# Longitudinal Effects of Prenatal Alcohol Exposure on Visual Neurodevelopment over Infancy

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**Abstract**

Prenatal alcohol exposure affects neurodevelopment in over 59 million individuals globally. Prior studies using dichotomous categorization of alcohol use and comorbid substance exposures provide limited knowledge of how prenatal alcohol specifically impacts early human neurodevelopment. In this longitudinal cohort study from Cape Town, South Africa, prenatal alcohol exposure is measured continuously – characterizing timing, dose, and drinking patterns (i.e., binge drinking). High-density electroencephalography (EEG) during a Visual-Evoked Potential (VEP) task was collected from infants ages 8 to 52 weeks with prenatal exposure exclusively to alcohol and matched on socio-demographic factors to infants with no substance exposure in-utero. First trimester alcohol exposure related to altered timing of the P1 VEP component over the first six months postnatally, and first trimester binge drinking exposure altered timing of the P1 VEP components such that increased exposure was associated with longer VEP latencies while increasing age was related to shorter VEP latencies (n=108). These results suggest alcohol exposure in the first trimester may alter visual neurodevelopmental timing in early infancy. Exploratory individual-differences analysis across infants with and without prenatal alcohol exposure tested the relation between VEP latencies and myelination for a subsample of infants with usable MRI T1w and T2w scans collected at the same time point as EEG (n=47). Decreased MRI T1w/T2w ratios (an indicator of myelin) in the primary visual cortex (n=47) were linked to longer P1 VEP latencies. Results from these two sets of analyses suggest that prenatal alcohol and postnatal myelination may both separately impact VEP latency over infancy.

**Public Significance Statement**

For infants prenatally exposed exclusively to alcohol, continuous measures of first trimester exposure and binge drinking exposure were associated with altered timing of visual neurodevelopment over the first six months postnatally. Myelination may serve as a partial

mechanism for these differences. The WHO ASSIST is an acceptable tool to detect alcohol risk in pregnant populations. This work can inform efforts to support pregnant people and their infants pre- and postnatally.

*Keywords:* prenatal alcohol exposure; infant neurodevelopment; fetal programming; Visual-Evoked Potential (VEP) Event-Related Potential (ERP); myelination

Alcohol is a known teratogen that affects central nervous system development *in utero* (Coles, 1994; Jones & Smith, 1973; Lemoine et al., 1968). Globally, 9.8% of pregnant people endorse alcohol use during their pregnancy, affecting neurodevelopment in over 59 million individuals (Popova et al., 2018). Rates and patterns of prenatal alcohol exposure vary by country, culture, and across communities. Certain communities report high incidences of prenatal alcohol exposure, including in South Africa (Popova et al., 2023). A recent study of 5,231 women receiving prenatal care and delivery in the Cape Town metropolitan area, South Africa found that up to 36.9% of the sample self-reported alcohol use during pregnancy and/or in the three months before pregnancy recognition (Petersen Williams et al., 2014).

**Contextualizing Prenatal Alcohol Exposure in South African Communities**

It is important to recognize the structural and cultural factors, including historical socio-political frameworks, that might contribute to the high prevalence of prenatal alcohol exposure present in certain South African communities. The colonial apartheid regime in South Africa created a system of significant economic and socio-political inequalities that served to disadvantage communities such as those in the current study sample (Marshall et al., 2022; Adebiyi et al., 2021; May et al., 2019; Jacobson et al., 2017). Additionally, in health systems with access to limited resources, more acutely life-threatening medical conditions may be prioritized over conditions that are more chronic and less immediately life-threatening, leading to less resource allocation to mental health and substance use disorder treatment and support (Williams & Wyatt, 2015). From an intersectional perspective (Crenshaw, 1989), some work suggests that intersecting identities for Black women in South Africa may lead to additional internal, external, and structural barriers in accessing, receiving, and completing treatment for alcohol use disorders (Pretorius et al., 2009). On an individual level, multiple explanations for alcohol use during pregnancy emerged from qualitative interviews conducted with peripartum women in informal settlements in the Cape Town metropole (Fletcher et al., 2018; Watt et al., 2014). Notably, one of the major themes identified was using drinking as a way to cope with

stressors, abuse, or other trauma, both those acutely related to the pregnancy and new child or other unrelated stressors. Women in both studies also cited drinking as a way to socialize, drinking during pregnancy being an acceptable social norm in their communities, and lack of attachment to the pregnancy or not wanting the pregnancy as reasons for their drinking.

