Prolonged Auditory Brainstem Response in Universal Hearing Screening of Newborns with Autism Spectrum Disorder

Oren Miron ⓘ, Rafael E. Delgado, Christine F. Delgado ⓘ, Elizabeth A. Simpson ⓘ, Kun-Hsing Yu ⓘ, Anibal Gutierrez, Guangyu Zeng ⓘ, Jillian N. Gerstenberger, and Isaac S. Kohane

Previous studies report prolonged auditory brainstem response (ABR) in children and adults with autism spectrum disorder (ASD). Despite its promise as a biomarker, it is unclear whether healthy newborns who later develop ASD also show ABR abnormalities. In the current study, we extracted ABR data on 139,154 newborns from their Universal Newborn Hearing Screening, including 321 newborns who were later diagnosed with ASD. We found that the ASD newborns had significant prolongations of their ABR phase and V-negative latency compared with the non-ASD newborns. Newborns in the ASD group also exhibited greater variance in their latencies compared to previous studies in older ASD samples, likely due in part to the low intensity of the ABR stimulus. These findings suggest that newborns display neurophysiological variation associated with ASD at birth. Future studies with higher-intensity stimulus ABRs may allow more accurate predictions of ASD risk, which could augment the universal ABR test that currently screens millions of newborns worldwide. *Autism Res* 2021, 14: 46–52. © 2020 The Authors. *Autism Research* published by International Society for Autism Research and Wiley Periodicals LLC.

Lay Summary: Children with autism spectrum disorder (ASD) have slow brain responses to sounds. We examined these brain responses from newborns' hearing tests and found that newborns who were later diagnosed with autism also had slower brain responses to sounds. Future studies might use these findings to better predict autism risk, with a hearing test that is already used on millions of newborns worldwide.

Keywords: auditory; event-related potential; biomarker; infants; children

Introduction

Autism spectrum disorder (ASD) is a neurodevelopmental disorder that includes deficits in social communication and social interaction, and restricted behavior [McPartland, Reichow, & Volkmar, 2012]. The rate of ASD is estimated to be approximately one in 59 children [Christensen et al., 2016], and although behavioral signs of ASD are present in many cases by the age of 18 months, diagnosis is not typically made before 3 to 4 years of age [Ozonoff et al., 2010; Zwaigenbaum et al., 2005, 2015]. Yet, earlier identification and intervention are critical for improving child outcomes and decreasing the economic costs associated with ASD [Dawson et al., 2010; National Research Council, 2001].

The auditory brainstem response (ABR) test—a test used for Universal Newborn Hearing Screening (UNHS)—may be an untapped opportunity to evaluate the risk of developing ASD [Mason & Herrmann, 1998; Morton & Nance, 2006]. The ABR test uses surface electrodes to measure the auditory nerve and brainstem responses to sounds [Brama & Sohmer, 1977]. The ABR waveform includes five waves of electrical activity within the brainstem, with the first wave (wave I) occurring in the auditory nerve, and the fifth wave (wave V) occurring in the lateral lemniscus [Cohen et al., 2013].

Previous studies identified abnormal ABR amplitude in 2- to 6-year-old children with ASD [Santos et al., 2017] and prolongation of the ABR (particularly wave V-positive) in children with ASD [e.g., 8- to 13-year-olds: Maziade et al., 2000; 0.2 months to 21-year-olds: Miron, Beam, & Kohane, 2018; 2- to 3-year-olds: Roth, Muchnik, Shabtai, Hildesheimer, & Henkin, 2011]. A similar ABR prolongation was also identified in infants from the neonatal intensive care unit (NICU) who were later diagnosed with ASD [average of 10 days old: Cohen

et al., 2013; 0.2 to 6.5 months old: Miron et al., 2015], suggesting these abnormalities may be present early in development. However, it remains unknown whether healthy newborns outside of the NICU, who will later develop ASD, would also show ABR differences compared to newborns who do not later develop ASD. Therefore, there is a need to examine ABRs in a larger and younger cohort of healthy infants.

Previous studies acquired ABRs using high sound intensities (80–85 dB normal hearing level (nHL)), which may be particularly well suited for detecting abnormalities related to ASD. In contrast, routine hearing screenings use low sound intensities (35 dB nHL) on most healthy newborns. It is, therefore, unclear whether this lower intensity screener may also identify infants who go on to develop ASD. If so, newborns could potentially be screened for ASD risk using existing universal ABR hearing tests that currently screen millions of newborns worldwide.

