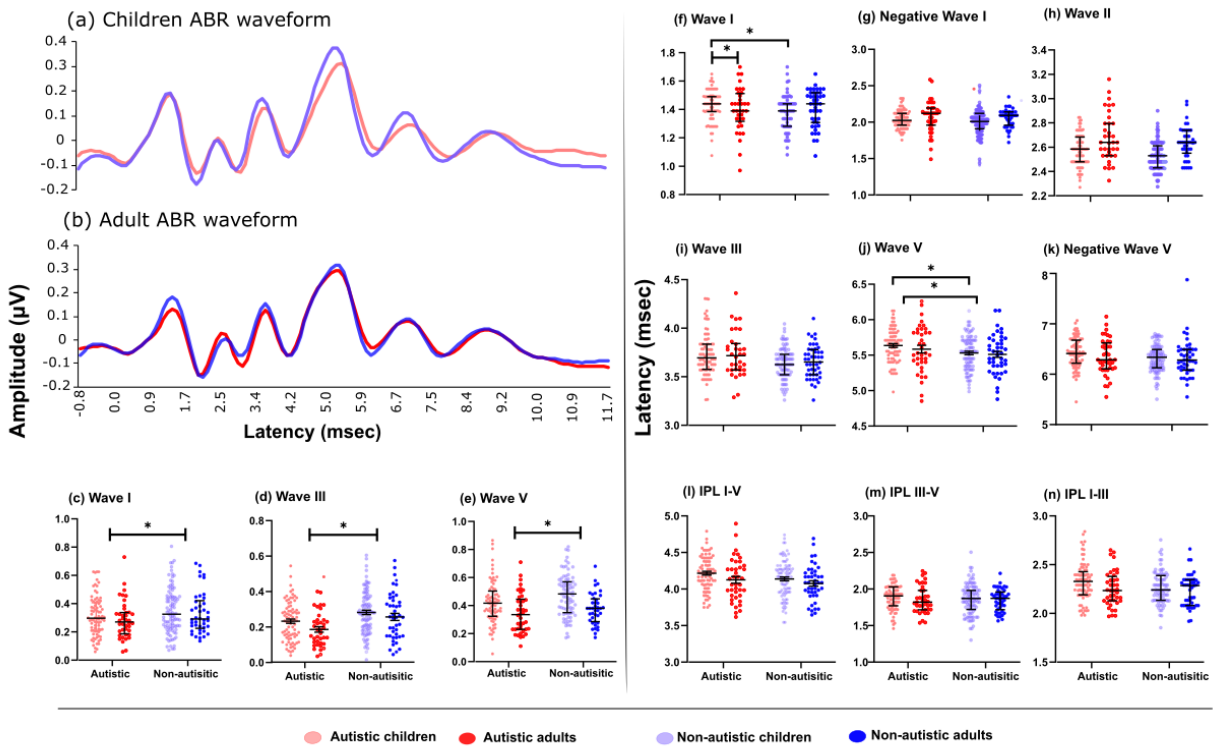


Figure 2: ABR results. (a) The averaged ABR waveform of the slow click-rate for the children sample. (b) The averaged ABR waveform of the slow click-rate for the adult sample. (c)There was a main effect of diagnostic group on amplitudes of waves I ($F_{(1,244)} = 4.16$, $p = 0.04$, $p_{adj}=0.043$). (d) There was a main effect of diagnostic group on amplitudes of waves III ($F_{(1,240)} = 4.44$, $p = 0.036$, $p_{adj}=0.043$). (e) There was a main effect of diagnostic group on amplitudes of waves V ($F_{(1,256)} = 5.27$, $p = 0.023$, $p_{adj}=0.043$). (f) There was a group by age interaction ($F_{(1,250)} = 6.28$, $p = 0.01$, $p_{adj}= 0.11$) on wave I absolute latency with a simple main effect of group for the children ($F_{(1,228)} = 4.26$, $p = 0.04$, $p_{adj}= 0.08$) with Autistic children having increased absolute latency. (g) Absolute negative wave 1 has no main effect of diagnostic group. (h) Absolute wave II has no main effect of diagnostic group. (i) Absolute wave III has no main effect of diagnostic group. (j) The absolute wave V latency had a main effect of diagnostic group ($F_{(1,234)} = 9.90$, $p = 0.0019$, $p_{adj}=0.016$) and two thee-way interactions of diagnostic group by sex by age ($F_{(1,234)} = 4.47$, $p = 0.036$, $p_{adj}=0.32$) and diagnostic group by rate by age ($F_{(1,234)} = 4.93$, $p = 0.027$, $p_{adj}=0.25$). The simple main effects revealed a main effect of diagnostic group on the absolute wave V of Autistic children ($F_{(1,234)} = 13.7$, $p_{adj}=0.0005$). (k) There was no main effect of diagnostic group on the absolute latency of negative wave V. (l) There was no main effect of diagnostic group on interpeak latencies I-V. There was a group by rate by age interaction for IPL I-V approaching significance ($F_{(1,240)} = 3.61$, $p = 0.059$). Probing this interaction revealed a simple main effect of group for children ($F_{(1,240)} = 5.94$, $p_{adj} = 0.032$). (m) There was no main effect of diagnostic group on interpeak latencies III-V. (n) There was no main effect of diagnostic group on interpeak latencies I-III.