**Prenatal Alcohol Exposure and Differences in Visual Processing During Childhood**

Prenatal alcohol exposure (PAE) may result in a spectrum of physical, sensory, and/or learning disabilities for exposed individuals. The spectrum of disabilities is commonly referred to as Fetal Alcohol Spectrum Disorders (FASD). Behavioral literature in school-aged children has established sensory differences as consistent outcomes of prenatal exposure, notably with respect to visual processing (National Institute on Alcohol Abuse and Alcoholism, 2000). Preschool and early school-age children with prenatal alcohol exposure have observed difficulties in visual-motor integration skills (Janzen, 1995; Mattson et al., 1998). Additional studies observed difficulties in visual-spatial processing and functioning in 9- to 16-year old children with prenatal alcohol exposure (Hunt et al., 1995; Mattson et al., 1996; Uecker & Nadel, 1996). One behavioral study conducted in infancy found alterations in visual fixation behavior for infants with PAE, which might be indicative of these visual differences emerging early in development (Jacobson et al., 1993). However, limited studies in human development examine brain phenotypes that may link the prenatal exposure to these later outcomes (Coles & Li, 2011; Donald et al., 2015; Wozniak & Muetzel, 2011).

Not only is early visual processing more obviously indicative of later visual-related abilities (Harter et al., 1977; Hartmann, 1995; MCculloch, 2007; Taylor et al., 1992), additional evidence suggests that early sensory processing, most notably in the visual domain, scaffolds later executive functioning via visual attention pathways (Amso & Scerif, 2015; Rosen et al., 2019; Werchan & Amso, 2017). Therefore, it is imperative to understand the emerging visual system in infants with prenatal alcohol exposure to better support later visual *and* cognitive development.

**Impact of Prenatal Alcohol Exposure on Visual Neuroplasticity Mechanisms**

One potential mechanism through which prenatal alcohol exposure may impact postnatal sensory development is via neuroplasticity regulation for sensory learning over the first year of life. Increased neuroplasticity results in rapid sensory learning during this window, which can be assessed in the human both functionally and structurally using non-invasive, *in-vivo* techniques such as electroencephalography (EEG; Barry-Anwar et al., 2020) and magnetic resonance imaging (MRI; Deoni et al., 2011; Goddings et al., 2021), respectively (Gabard-Durnam & McLaughlin, 2020).

In the human, this visual learning and plasticity can be indexed neurally through a Visual-Evoked Potential (VEP) task using Electroencephalography (EEG; Barry-Anwar et al., 2020). This type of visual Event-Related Potential (ERP) provides a time-locked response to a visual stimulus which can be used as a marker of the underlying learning and development (Lippé et al., 2007; Lippé et al., 2009). For example, as learning accumulates and the brain develops, sensory processing speeds up and neural responses come to resemble mature spatio-temporal patterns (Barry-Anwar et al., 2020). Specifically, the VEP comprises three main components: a negative N1 peak, a positive P1 peak, and a negative N2 peak. As infants gain more visual experience and their brain develops, these VEP components occur more quickly in response to visual stimuli; that is, the latency to each component's peak amplitude becomes shorter (Lippé et al., 2009). These changes occur rapidly over infancy, with the adult pattern of response emerging around 24 months of age (Lippé et al., 2009). The VEP response (when measured via magnetoencephalography) as used in this study has been localized to activity within the visual cortex from primarily two sources, the primary visual cortex (V1; components N1, P1, N2) and the middle temporal visual area (i.e, MT or V5; components P1, N2; Barnikol et al., 2006).

Prior studies in human infancy suggest the VEP in early life is sensitive to prenatal alcohol exposure. A study with a sample of human infants with heterogeneous prenatal

substance exposures found that infants with more first trimester alcohol exposure (quantified as drinks per week within the first trimester) demonstrated prolonged P1 Visual-Evoked Potential latencies at 1 month of age when compared to those without first trimester alcohol exposure (Scher et al., 1998). Analyses examining second or third trimester use in that same study did not find the same effects on the P1 latency as the first trimester exposure (though other substances also impacted the P1 latency), indicating the need to examine prenatal alcohol use in terms of both timing and dose with respect to VEP development. Although the heterogeneous substance use precludes conclusions about the specificity of this effect to alcohol, and effects of subsequent trimesters may have been confounded by prior trimester(s) exposure (unaccounted for in models), the longer P1 latencies observed are consistent with delayed early visual development following alcohol exposure. Another study in neonates with PAE has also demonstrated differential electrophysiological responses following a visual stimulus when compared to neonates without exposure, though exposure was quantified categorically rather than by dose (Olegård et al., 1979). Indeed, the majority of prior literature on PAE has measured exposure categorically (comparing those with high exposure levels to those with no prenatal exposure (Coles & Li, 2011) so dose-related effects as a function of trimester timing remain poorly understood despite early evidence this approach matters for interrogating visual neurodevelopment.