The current study, therefore, examined newborn infants from an entire birth cohort (not only NICU) *and* used lower sound intensity (35 dB nHL) hearing screening tests. We hypothesized that newborns later diagnosed with ASD would exhibit ABR prolongation compared to newborns the non-ASD group, reflecting a possible new biomarker for ASD at birth.

Method
Study Groups

ABR data from UNHS testing and ASD data were linked and de-identified in a retrospective case–control design. The case group included newborns later diagnosed with ASD (n = 321) and the control group consisted of newborns who did not receive an ASD diagnosis (n = 138,844). Phase was calculated on the entire sample, while Vn latency was calculated for cases that had waveform data (93% of total sample), which included 286 newborns in the ASD case group and 129,360 newborns in the non-ASD control group. The mean ABR testing age for the newborns who were later diagnosed with ASD was 1.76 days (Range = 0–26, SD = 3.08) and the mean ABR testing age for newborns in the non-ASD group was 1.86 days (Range = 0–31, SD = 3.22). The ASD group was 76.95% male, while the non-ASD group was 51.11% male. The rate of NICU admission was 7.79% in the ASD group and 9.72% in the non-ASD group.

Auditory Brainstem Response Data

This study was approved by the Institutional Review Board at the University of Miami. The ABR data were obtained from UNHS from tests performed in the state of Florida, United States, between 2009 and 2015. The ABR testing was performed by the Pediatrix Medical Group (a MEDNAX® company), which screens 850,000 newborns a year for hearing impairment in the United States. The Smart Screener-Plus from Intelligent Hearing Systems Corp was the ABR device used to collect these data. The test included placing an earpiece in the newborn's ear and delivering click sounds at 35 dB above nHL at a rate of 77 clicks per second in the right ear and 79 clicks per second in the left ear. The clicks create an electrical activity, which is recorded by a surface electrode and used to extract the ABR waveform. The series of 77/79 clicks were repeated to increase the accuracy of the measurement until the algorithm determined if the brainstem responded to sounds. This hearing screening test was performed in the hospital in the first week after birth unless it was postponed due to a medical condition.

The UNHS testing used low intensities, which makes it more difficult to differentiate most wave components. To address this challenge, we focused on two measures: the negative wave that follows wave V positive (V-negative latency), which is easier to detect than the positive wave, and the phase of the ABR response. The V-negative latency is based on the waveform that includes 61 samples from 0.25 milliseconds after the start of each sound stimulus to 15.25 milliseconds after the end of each stimulus presentation. The earphone sound travel time is 0.9 milliseconds, which is subtracted from the latency from sound presentation to calculate the final latency, from the sound onset. V-negative latency was determined for each waveform by searching for the minimum amplitude between 6.35 and 14.35 milliseconds (Fig. 1). The latencies of wave V-negative and the other waves impact the ABR phase, which means a delay in those waves will also cause a delay in the phase. The phase itself was calculated based on the Fast Fourier Transform [Tlumak, Durrant, Delgado, & Boston, 2011] of 1.024-sec-long averaged ABR response sequences.

Autism Spectrum Disorder Data

We obtained children's ASD status from the Florida Department of Education Children's Registry and Information System (CHRIS). This database contains information on ASD eligibility determination for children ages 3 to 5 years who were referred to the Florida Diagnostic and Learning Resources System.