ASR: Startle Magnitude

Startle magnitude was higher for the Autistic participants with a main effect of group (Figure 3; $F_{(1,709)} = 5.75$, $p = 0.017$) on acoustic reactivity. To probe the main effect of group, we compared autistic and non-autistic participants separately within children and adults using nonparametric Wilcoxon rank-sum tests. A significant group difference was found in adults ($W = 7634$, $p = .0023$, $p_{adj} = 0.005$) but not in children ($W = 17386$, $p = 0.088$, $p_{adj} = 0.088$). There was also a main effect of stimuli intensity ($F_{(1,709)} = 7.2$, $p < 0.001$) and age ($F_{(1,709)} = 36.3$, $p < 0.001$). The autistic participants driven by the adults presented with hyper-reactivity to auditory stimuli.

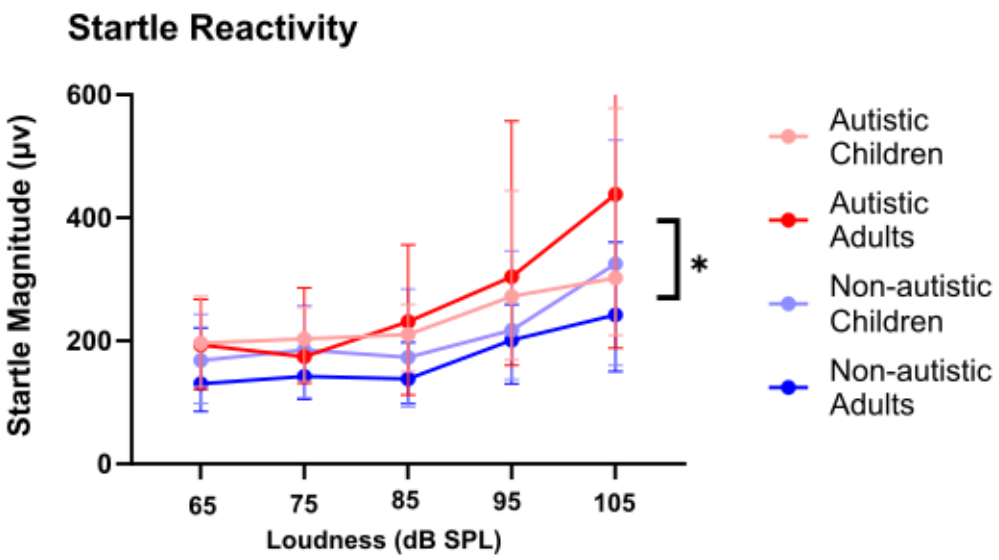


Figure 3: The acoustic startle reactivity magnitude. There was a main effect of diagnostic group on startle magnitude which was mainly driven by the adults.

ASR: Startle Latency

There was a three-way interaction of group by age by sex on startle latency ($F_{(1,615)} = 5.86$, $p = 0.016$) and startle onset ($F_{(1,620)} = 9.62$, $p = 0.002$) but when probing for simple main effects there was no simple main effect of group.

Associations with Age

The ASR reactivity was only negatively correlated with age for the non-Autistic participants (Table 1). The ABR wave latencies were positively correlated with age, while amplitudes were negatively correlated (Figure 4). When splitting the groups by diagnosis some associations were only significant for one of the groups (Table 1), for example wave I latency was positively correlated with age for the non-autistic participants, a relation not established for the autistic participants. There were no significant associations of age and startle latency or startle onset.

Table 1: Spearman's rank correlation of ABR features, ASR reactivity, and age, and difference in correlations between groups.

	Autistic		*Non-autistic*		*Group difference*
	ρ[CI]	p	ρ[CI]	p	Δ ρ[CI]
ABR Wave I latency and Age	-0.02[-0.30 0.25]	0.90	0.33[0.07 0.54]	0.006	-0.35[-0.72 -0.01]
ABR Wave I Negative latency and Age	0.15[-0.14 0.45]	0.27	0.36[0.13 0.57]	0.003	-0.22[-0.56 0.15]
ABR Wave II latency and Age	0.31[0.01 0.58]	0.02	0.42[0.23 0.60]	0.0004	-0.11[-0.48 0.23]
ABR Wave I amplitude and Age	-0.28[-0.51 0.01]	0.03	-0.30[-0.53 -0.06]	0.01	-0.03[-0.35 0.38]
ABR Wave V amplitude and Age	-0.27[-0.51 -0.01]	0.04	-0.23[-0.49 0.05]	0.06	-0.04[-0.40 -0.34]
ASR reactivity and Age	-0.08[-0.21 0.07]	0.26	-0.12[-0.24 -0.00]	0.04	0.04[-0.15 0.23]
ABR Wave I and ASR magnitudes	-0.09[-0.23 0.04]	0.17	0.15[0.03 0.27]	0.018	-0.24[-0.42 -0.07]
ABR Wave III and ASR magnitudes	-0.01[-0.16 0.14]	0.90	-0.01[-0.13 0.11]	0.93	-0.003[-0.21 -0.18]
ABR Wave V and ASR magnitudes	-0.003[-0.14 0.13]	0.96	0.22[0.11 0.34]	0.0004	-0.23[-0.41 -0.05]

ABR and acoustic reactivity associations

ABR amplitudes and reactivity associations are in opposite directions for Autistic and non-Autistic participants (Table 1). Wave I and wave V amplitudes were positively correlated with reactivity for the non-autistic participants while they were negatively correlated with reactivity for the autistic participants. The associations were significantly different from each other.