Visual plasticity is also regulated by structural changes in myelin over early development that can be detected with MRI. Specifically, myelination happens most rapidly in infancy, with occipital lobes largely myelinated by 5-6 months (Dean et al., 2014). Among its many other roles, myelination serves as an active player in the regulation of sensory neuroplasticity by acting as both a “brake” on this plasticity and a “shield” protecting prior functional learning from future insults (e.g. by preserving the structural connections serving learned circuit function; Nelson & Gabard-Durnam, 2020). Myelination also increases nerve conduction velocity, and is therefore associated with changes in VEP latency in clinical populations and in animal models at

other life stages, such that increased myelination is associated with shorter latencies (Heidari et al., 2019; Klistorner & Graham, 2021; Walt et al., 2015). It has long been hypothesized that increased myelination in the visual cortex over infancy is also partly responsible for the latency shifts in VEP over infancy, thus indicating preservation of the visual learning and efficient visual cortex responsivity.

Animal model literature and human literature beginning in childhood have characterized the effects of alcohol exposure on myelination (e.g. Darbinian & Selzer, 2021). Human histological studies also provide evidence for the impact of PAE on dysmyelination (Darbinian & Selzer, 2021). Human neuroimaging studies using MRI found white matter microstructural alterations, including in the occipital lobe, in individuals as young as 5 years of age with prenatal alcohol exposure and/or FASD (Donald et al., 2015; Ghazi Sherbaf et al., 2019; McLachlan et al., 2019; Wozniak & Muetzel, 2011). However, it is important to characterize these microstructural differences in infancy, during critical windows of myelination and functional learning to understand how and when these differences emerge.

In summary, sensitive periods are tightly regulated by biological mechanisms including by "brakes" which punctuate these sensitive periods and preserve/protect the learning that has occurred (Gabard-Durnam & McLaughlin, 2020; Hensch & Bilimoria, 2012; Reh et al., 2020; Takesian & Hensch, 2013). Exposure to alcohol prenatally impacts sensitive period regulators such as myelination (Almeida et al., 2020; Darbinian & Selzer, 2021; Donald et al., 2015; Wozniak & Muetzel, 2011), which is a key "brake" on sensitive periods. By disrupting regulators such as myelin, alcohol exposure may shift the timing of postnatal sensitive periods, leading to downstream, potentially disordered, consequences on cognition and behavior (e.g. Hensch, 2005; Ivanaov, 2021; Makinodan et al., 2012; Marín, 2016; Takesian & Hensch, 2013).

**The Importance of Considering Timing and Dose Effects of Prenatal Alcohol Exposure**

It is important to consider the substantial heterogeneity in presentation for individuals with PAE. Timing, dose, and drinking pattern differences contribute to this heterogeneity in

postnatal disabilities and underlying neural substrates (O'Leary et al., 2010; Subramoney et al., 2018; Pini et al., 2019). Trimester timing effects are perhaps the best characterized of these factors to date, in which those with PAE during first trimester organogenesis display craniofacial abnormalities such as thin upper lip, smooth philtrum, and short palpebral fissure (Popova et al., 2023), and those with exposure after 8 weeks gestation are less likely to present with this facial phenotype that is typically associated with PAE. However, those with later exposure are not immune to the effects of alcohol and exhibit alterations in brain development. Notably, delayed P1 Visual-Evoked Potential latencies at 1 month of age have been found in those with alcohol exposure in the first trimester (Scher et al., 1998).

Multiple studies of human behavior have characterized dose-related effects of PAE (e.g., Smith et al., 1986; Streissguth et al., 1989; Testa et al., 2003), but few studies examining neurodevelopment in humans have examined prenatal alcohol exposure with dose-specific information (Almeida et al., 2020; Jacobson et al., 2017). One neurodevelopmental study in 10-year old children with heterogeneous prenatal substance exposure found increased PAE dose was linked to greater cerebral volume and had non-linear associations with regional brain volumes including the occipital lobes (Lees et al., 2020). In a more general sense, the majority of human studies use clinical samples and therefore highlight the effects of PAE at high doses, but there is a need for more human work characterizing the effects at more moderate levels of PAE (Dejong et al., 2019).