Analysis of ASD and ABR Data

We linked the ABR records and the ASD records using Structured Query Language. A match required the ABR and ASD records to have a perfect match on the date of birth, sex, and name. The ABR records that were not matched to the ASD records were included in the non-ASD group. After completing the linkage, the data were de-identified to ensure confidentiality.
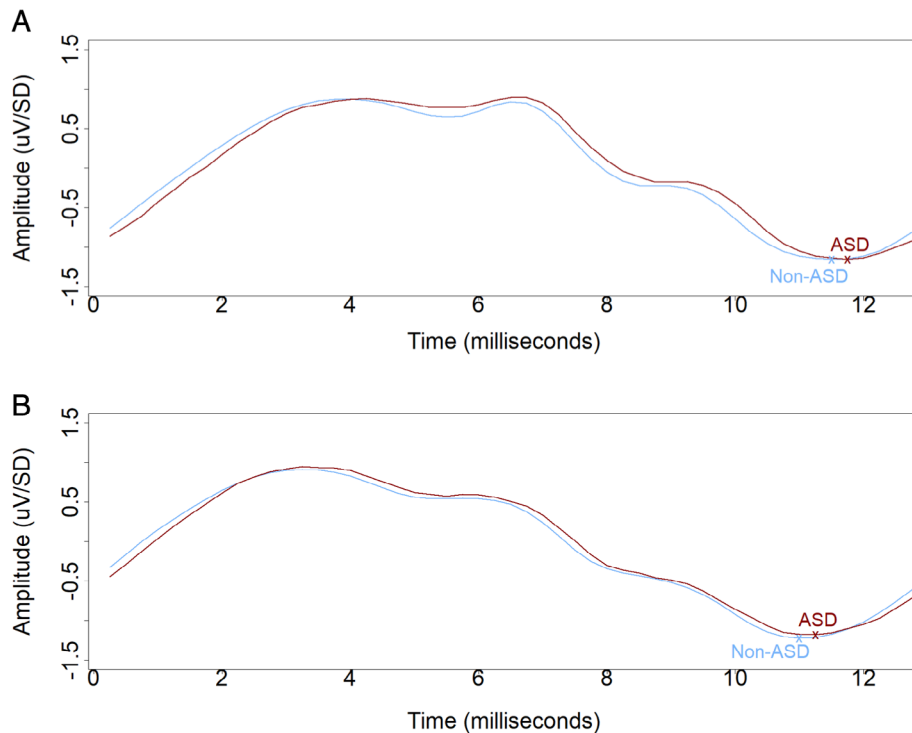
Figure 1. Averaged waveform in ASD vs. Non-ASD. (A and B) Averaged waveform with millivolt divided by the standard deviation of the waveform. "x" indicates the V-negative point. Vn in the ASD group (dark red line) is prolonged compared to the non-ASD group (light blue line).

For the wave V-negative (Vn) analysis, we excluded cases that did not have a waveform from both the right and left ears. Phase and V-negative latency were analyzed separately to compare the ASD and non-ASD groups. We performed these tests for each ear separately. For this comparison, we used an Analysis of Covariance (ANCOVA) that accounted for the covariance of testing age (in days), sex (male, female), NICU status (NICU, non-NICU infant), click amount, and rejected click amount, with statistical significance defined as $ps < 0.05$, two-tailed (Tables S1 and S2).

Results

The mean number of averaged stimulus presentations (sweeps) in a test for the ASD group was 16,614 (SD = 10,397) for the right ear and 17,016 (SD = 10,591) for the left ear, and in the non-ASD group it was 16,470 (SD = 10,503) for the right ear and 16,893 (SD = 10,764) for the left ear. The mean number of rejected sweeps (unreliable result) in a test for the ASD group was 3,528 (SD = 4,380) for the right ear and 3,595 (SD = 4,475) for the left ear, and in the non-ASD group it was 3,010 (SD = 3,959) for the right ear and 3,087 (SD = 4,058) for the left ear.

ABR phase in the right ear was statistically significantly prolonged in the ASD group (M = 152.53°; SD = 35.62) compared to the non-ASD group (M = 143.54; SD = 33.86; ANCOVA $F(1, 139158) = 12.94$, $P < 0.001$; Fig. 2A). ABR phase in the left ear was also statistically significantly prolonged in the ASD group (M = 132.30; SD = 35.3) compared to the non-ASD group (M = 125.5; SD = 35.5; ANCOVA $F(1, 139158) = 5.33$, $P = 0.021$; Fig. 2B).

Vn latency in the right ear was statistically significantly prolonged in the ASD group (M = 10.77 milliseconds; SD = 1.44) compared to the non-ASD group (M = 10.51; SD = 1.54; ANCOVA $F(1, 129639) = 3.92$, $P = 0.048$; Fig. 3A). We did not detect a prolongation in Vn latency in the left ear in the ASD group (M = 10.18, SD = 1.53) compared to the non-ASD group (M = 10.06, SD = 1.56; ANCOVA $F(1, 129639) = 0.29$, $P = 0.591$; Fig. 3B).