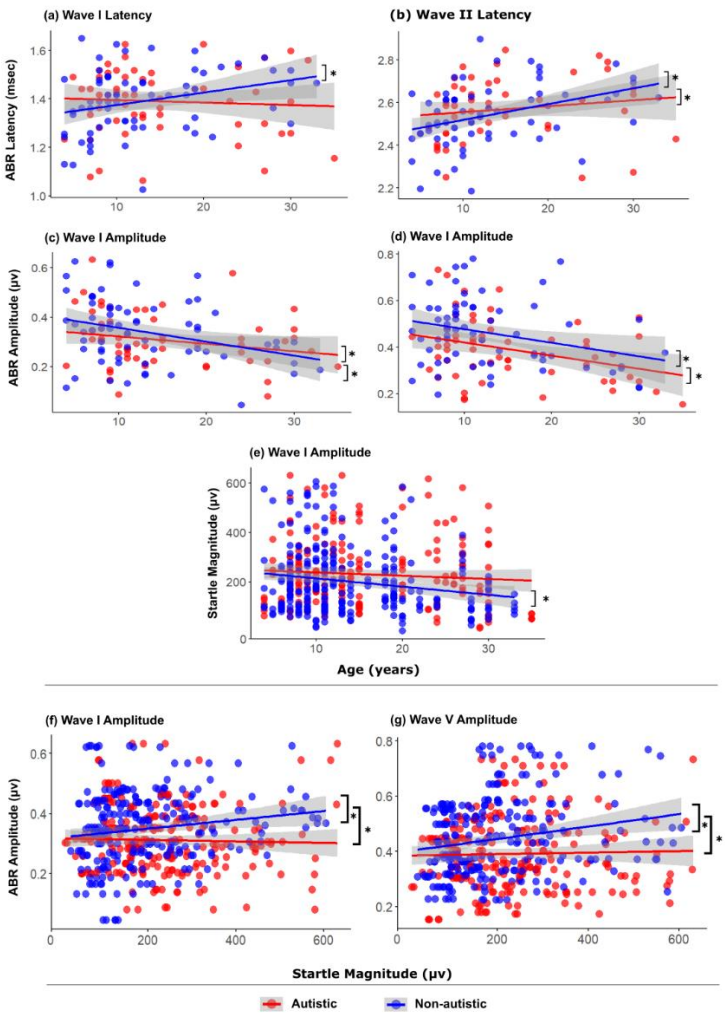


Figure 4: ABR associations with age and startle reactivity. (a) Absolute ABR waves I latency was positively correlated with age (ρ= .18, p = .0498, padj = 0.006) across diagnostic groups. After splitting by diagnostic groups, the association was significant for the non-autistic group (ρ = .32, p = .00012, padj = 0.0004) but not the autistic group (ρ = .008, p = .93, padj = .95). (b) Absolute ABR waves II latency was positively correlated (ρ = .36, p <0.0001, padj <0.0001) with age across diagnostic groups. When probing for diagnostic groups effect, the association remained significant for the non-autistic group (ρ = .42, p <0.0001, padj < 0.0001) and the autistic group (ρ = .27, p = .0017, padj < 0.015). (c) ABR waves I amplitude negatively correlated I (ρ = - .23, p = .00018, padj =0.03) with age across diagnostic groups and within diagnostic groups. (d) ABR waves V amplitude negatively correlated (ρ = - .27, p < 0.0001, padj = 0.01) with age across diagnostic groups and within diagnostic groups. (e) Startle reactivity was negatively correlated with age for the non-autistic participants (ρ = - .13, p = 0.025), but not the autistic participants (ρ = .03, p = 0.62). (f) ABR waves I amplitude positively correlated with startle reactivity for the non-autistic participants and trended towards negative correlation for the autistic participants. The correlations were significantly different. (g) ABR waves V amplitude positively correlated with startle reactivity for the non-autistic group. The correlations were significantly different.

Discussion

We investigated whether Autistic children and adults experienced a combination of brainstem-mediated auditory hypo-responsiveness and hyper-reactivity. Participants completed a passive paradigm of auditory tasks which included ABR as a measure of neural responsivity and ASR as a measure of behavioral reactivity. We hypothesized that the Autistic children would present with altered auditory brainstem neural responsivity while this responsivity would have normalized in adults. At the same time, we expect no diagnostic difference in behavioral reactivity between Autistic and non-Autistic children but predicted an increase in reactivity in Autistic adults relative to their non-Autistic peers. This auditory profile could be due to compensatory adaptive auditory gain formed during critical windows of experience induced plasticity in Autistic children that impacts their reactivity to sound in the long term. The auditory system expresses increased experience-induced plasticity in younger age. For example, in congenitally deaf children, the maturation of the auditory evoked potentials depended on the age of cochlear implantation with optimal development occurring in implantation before the age of 3.5 years[34]. Therefore, the hypo-responsivity experienced in early-life of Autistic children could have cascading effects on the long-term development of their auditory system.

Indeed, Autistic children showed reduced neural responsivity and slower neurotransmission in their ascending auditory pathway, evidenced by increased latencies and reduced amplitudes of ABR waves. Autistic children presented with increased ABR absolute latencies in the ascending brainstem auditory pathway starting at the auditory nerve, lateral lemniscus, and the inferior colliculus. In contrast, Autistic adults did not show the same extent of increased latency, meaning the ascending auditory pathway could be experiencing a perturbation in development that eventually normalizes, a finding consistent with multiple studies (for review, see[20,35]).

In addition, one of the possible cascading effects of hypo-responsivity in the ascending auditory pathway during development could be the long-term hyper-reactivity to sounds, which we measured using the ASR. Autistic participants had an increased reactivity to sound which was driven by the autistic adults. The increased reactivity was in combination with reduced responsivity in the brainstem auditory pathway, a contradiction posed previously and stated in the pre-registration. An explanation for this observation is the presence of a compensatory gain that is mediating the startle response. The primary startle pathway includes the cochlear nerve and the cochlear nucleus in the brainstem that relays the signal to the caudal pontine reticular nucleus (PnC), which coordinates motor responses via projections to spinal and cranial motor neurons. It is modulated by midbrain circuits such as the inferior colliculus, amygdala, and periaqueductal gray to adjust the reflex based on sensory or emotional context.