There is also evidence that exposure matters not only in terms of timing or amount, but also in the way in which these two factors interact to produce different drinking patterns. Binge drinking for women, according to the U.S. Centers for Disease Control and Prevention, is defined as drinking 4 or more drinks in a drinking episode (*Binge Drinking | CDC*, 2022). Animal models observed more severe consequences with regards to brain growth (i.e., more severe microcephaly) for rats with alcohol exposure following a binge drinking pattern as opposed to those exposed to an equal amount of alcohol following a more uniform pattern (Kelly et al.,

1987; West et al., 1987). Other studies have reported increased difficulties in regulation of executive functioning for school-age children with prenatal binge drinking exposures (Bailey et al., 2004; Jacobson et al., 2021; Nulman et al., 2004). However, it remains unknown how binge exposures impact human neurodevelopment underlying these behavioral differences. Thus, in studies investigating the effects of prenatal alcohol exposure, it is imperative to consider timing across trimesters, dose effects, and pattern of drinking exposure (i.e., binge drinking), as these may differentially impact the neural substrates of early development.

**Current Study**

The current study addresses limitations in the current literature regarding how and when prenatal alcohol exposure is biologically embedded to shape postnatal brain development and result in sensory behavioral outcomes using longitudinal data from an ongoing project with 394 families recruited from Gugulethu, an informal settlement, in Cape Town, South Africa. This study may contribute to the field's understanding of how alcohol teratogenesis impacts postnatal neurodevelopment in several ways. First, prenatal alcohol exposure was quantified by trimester (to account for fluctuations across trimesters) using a continuous rather than dichotomous index. Distinguishing alcohol exposures by trimester facilitates the investigation of how the timing, dose, and pattern of prenatal alcohol exposure engender teratogenesis. Second, the homogeneous alcohol exposure measured in the current sample removes confounding effects of substance comorbidity found in prior studies. Furthermore, the multimodal longitudinal design of the current study allows for indexing alcohol's effects on functional neurodevelopmental changes via EEG Visual-Evoked Potential (VEP) components, and exploring potential structural changes (via MRI T1w/T2w ratios to estimate myelination; Glasser & Essen, 2011) that may in part contribute to functional neurodevelopment during the period of rapid development that has been historically under-studied. Specifically, this study set out to test 1) how prenatal alcohol exposure (i.e., timing, dose, and binge behavior) impacts electrophysiological responses to visual stimuli, as indexed by latencies of the EEG Visual-Evoked Potential (VEP) components.

We predicted that earlier exposure as well as increased dose and binge episodes would result in longer VEP latencies, indicating delayed neurodevelopment or less-efficient visual learning. We also investigated 2) how changes in VEP component latencies in this sample relate to underlying differences in myelination during this critical window. We predicted that shorter latencies would be associated with increased myelination of the primary visual cortex (V1) and middle temporal visual area (MT/V5), as indexed by T1w/T2w ratios from MRI.

## Method

### Participants

Participants for the current study come from an ongoing prospective longitudinal project with 394 mothers and their infants recruited from Gugulethu, an informal settlement, in Cape Town, South Africa (for study description, see Zieff et al., *in prep*). The first language of the majority of residents in this area is Xhosa. 329 mothers were recruited prenatally, and 65 mothers were recruited postnatally from local community clinics. One infant was excluded after birth using *a priori* exclusion criteria due to extreme prematurity (born 8 weeks premature). This study was approved by the relevant university Health Research Ethics Committees. Informed consent was collected from mothers on behalf of themself and their infants.

### Study Design

All questionnaires were administered by an interviewer (trained study nurse or research assistant). Questionnaires and procedures were offered/explained in English or Xhosa depending on language preference of the mother. At the enrollment study visit, mothers completed a series of questionnaires including the Alcohol Exposure Questionnaire (AEQ), World Health Organization Alcohol, Smoking and Substance Involvement Screening Test (WHO ASSIST), and Edinburgh Postnatal Depression Scale (EPDS; Cox et al., 1987; Kwiatkowski et al., 2018; WHO ASSIST Working Group, 2002). Demographic information including maternal place of birth, primary spoken language, maternal age at enrollment, maternal educational

attainment, and maternal income were also collected at the enrollment visit.

Families were invited to participate in two in-lab study visits over their infant's first year of life, one visit in the first 6 months and another visit from 6 to 12 months of age. At the first in-lab study visit (hereafter Visit 1), occurring when infants were between approximately 2 months and 6 months of age (age in weeks: *M*=15.798, *SD*=3.719, range=8.286-24.571), the following data were collected: the infants' age (in weeks), sex, infant electroencephalography (EEG), and magnetic resonance imaging (MRI). For a infants who were unable to complete both EEG and MRI on the same day, EEG and MRI were collected on different days (for those with multimodal data included in the current study, median age difference=1.571 weeks).