Discussion

We identified phase increases and latency prolongation in the ABRs of newborns later diagnosed with ASD, which were especially pronounced in the right ear. These findings are similar to the reported prolonged ABR latencies in older infants and children with ASD [7- to 13-year-olds: Fujikawa-Brooks, Isenberg, Osann, Spence, & Gage, 2010; 0.2- to 6.5-month-olds: Miron et al., 2016;
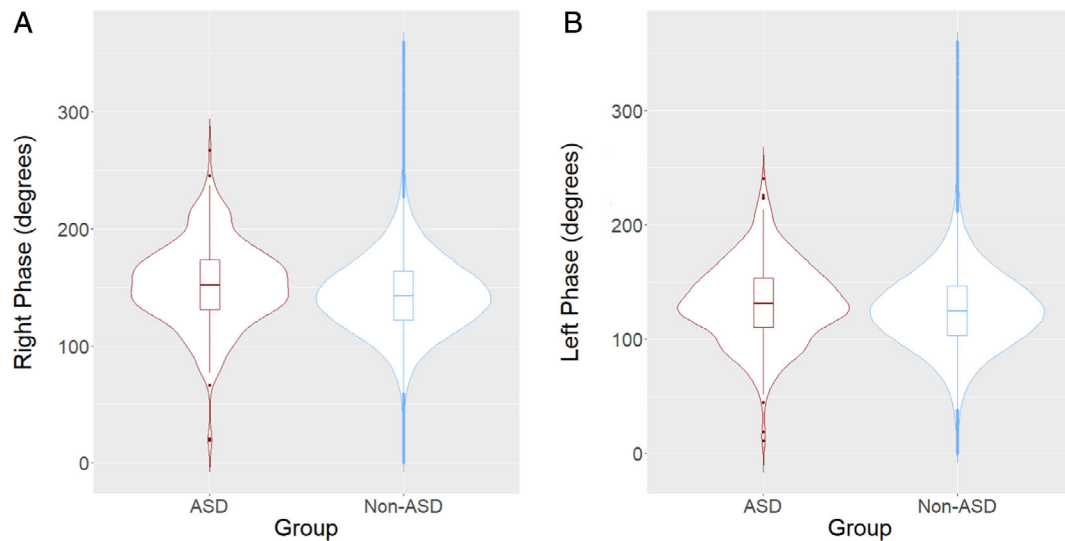
Figure 2. ABR Phase in ASD vs. non-ASD. (A) *Y*-axis indicates the right ear phase (in degrees) in the ASD group (dark red) vs. the non-ASD group (light blue). The distribution of the values is indicated by the violin shape, the median is indicated by the middle line and the 25% and 75% quartile are indicated by the bottom and top of the box. (B) Same as part A for the left ear.

Figure 3. Vn latency in ASD vs. non-ASD. (A) *Y*-axis indicates the right ear Vn latency (milliseconds) in the ASD group (dark red) vs. the non-ASD group (light blue). The distribution of the values is indicated by the violin shape, the median is indicated by the middle line and the 25% and 75% quartile are indicated by the bottom and top of the box. (B) Same as part A for the left ear.
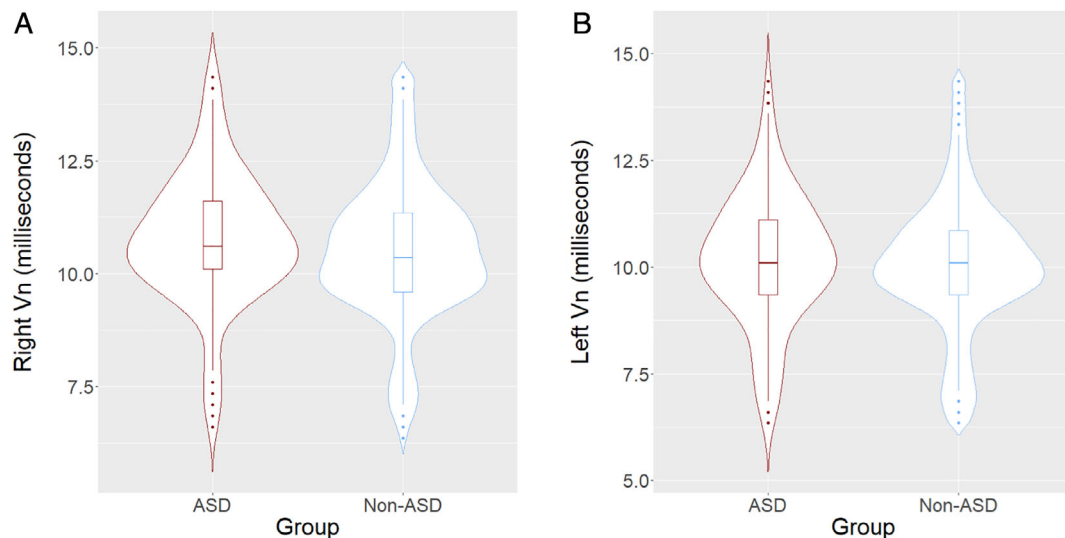
0.2 months to 21 years old: Miron et al., 2018; 2- to 3-year-olds: Roth et al., 2011; 4- to 12-year-olds: Wong & Wong, 1991]. We found that the prolongation of phase and V-negative latency in ASD was greater in the right ear compared to the left ear, similar to the reports in older infants who are later diagnosed with ASD [Miron et al., 2016].