The ABR and ASR pathways directly intersect at the cochlear nerve and the cochlear nucleus. They also indirectly intersect through the midbrain ASR modulatory pathways (inferior colliculus - superior colliculus - Pedunculopontine Tegmental Nucleus) that adjust the response intensity based on the sensory context. The increased magnitude of the startle response associated with Autism is regulated by the excitability, size and number of recruited PnC giant neurons, as recently

shown in an *Cntnap2* knock-out Autism rat model[36]. A similar modulation in the sensorimotor interface of those pathways is most likely causing the increased reactivity to sound in Autistic individuals.

Autistic participants with more severe disruptions in the ascending auditory pathway also experience higher hyper-reactivity. There is an intuitive understanding of an increase in responsivity inducing hyper-reactivity. However, the negative relationship observed in the Autistic participants is an indication that disruptions in the ascending auditory pathway highlight systematic differences in the auditory brainstem. The change in the auditory responses across development hint toward compensatory changes in the auditory gain.

The idea of maladaptive sensory gain stems from reports of reduced neurotransmission of the ascending auditory pathway of newborn babies later diagnosed with Autism[24,37]. During early postnatal development neural circuits, both structural and functional, are shaped by exposure to sensory input. These periods of heightened plasticity play a crucial role in the maturation of sensory representations in both subcortical and cortical regions, influencing the development of behavior and cognition. In the auditory system, this period of rapid refinement supports the acquisition of specialized skills such as phoneme recognition, language learning, absolute pitch, and musical ability, but it also marks a time of heightened vulnerability to sensory deprivation. The early reduced responsivity and slowed neurotransmission regardless of the molecular reason could induce adaptive excitatory gain or reduced inhibitory gain within the auditory system. As evidenced in our results, the ABR pathway partially recovers its output and therefore what was considered adaptive would induce maladaptive gain, leading to behavioral hyper-reactivity.

Mechanistically, the ABR differences could be linked to possible molecular and cellular changes. Slower neural transmission could be due to dysfunction in myelination, pathway length, axonal diameter, the (a)synchronization of neuronal firing, or synaptic efficacy[38]. Those factors have not been directly or consistently linked to Autism as there are opposing findings within the central nervous system. For example, diffusion tensor imaging mean diffusivity of white matter microstructure findings has shown both increases[39] and decreases[40] in Autism. While ABRs do not directly measure white matter microstructure, ABRs are sensitive to changes in axonal morphology and myelination, axon bundle density and fiber orientation distribution, and other intra- and extra-cellular processes.

In addition, Autistic children had reduced amplitude of waves I, III and V, which implies reduced neural responsivity across the ascending auditory pathway starting at the dorsal end of the auditory nerve through the cochlear nucleus and the superior olivary complex to the lateral lemniscus and inferior colliculus. The ABR wave amplitudes are related to synchronized neural activity [41], and a reduced amplitude could be due to numerous reasons such as desynchrony in the neural firing, reduced number of firing neurons, or synaptic dysfunction. Amplitudes are not commonly examined clinically and in research, and results are limited and contradictory. For example, wave III has been previously reported to have reduced [30] or increased amplitude [42] in Autism. Moreover, typically ABR wave amplitudes reduce with age, and this association is observed in our sample.

Unlike the children, there were no latency differences between Autistic and non-Autistic adult groups, therefore it seems like the speed of conduction recovers with age. For the non-Autistic group, latency of ABR wave I was positively associated with age as aging increase ABR peak latencies, with significant shifts limited to early waves[43]. Despite the fact that the Autistic participants do not have the same developmental trajectory, by adulthood, Autistic adults have an ABR waveform that is not significantly different from their non-Autistic peers. Although neuronal responsivity and conduction may recover by adulthood, the cascading and lasting effects of these initial reductions could extend well beyond this period.

The atypical development of the ascending auditory pathway is also supported by differences in the brainstem being more often reported in Autistic children than Autistic adults[20]. Longitudinal ABR studies also report a decline in the group differences between Autistic and non-Autistic children across age[25,44].

There is no consensus on the structural or synaptic condition within the ascending brainstem pathways in Autism therefore it is difficult to disentangle the specific reasons for the reduction in action potential velocity and responsivity. One interpretation of our findings is that the ascending auditory pathway has a combination of abnormalities such as reduced number of cellular nuclei, reduced axonal diameter, asynchronization of neuronal firing and changes in myelination that results in this unique ABR profile in Autism. Those kinds of changes are observed in post-mortem analysis of the superior olivary complex in which the nuclei have altered volumes[45,46], shapes[47], and number[47] (for a review, see[20]). Some of those features undergo tuning based on sensory experiences and this experience-based tuning may lead to cascading sensory and hyper-reactivity differences in Autistic children and adults.

Addressing ABR methodology heterogeneity

While the atypical ABR finding is not unique to our study, results in the field have been highly heterogeneous. Three factors have been previously suggested to contribute to the heterogeneity of the literature which our current design addresses. The first factor of variation is age[35,48] in which some studies have age-specific ranges, while others examine ABR across less-defined age ranges. In this study, we age-matched and included an age analysis. Another factor is the inclusion of participants with possible peripheral hearing loss - we controlled for this using a battery of periphery hearing evaluation (Tables 3 and 4). Finally, another common confounding factor is the ABR click-rate, since it could be used to exert varying levels of challenge to the auditory nerve, with faster click rates eliciting longer wave latencies[48–50].This study employed a slow click-rate and a fast click-rate to eliminate the click-rate related variation. While there was a main effect of click-rate on ABR wave features, there was no group by rate interactions. All in, we present robust evidence for altered ABR waves of Autistic children and adults.