At the second study visit (hereafter Visit 2), occurring when infants were between approximately 6 months and 12 months of age (age in weeks: *M*=36.489, SD=6.337, range=26.000-51.857), infant EEG and MRI data were collected again. Not all infants had EEG and MRI data collected at both timepoints or contributed usable data at both timepoints.

Prior work has explored validity of the standardized clinical cut offs within the context of diverse African and South African communities (Donald et al., 2019; Lawrie et al., 1998; Pelowski et al., 2023; Tsai et al., 2013) and have validated the scale in the Xhosa language (Lawrie et al., 1998), the primary language of the participants in this study. Using the recommended clinical cut-off of 13 (Cox et al., 1987), which has been used in prior studies in South Africa (Donald et al., 2019; Pelowski et al., 2023), 18.52% of the participants included in the main analysis at either time point had EPDS scores of 13 or greater (24.62% of participants in the group that endorsed alcohol use at any point in pregnancy met this cutoff and 14.43% of participants in the group that did not endorse alcohol use met this cutoff). The study validating the scale in Xhosa has a recommended cutoff of 12 or greater (Lawrie et al., 1998), and using this criterion, 20.37% of the participants included in the main analysis at either time point had EPDS scores of 12 or greater (29.23% of participants in the group that endorsed alcohol use at any point in pregnancy met this cutoff and 14.43% of participants in the group that did not

endorse alcohol use met this cutoff).

All enrolled infants received a comprehensive medical exam at each visit, which included assessments of eye-related conditions. Several infants (n=3) were identified as having eye-related anomalies during the medical exam, and they were excluded from any further analyses (note that none of these infants had reported prenatal alcohol exposure).

**Substance Exposure Measurement**

Alcohol use during pregnancy was measured by maternal self-report using the Alcohol Exposure Questionnaire (AEQ) collected at enrollment (Kwiatkowski et al., 2018). Due to the personal nature of these questions and learning from prior experience working with this population, these questions were asked as multiple-choice options of ordinal ranges. During this questionnaire, mothers were asked to estimate, on average, how many times they drank per week in each trimester (i.e., “Once per week or less,” “2 to 3 times per week,” “4 to 6 times per week,” “Daily”). These responses were coded as the midpoint of the range unless the multiple choice option specified a specific number (i.e., 0.5 weekly drinking episodes, 2.5, 5, or 7, respectively). They were then asked an additional question about how many drinks were consumed per drinking episode in that trimester (i.e., “Fewer than 2”, “2 to 3”, “4 or more,” or “Not sure”). Responses were coded as the midpoint of the range for the first two options (i.e., 1, 2.5, respectively). Those selecting “4 or more” were asked to write in a specific number which was used as the number of drinks per episode and those selecting “Not sure” wrote in drink types such as “4 ciders” or “6x330mL” which were later coded for alcohol units with a standardized system developed based on the Drakenstein study and the alcohol unit conversion scale from the South African Association for Alcohol Responsibility and Education (AWARE) and in consultation with an expert in prenatal alcohol exposure in South Africa (Stein et al., 2015; The Association for Alcohol Responsibility and Education, n.d.).

As our continuous measure for level of prenatal alcohol exposure, we extracted the number of standardized alcohol units consumed per week in each trimester from the AEQ

(number of drinking episodes per week in each trimester x number of drinks consumed per episode). We also developed a continuous measure of exposure to binge drinking in each trimester (with a binge drinking episode defined as drinking 4 or more drinks in a particular episode) by extracting the number of drinking episodes per week for those who endorsed 4 or more drinks per episode in that trimester (*Binge Drinking | CDC*, 2022). See Figures 1 and 2 for frequency distributions of the number of drinks per week by trimester and of binge episodes per week by trimester, respectively and Table 1 for descriptive statistics of alcohol endorsement.