The current study's findings of prolonged brainstem response to sound in ASD are consistent with previous findings of prolonged and abnormal cortical response to sound in 7- to 14-year-old children with ASD [Roberts

et al., 2010] and 2- to 3-year-old boys with ASD and megalencephaly [De Meo-Monteil et al., 2019]. The brainstem prolongation could be due to anatomical abnormalities in the brainstems of those with ASD, such as those observed in the olivary complex at 2 to 36 years of age [Kulesza, Lukose, & Stevens, 2011].

The current study's strengths and limitations both derive from our reliance on existing data from UNHS. One strength of our approach is that the UNHS data allowed us to examine a larger, younger, and healthier sample compared to previous studies. However, the

UNHS ABR data were from tests using 35 dB nHL, which is a lower sound intensity than those used in most ASD studies, which more often use 85 dB nHL [Miron et al., 2016]. Our use of these lower intensity stimuli resulted in a weaker signal, and a lower signal-to-noise ratio compared to higher intensity stimuli, preventing us from precise labeling wave V-positive. Thus, we focused on wave V-negative, which is easier to detect, and ABR phase, whose timing is affected by V-positive and other waves and is more robust in noisy conditions. V-negative and phase have not been studied as widely as wave-V and the current study is the first study to show V-negative and phase abnormalities are associated with ASD. There is, therefore, a need to replicate our findings in future studies of V-negative and phase to determine whether they are clinically useful. In addition, the smaller signal-to-noise ratio likely led to a larger standard deviation in our latencies compared to previous ASD studies. As a result, even though the mean latency difference between ASD and non-ASD in our study was comparable to previous studies, the signal in our study was insufficient to support a robust clinically useful classification model. Even if future studies achieve sufficient accuracy for detecting ASD risk, it will still be important for doctors to rule out other diagnoses that affect ABR, such as hearing impairment, and to refer infants for a behavioral ASD diagnosis.

Future studies should test newborns who have a family history of ASD and are, therefore, at elevated genetic risk of ASD [Ciarrusta et al., 2020; Grove et al., 2019]—as well as toddlers who have already been diagnosed with ASD. Such studies should use higher stimulus intensities and high-rate stimulation techniques to determine the ABR settings that can most accurately identify children who later develop ASD [Delgado & Ozdamar, 2004]. Studies could also test for relations among prolonged ABR in ASD with other biomarkers of ASD, such as genetic markers [Bruneau, Bonnet-Brilhault, Gomot, Adrien, & Barthelemy, 2003; Luo, Zhang, Jiang, & Brouwer, 2018]. Combining ABR and genetic biomarkers could improve the classification of ASD and our understanding of ASD's early development. The existence of ABR biomarkers of ASD in the first weeks after birth, as reported here, is an evidence that, for a large group of these individuals, the disorder is likely present before birth [Kong et al., 2012].

Further studies could also benefit from examining the relationship among ABR markers, measures of ASD severity (e.g., Autism Diagnosis Observation Schedule) and information on specific symptoms, such as hyperreactivity to sound. Such analysis could also assist in identifying whether specific ASD severity levels or symptoms are better classified by ABR markers, given the heterogeneity of ASD, which consists of numerous sub-groups. Such studies might therefore create ABR markers that accurately predict risk for one or more sub-groups of ASD. Early detection of ASD risk could result in an earlier diagnosis that may lead to earlier treatments and therefore better outcomes [Dawson et al., 2010].

Author Contributions

Experimental/methodological design, O.M., R.E.D., C.F.D., E.A.S., A.G., and I.S.K.; data processing, O.M., C.F. D, R.E.D., Z.G., and J.N.G.; data and statistical analysis, O.M., C.F.D., and Z.G.; statistical interpretation, O.M., R.E.D., C.F.D., K.H.Y., Z.G., and I.S.K., manuscript preparation, O.M., R.E.D, C.F.D, E.A.S, A.G., K.H.Y., Z.G., J.N. G, and I.S.K.

Conflict of Interest

The authors have no conflict of interest to declare.

Data Availability Statement

The data and materials are detailed in the main text and Tables S1 and S2 in as much detail as we are allowed given its medical and educational nature.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. ANCOVA of Phase in ASD vs. Non-ASD in Right and Left Ears

Table S2. ANCOVA of Vn Latency in ASD vs. Non-ASD in Right and Left Ears