Limitations

This study has several limitations that warrant consideration when interpreting the findings. First, the exclusion of Autistic participants with the most severe auditory and sensory sensitivities introduces a potential selection bias. Some participants, particularly those with significant tactile sensitivities, were unable to complete the study due to discomfort caused by the conductive gel,

despite the study being focused on auditory processing. This limits the generalizability of the results, as the participants who completed the study may represent a subset of the Autistic population with milder sensory challenges. Second, the underrepresentation of Autistic females in the study, combined with the fact that many Autistic women included were diagnosed in adulthood, introduces additional biases. Late-diagnosed women often present with less pronounced sensory differences due to either a milder symptom profile or the development of coping strategies over time. These factors may have skewed the findings, as the sample may not accurately reflect the broader variability of sensory experiences in Autistic individuals, particularly among females. Future studies should strive for greater inclusion of individuals with severe sensory sensitivities and ensure more representative sex diversity to better capture the full spectrum of sensory processing differences in Autism.

Conclusion

In conclusion, this study provides robust evidence for altered auditory brainstem responses in Autistic children and adults, highlighting unique patterns of neural responsivity and behavioral reactivity. Our findings suggest that disrupted auditory processing in childhood may have long-term effects on sensory integration and reactivity, potentially due to maladaptive neural plasticity during critical developmental periods. These disruptions likely involve a combination of factors, including changes in synaptic efficiency, neural synchronization, axonal properties, and brainstem nuclei structure, as supported by both our findings and the broader literature. While the normalization of ABR latency in Autistic adults suggest recovery in conduction speed, the persistence of hyper-reactivity underscores the lasting impact of early auditory pathway disruptions. These results not only deepen our understanding of auditory processing differences in Autism but also highlight the importance of investigating the interplay between brainstem-mediated sensory responsivity and higher-order cognitive and sensory functions. Overall, these findings underscore the atypical auditory brainstem profile of hypo-responsivity and hyper-reactivity in Autistic individuals and the partial recovery of the ascending auditory pathway across development.

Financial Disclosures / Acknowledgements. RAS is funded through a Dorothy Killam Fellowship, an NSERC Discovery Grant (RGPIN-2024-06233), two SSHRC Insight Grants (435-2017-0936 & 435-2024-1375), a CIHR Project Grant (487850), the University of Western Ontario Faculty Development Research Fund, and a Canadian Foundation for Innovation John R. Evans Leaders Fund (37497), and through a grant from the Canada First Research Excellence Fund (BrainsCAN). RS would also like to thank the Garvey sisters for appropriately dealing with JP. The study was also supported by a CIHR project grant to SS (168866) and the Simons Foundation for Autism Research (SFARI). The remaining authors reported no biomedical financial interests or potential conflicts of interests.

CRediT Statement. AS – Conceptualization, Methodology, Formal analysis, Investigation, writing – original draft, writing – review and editing, Visualization. RG – Investigation, MR – Investigation, CM – Investigation, KS – Investigation, RAS – Conceptualization, resources, data

curation, writing – review and editing, supervision, visualization, project administration, funding acquisition. SES – Conceptualization, methodology, investigation. SS Conceptualization, review and editing.

References:

1. *merican Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders: DSM-5*. (2013).
2. Dwyer, P., Takarae, Y., Zadeh, I., Rivera, S. M. & Saron, C. D. A multidimensional investigation of sensory processing in autism: Parent- and self-report questionnaires, psychophysical thresholds, and event-related potentials in the auditory and somatosensory modalities. *Front. Hum. Neurosci.* **16**, 811547 (2022).
3. Stevenson, R. A. *et al.* The cascading influence of multisensory processing on speech perception in autism. *Autism* **22**, 609–624 (2018).
4. Stevenson, R. A., Segers, M., Ferber, S., Barense, M. D. & Wallace, M. T. The impact of multisensory integration deficits on speech perception in children with autism spectrum disorders. *Front. Psychol.* **5**, 379 (2014).
5. Ashburner, J., Ziviani, J. & Rodger, S. Sensory processing and classroom emotional, behavioral, and educational outcomes in children with autism spectrum disorder. *Am. J. Occup. Ther.* **62**, 564–573 (2008).
6. Pierce, K. & Courchesne, E. Evidence for a cerebellar role in reduced exploration and stereotyped behavior in autism. *Biol. Psychiatry* **49**, 655–664 (2001).
7. Schulz, S. E. *et al.* Sensory processing patterns predict problem behaviours in autism spectrum disorder and attention-deficit/hyperactivity disorder. *Adv. Neurodev. Disord.* (2022) doi:10.1007/s41252-022-00269-3.
8. Cardon, G., Cate, M., Cordingley, S. & Bown, B. Auditory brainstem response in autistic children: Implications for sensory processing. *Hearing Balance Commun.* **21**, 224–232 (2023).
9. Rozenkrantz, L. *et al.* A mechanistic link between olfaction and autism spectrum disorder. *Curr. Biol.* **25**, 1904–1910 (2015).