103 (26.2%) mothers endorsed alcohol use at any point in pregnancy out of the 393 mothers for whom that information was collected. Notably, for participants contributing to Visit 1 and/or Visit 2 data, there were no statistically significant differences between reported alcohol use patterns in mothers who endorsed alcohol use (without comorbid non-alcohol substance use) who were recruited prenatally compared to those recruited postnatally for drinks consumed per week in any trimester (Visit 1 sample: tri 1: $p>.05$, n=52; tri 2 and 3 $p>.05$, n=53; Visit 2 sample: tri 1, 2, and 3 $p>.05$, n=50) or for binge episodes in trimester 1 (Visit 1 sample: $p>.05$, n=52; Visit 2 sample: $p>.05$, n=50), indicating consistent reporting across recruitment windows in those who endorsed alcohol use. There were also no statistically significant differences between reported alcohol use patterns as a function of pre- compared to postnatal recruitment in the overall sample of mothers whose infants participated in EEG visits (both those who endorsed and those who did not endorse prenatal alcohol use; without comorbid non-alcohol substance use) for any trimester (Visit 1 sample: tri 1: $p>.05$, n=227; tri 2, and 3 $p>0.05$, n=228; Visit 2 sample: tri 1, 2, and 3 $p>0.05$, n=226) or for binge episodes in trimester 1 (Visit 1 sample: $p>0.05$, n=227; Visit 2 sample: $p>0.05$, n=226), suggesting consistent endorsement patterns in the overall sample regardless of recruitment timing.

In addition, alcohol risk scores were calculated using the World Health Organization Alcohol, Smoking and Substance Involvement Screening Test (WHO ASSIST; WHO ASSIST Working Group, 2002). This is a screening tool developed by a group of international

researchers for use in identifying those most at risk of substance use problems in primary care settings (WHO ASSIST Working Group, 2002). To the best of our knowledge, there have not been any studies empirically evaluating its use in measuring alcohol risk in pregnant populations. In an effort to better predict which mothers would be at increased risk of alcohol use during pregnancy, we looked at whether the alcohol risk score calculated by the World Health Organization Alcohol, Smoking and Substance Involvement Screening Test (WHO ASSIST; WHO ASSIST Working Group, 2002) was related to reported drinking behavior and patterns across trimesters. We looked at correlations between the continuous alcohol risk score (measured from 0 to 39) and the number of drinks per week in each trimester and also the number of binge episodes per week in each trimester for the subsample of infants whose mothers completed both alcohol measures (n=392; Figure 3).

The continuous alcohol risk score calculated using the WHO ASSIST significantly correlated with whether alcohol was dichotomously endorsed at any point during pregnancy ($r(390)=.44$, $p<.001$), as well as the continuous measure of number of drinks per week endorsed in each trimester (tri 1: $r(390)=.31$, $p<.001$; tri 2: $r(390)=.45$, $p<.001$; tri 3: $r(390)=.22$, $p<.001$) and the continuous measure of number of binge episodes per week in each trimester (tri 1: $r(390)=.21$, $p<.001$; tri 2: $r(390)=.41$, $p<.001$; tri 3: $r(390)=.21$, $p<.001$). These analyses indicate consistent reporting across measures of alcohol use behavior in the sample. Although only 16 mothers scored within the moderate- or high-risk ASSIST categories, 100% of these mothers endorsed drinking during pregnancy, indicating the ASSIST scores may be useful for identifying those who will drink during pregnancy to facilitate targeting counseling and other support services concerning the effects of prenatal alcohol exposure.

Non-alcohol comorbid substance use endorsement in the three months prior to enrollment was collected using the World Health Organization Alcohol, Smoking and Substance Involvement Screening Test (WHO ASSIST Working Group, 2002). Mothers reported on how often they endorsed tobacco, cannabis, cocaine, amphetamine, inhalant, sedative/sleeping pill,

hallucinogen, opioid, or other drug use in the past three months prior to enrollment. Those who endorsed use of these nine substance categories in any amount or at any point in the past three months were coded as having comorbid substance use. Those who did not endorse use of any of these substances in the past three months were considered to have no comorbid substance use during pregnancy. An additional 21 of the 103 infants with prenatal alcohol exposure also had prenatal comorbid non-alcohol substance exposure and were excluded from further analyses for the purposes of this project.

### *Matching Comparison Participants for Primary Analyses*

For each timepoint (i.e., Visit 1 or Visit 2), a matched subsample of infants without prenatal alcohol exposure or other substance exposure with usable EEG data were selected for comparison to the infants with prenatal alcohol exposure using the *MatchIt* package in R (Ho et al., 2011). These samples were matched with *MatchIt* based on infant age at EEG data collection. At each timepoint, matched samples without prenatal alcohol exposure were not significantly different from those with exposure on the following potential confounding variables: infant age at EEG visit, maternal reports of depression as indexed by the Edinburgh Postnatal Depression Scale, maternal age at infant birth, maternal education, and maternal income (Student's t-tests, all $p>.05$). Additionally, chi-square tests indicated there was no significant difference in infant sex for those with alcohol exposure or without for each matched sample (both $p>.05$). See Figure 4 for a representation of how the final study sample across visits was determined. Demographic characteristics of the sample who contributed data at either time point are summarized in Table 2.