10. Puts, N. A. J., Wodka, E. L., Tommerdahl, M., Mostofsky, S. H. & Edden, R. A. E. Impaired tactile processing in children with autism spectrum disorder. *J. Neurophysiol.* **111**, 1803–1811 (2014).
11. Bonnel, A. *et al.* Enhanced pitch sensitivity in individuals with autism: a signal detection analysis. *J. Cogn. Neurosci.* **15**, 226–235 (2003).
12. Simmons, D. R. *et al.* Vision in autism spectrum disorders. *Vision Res.* **49**, 2705–2739 (2009).
13. Stevenson, R. A. *et al.* Brief report: Arrested development of audiovisual speech perception in autism spectrum disorders. *J. Autism Dev. Disord.* **44**, 1470–1477 (2014).
14. Stevenson, R. A. *et al.* Evidence for diminished multisensory integration in autism spectrum disorders. *J. Autism Dev. Disord.* **44**, 3161–3167 (2014).
15. Wallace, M. T., Woynaroski, T. G. & Stevenson, R. A. Multisensory integration as a window into orderly and disrupted cognition and communication. *Annu. Rev. Psychol.* **71**, 193–219 (2020).
16. Woynaroski, T. G. *et al.* Multisensory speech perception in children with autism spectrum disorders. *J. Autism Dev. Disord.* **43**, 2891–2902 (2013).
17. Litovsky, R. Development of the auditory system. *Handb. Clin. Neurol.* **129**, 55–72 (2015).
18. Uylings, H. B. M. Development of the human cortex and the concept of “critical” or “sensitive” periods. *Lang. Learn.* **56**, 59–90 (2006).
19. Gonçalves, A. M. & Monteiro, P. Autism Spectrum Disorder and auditory sensory alterations: a systematic review on the integrity of cognitive and neuronal functions related to auditory processing. *J. Neural Transm.* **130**, 325–408 (2023).
20. Seif, A., Shea, C., Schmid, S. & Stevenson, R. A. A Systematic Review of Brainstem Contributions to Autism Spectrum Disorder. *Front. Integr. Neurosci.* **15**, 760116 (2021).
21. Wegiel, J. *et al.* Brain-region-specific alterations of the trajectories of neuronal volume growth throughout the lifespan in autism. *Acta Neuropathol. Commun.* **2**, 28 (2014).

22. Scott, K. E. *et al.* Altered Auditory Processing , Filtering , and Reactivity in the Cntnap2 Knock-Out Rat Model for Neurodevelopmental Disorders. *J. Neurosci.* **38**, 8588–8604 (2018).

23. Young, A., Cornejo, J. & Spinner., A. *Auditory Brainstem Response*. (Treasure Island (FL): StatPearls Publishing, 2023).

24. Miron, O. *et al.* Prolonged Auditory Brainstem Response in Universal Hearing Screening of Newborns with Autism Spectrum Disorder. *Autism Res.* **14**, 46–52 (2021).

25. Li, A. *et al.* Continued development of auditory ability in autism spectrum disorder children: A clinical study on click-evoked auditory brainstem response. *Int. J. Pediatr. Otorhinolaryngol.* **138**, 110305 (2020).

26. Kamita, M. K. *et al.* Brainstem auditory evoked potentials in children with autism spectrum disorder ☆ , ☆☆. *J. Pediatr. (Rio J.)* 7 (2019).

27. Takahashi, H. *et al.* Hyperreactivity to weak acoustic stimuli and prolonged acoustic startle latency in children with autism spectrum disorders. *Mol. Autism* **5**, 23 (2014).

28. Sinclair, D., Oranje, B., Razak, K. A., Siegel, S. J. & Schmid, S. Sensory processing in autism spectrum disorders and Fragile X syndrome—From the clinic to animal models. *Neurosci. Biobehav. Rev.* **76**, 235–253 (2017).

29. Zheng, A. & Schmid, S. A review of the neural basis underlying the acoustic startle response with a focus on recent developments in mammals. *Neurosci. Biobehav. Rev.* **148**, 105129 (2023).

30. Källstrand, J., Olsson, O., Nehlstedt, S. F., Sköld, M. L. & Nielzén, S. Abnormal auditory forward masking pattern in the brainstem response of individuals with Asperger syndrome. *Neuropsychiatr. Dis. Treat.* **6**, 289–296 (2010).

31. Wechsler, D. Wechsler Abbreviated Scale of Intelligence–Second Edition (WASI-II). *San Antonio, TX: NCS Pearson.* (2011) doi:10.1037/t15171-000.

32. Kaufman, A. S., & Kaufman, N. L. Kaufman Brief Intelligence Test, Second Edition (KBIT-2). (2004).

33. Lord, C. Autism diagnostic observation schedule: ADOS-2. Preprint at (2012).

34. Irvine, D. R. F. Plasticity in the auditory system. *Hear. Res.* **362**, 61–73 (2018).

35. Pillion, J. P., Boatman-Reich, D. & Gordon, B. Auditory brainstem pathology in autism spectrum disorder: A review. *Neuropsychiatry Neuropsychol. Behav. Neurol.* **31**, 53–78 (2018).

36. Zheng, A., Mann, R. S., Solaja, D., Allman, B. L. & Schmid, S. Properties of the caudal pontine reticular nucleus neurons determine the acoustic startle response in Cntnap2 KO rats. *J. Integr. Neurosci.* **23**, 63 (2024).

37. Delgado, C. F., Simpson, E. A., Zeng, G., Delgado, R. E. & Miron, O. Newborn auditory brainstem responses in children with developmental disabilities. *J. Autism Dev. Disord.* **53**, 776–788 (2023).

38. Eggermont, J. J. On the rate of maturation of sensory evoked potentials. *Electroencephalogr. Clin. Neurophysiol.* **70**, 293–305 (1988).

39. Gibbard, C. R. *et al.* White matter microstructure correlates with autism trait severity in a combined clinical-control sample of high-functioning adults. *NeuroImage Clin.* **3**, 106–114 (2013).

40. Surgent, O. *et al.* Brainstem white matter microstructure is associated with hyporesponsiveness and overall sensory features in autistic children. *Mol. Autism* **13**, 48 (2022).

41. Rouillon, I., Parodi, M., Denoyelle, F. & Loundon, N. How to perform ABR in young children. *Eur. Ann. Otorhinolaryngol. Head Neck Dis.* **133**, 431–435 (2016).

42. Claesdotter-Knutsson, E., Åkerlund, S., Cervin, M., Råstam, M. & Lindvall, M. Abnormal auditory brainstem response in the pons region in youth with autism. *Neurology, Psychiatry and Brain Research* **32**, 122–125 (2019).

43. Konrad-Martin, D. *et al.* Age-related changes in the auditory brainstem response. *J. Am. Acad. Audiol.* **23**, 18–35; quiz 74–5 (2012).

44. Chen, J. *et al.* Atypical longitudinal development of speech-evoked auditory brainstem response in preschool children with autism spectrum disorders. *Autism Res.* **12**, 1022–1031 (2019).

45. Kulesza, R. J. & Mangunay, K. Morphological features of the medial superior olive in autism. *Brain Res.* **1200**, 132–137 (2008).

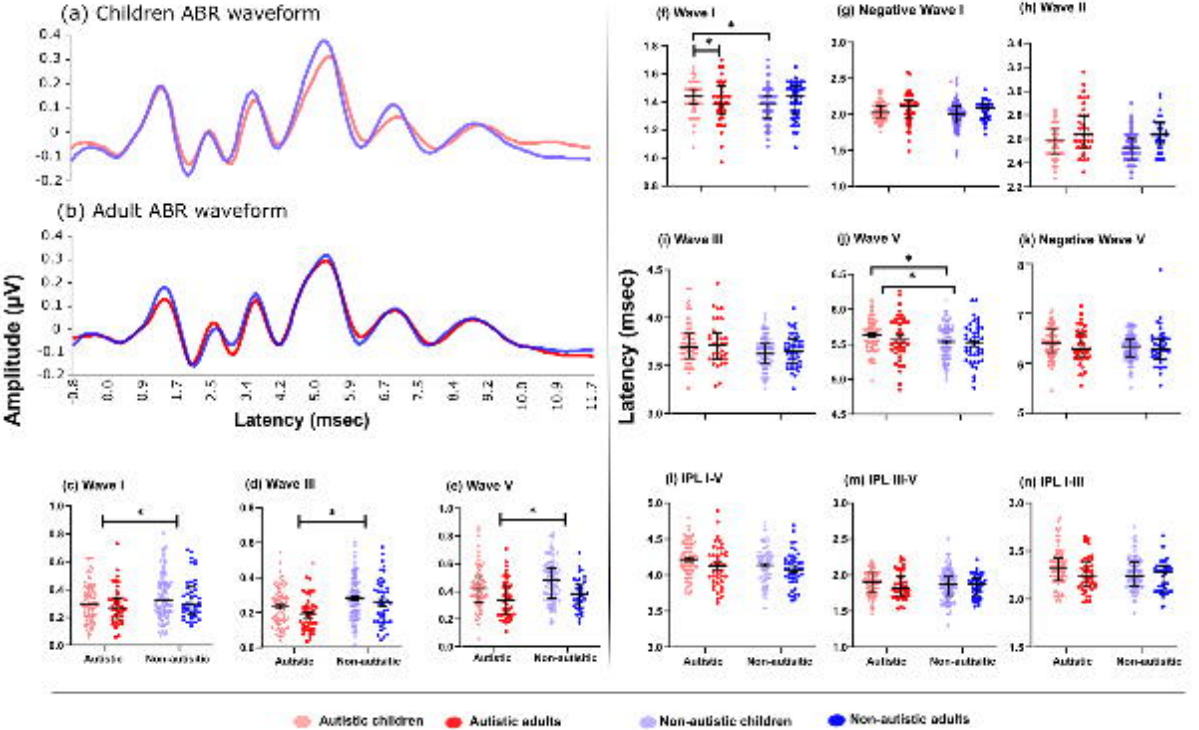
46. Mansour, Y. & Kulesza, R. Three dimensional reconstructions of the superior olivary complex from children with autism spectrum disorder. *Hear. Res.* **393**, 107974 (2020).

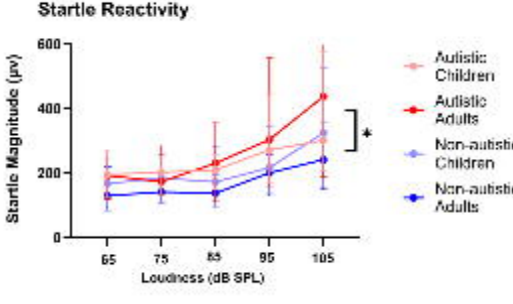
47. Kulesza, R. J., Lukose, R. & Stevens, L. V. Malformation of the human superior olive in autistic spectrum disorders. *Brain Res.* **1367**, 360–371 (2011).