### EEG Data Acquisition

Electroencephalography (EEG) data were acquired at both Visit 1 and Visit 2 from infants while they were seated in their caregiver's lap in a dimly-lit, quiet room using a 128-channel high density HydroCel Geodesic Sensor Net (EGI, Eugene, OR), amplified with a NetAmps 400 high-input amplifier, and recorded via an Electrical Geodesics, Inc. (EGI, Eugene,

OR) system with a 1000 Hz sampling rate. EEG data were online referenced to the vertex (channel Cz) through the EGI Netstation software. Impedances were kept below 100KΩ in accordance with the impedance capabilities of the high-impedance amplifiers. Geodesic Sensor Nets with modified tall pedestals designed for improving inclusion of infants with thick/curly/tall hair were used as needed across participants. Shea moisture leave-in castor oil conditioner was applied to hair across the scalp prior to net placement to improve both impedances and participant comfort. This leave-in conditioner contains insulating ingredients so there is no risk of electrical bridging and has not been found to disrupt the EEG signal during testing (unpublished data). Conditioning hair in this way allows for nets to lay closer to the scalp for Afro-textured hair types and makes for far more comfortable net removal at the end of testing.

The Visual-Evoked Potential (VEP) task was presented using Eprime 3.0 software (Psychology Software Tools, Pittsburgh, PA) on a Lenovo desktop computer with an external monitor 19.5 inches on the diagonal facing the infant (with monitor approximately 65 cm away from the infant). A standard phase-reversal VEP was induced with a black and white checkerboard (1cm x 1 cm squares within the board) stimulus that alternated presentation (black squares became white, white squares became black) every 500 milliseconds for a total of 100 trials. Participant looking was monitored by video and by an assistant throughout data collection. If the participant looked away during the VEP task, the task was rerun.

**EEG Data Pre-Processing**

VEP data were exported from native Netstation .mff format to .raw format and then pre-processed using the HAPPE+ER pipeline within the HAPPE v3.3 software, an automated open-source EEG processing software validated for infant data (Monachino et al., 2022). A subset of the 128 channels were selected for pre-processing that excluded the rim electrodes as these are typically artifact-laden (channels excluded from pre-processing included in Table 3). The HAPPE+ER pipeline was run with user-selected specifications that are outlined in Table 3.

Pre-processed VEP data were considered usable and moved forward to VEP extraction

if HAPPE pre-processing ran successfully, at least 15 trials were retained following bad trial rejection, and at least one good channel was kept within the visual ROI. Note that channels marked bad during pre-processing had their data interpolated as part of standard pre-processing pipelines for ERPs (Monachino et al. 2022). Interpolated channels were included in analyses here as is typically done in developmental samples and given the low overall rates of interpolation present (e.g., all groups at all visits had an average of between 4 to 5 of 5 possible good channels in the region of interest retained). At this point, 6 infants with PAE were excluded from further analysis from the Visit 1 time point, and 1 infant with PAE was excluded from the Visit 2 time point. Student's t-tests confirmed that there were no significant differences in any data quality measure between the alcohol-exposed and non-exposed groups at either time point (all $p>0.05$) with the exception of number and percent of retained channels in the ROI for the alcohol-exposed vs. non-exposed groups at Visit 2 (both $t(100)=2.37$, $p=.020$). Regression models for Visit 2 were run including the number of useable channels within the ROI as a covariate. However, since this variable was not a significant predictor of any VEP outcome measure (all predictors $p>.05$) nor did it change any of the other model and/or predictors' significance or any pattern of results in these analyses, it was removed from the final regression models reported in the results section to preserve model parsimony and statistical power. All EEG data quality metrics for included files at each age for each group (prenatal alcohol exposure or no exposure) are summarized in Table 4.

***Visual-Evoked Potentials (VEPs)***

VEP waveforms were extracted and quantified using the HAPPE v3.3 Generate ERPs script (Monachino et al., 2022). Electrodes in the occipital region were selected as a region of interest (i.e., E70, E71, E75, E76, E83). The VEP waveform has three main components to be quantified: a negative N1 peak, a positive P1 peak, and a negative N2 peak. The feature of interest for these components was peak latency. The window for calculating peak latencies for the N1 component was 40-100 ms, 75-175 ms for the P1 component, and 100-325 ms for the

N2 component. HAPPE parameters used in extracting the ERPs are summarized in Table 5.