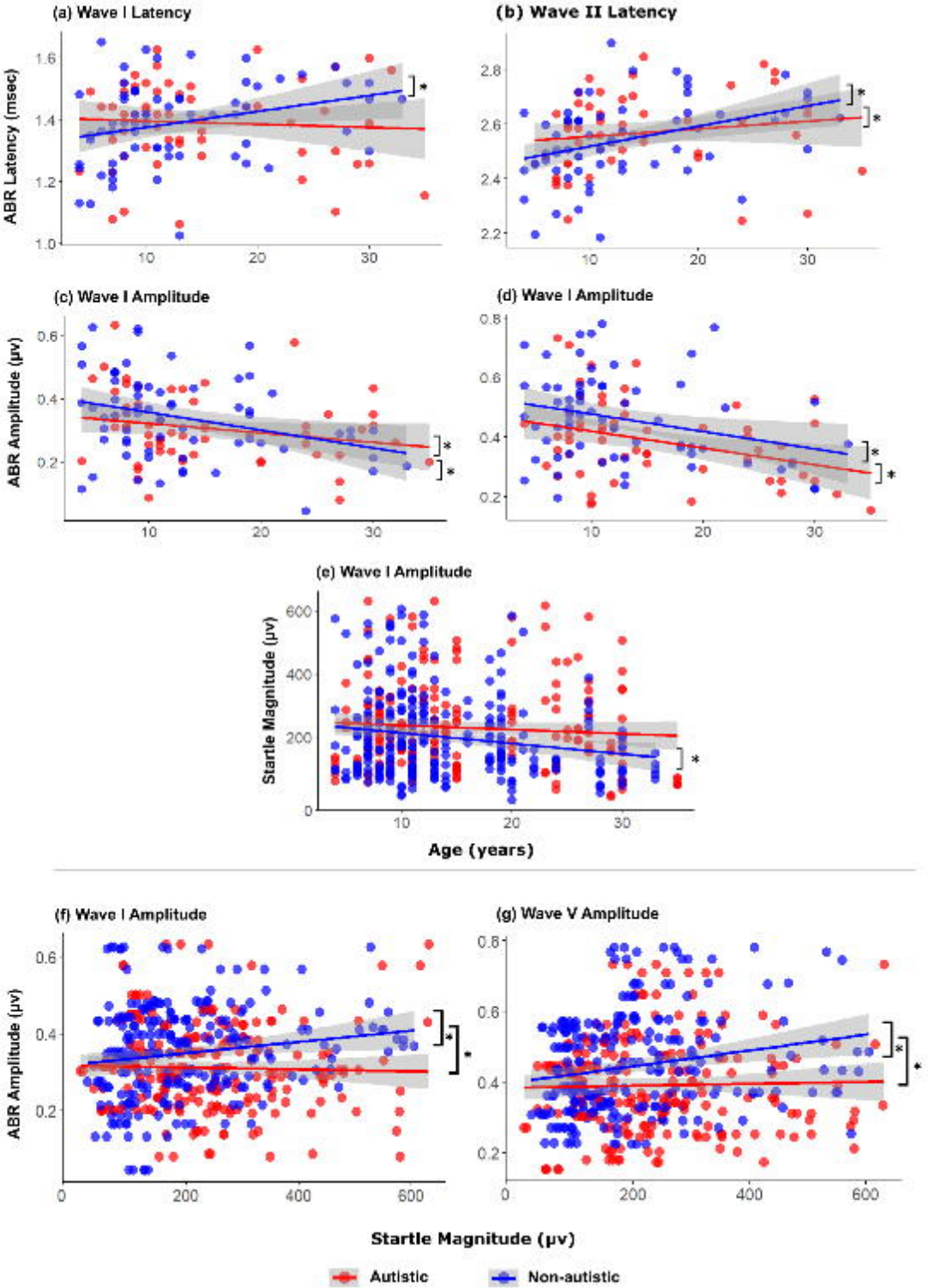
48. Talge, N. M., Tudor, B. M. & Kileny, P. R. Click-evoked auditory brainstem responses and autism spectrum disorder: A meta-analytic review. *Autism Res.* **11**, 916–927 (2018).

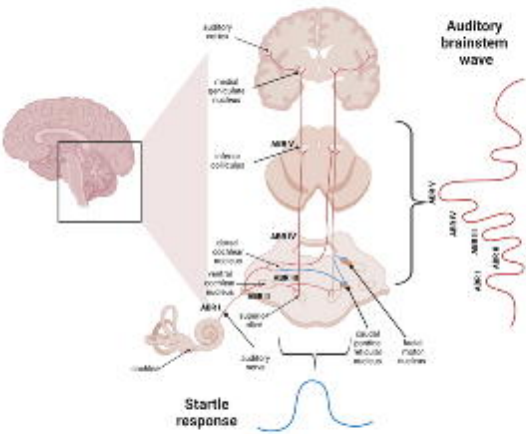
49. Jacobson, J. T., Murray, T. J. & Deppe, U. The effects of ABR stimulus repetition rate in multiple sclerosis. *Ear Hear.* **8**, 115–120 (1987).

50. Jiang, Z. D., Brosi, D. M. & Wilkinson, A. R. Auditory neural responses to click stimuli of different rates in the brainstem of very preterm babies at term. *Pediatr. Res.* **51**, 454–459 (2002).


(a) Children ABR waveform
(b) Adult ABR waveform
Amplitude (μV)
Latency (msec)
(c) Wave I
(d) Wave III
(e) Wave V
(f) Wave I
(g) Negative Wave I
(h) Wave II
(i) Wave III
(j) Wave V
(k) Negative Wave V
(l) IPL I-V
(m) IPL III-V
(n) IPL I-III
Latency (msec)
Autistic
Non-autistic
Autistic children
Autistic adults
Non-autistic children
Non-autistic adults


Startle Reactivity
Startle Magnitude (µv)
600
400
200
0
65
75
85
95
105
Loudness (dB SPL)
*
Autistic Children
Autistic Adults
Non-autistic Children
Non-autistic Adults


(a) Wave I Latency
ABR Latency (msec)
(b) Wave II Latency
(c) Wave I Amplitude
ABR Amplitude (µv)
(d) Wave I Amplitude
(e) Wave I Amplitude
Startle Magnitude (µv)
Age (years)
(f) Wave I Amplitude
ABR Amplitude (µv)
(g) Wave V Amplitude
Startle Magnitude (µv)
Autistic
Non-autistic


Auditory brainstem wave
Startle response