All VEPs were visually inspected to ensure that the automatically extracted values were correct and were adjusted if observable peaks occurred outside the automated window bounds. Participants were considered to have failed this visual inspection and were subsequently removed from the data set if their VEP did not produce three discernible peaks.

Of the alcohol-exposed group, no infants from Visit 1 failed visual inspection, and 7 infants from Visit 2 failed visual inspection. This left a final sample of 54 infants with PAE and no comorbid substance exposure with usable EEG data at Visit 1, and 51 infants with these exposure characteristics with usable EEG data at Visit 2 (40 of these infants also provided usable EEG data at Visit 1). The determination process for usable EEG is detailed in Figure 4. Data quality for included files at each age for each group (prenatal alcohol exposure or no exposure) are summarized in Table 4. See Figure 5 for visualizations of the average Visual-Evoked Potential ERP waveforms at both visit time points.

***Sample Included in VEP Analyses***

After exclusions during processing, the final sample size for Visit 1 was 108 infants (54 with prenatal alcohol exposure, 54 matched comparisons with no prenatal substance exposure). There were no statistically significant differences between infants with PAE who contributed usable VEP and those who did not (either because VEP was not collected or because it was deemed unusable) with respect to maternal EPDS scores, education level, income, maternal age at birth (Student's t-tests, all $p>.05$), or infant sex (Pearson's Chi-Squared test, $p>.05$). Through the matching process described above, the comparison infants without PAE included in analyses from the larger pool of data were not statistically different from the infants with PAE on any of these variables, either.

The final sample size for Visit 2 was 102 infants (51 with prenatal alcohol exposure, 51 matched comparisons with no prenatal substance exposure). Again, there were no statistically significant differences between infants with PAE who contributed usable VEP and those who did

not (either because VEP was not collected or because it was deemed unusable) with respect to maternal EPDS scores, income, age at birth, or infant sex (all p>.05). Infants with PAE included in VEP analyses had significantly higher maternal education levels than those that did not contribute ($t$(80)=-2.45, p=.017)) though both groups had mean education levels corresponding to the same rank of schooling completion (i.e., Standard 6 (Grade 8) to Standard 9 (Grade 11)). Through matching, the comparison infants without PAE were not statistically different from the infants with PAE on any of these variables.

***Participants Included in Exploratory MRI Analyses***

To better explore the potential of myelination as a mechanism explaining VEP latencies with limited MRI data, participants were not split into Visit 1 or Visit 2 groups for these analyses. Instead, our sample was composed of infants who had usable data for both EEG and MRI at either time point. 34 infants had usable EEG and MRI data from the Visit 1 time point, and 14 infants had usable EEG and MRI data from the Visit 2 time point. Only one infant had usable EEG and MRI data at both of their visits, so we selected one of their visits (Visit 2) for inclusion in this analysis (note that results remained the same regardless of which visit was included for this individual, if both visits were included, or if neither was included; final $n$=47 measurements modeled). In the sample for this exploratory analysis, 19.15% of infants ($n$=9) had prenatal alcohol exposure, while the remainder (80.85%, $n$=38) had no prenatal alcohol exposure. Infants with usable neuroimaging data were included in the sample regardless of prenatal substance exposure (i.e., alcohol or no alcohol exposure) as the sample was not powered to explicitly explore the impact of PAE on this relation.

**MRI Data Acquisition**

Magnetic Resonance Imaging (MRI) data including T1- and T2-weighted scans were collected during natural sleep on a 3T Siemens MAGNETOM Skyra scanner (Siemens Medical Solutions USA, Inc., Malvern, Pennsylvania) with a 16-channel neonatal head coil. Infants were scheduled for MRI scans during their normal nap time whenever possible. Before scanning,

infants were fed and then swaddled according to the feed and wrap technique and then soothed to sleep by their caregiver (Antonov et al., 2017). Ear cushions and headphones were worn by infants to adequately protect their hearing. If the infant woke up or was distressed at any point, scanning would immediately stop and start again once the infant was settled and if the caregiver wished to continue. Each participant had structural imaging, acquired with the following parameters: $T_1$ MPRAGE [echo time (TE) = 1.69 ms, repetition time (TR) = 2,400 ms, 1 mm isotropic voxel, 256 × 256 mm field of view, inversion time (TI) = 1,450 ms, flip angle = 7°, 4 min 10 sec scanning time], $T_2$ (TE = 561 ms, TR = 3,200 ms, TI = 1800 ms, 1 mm isotropic voxel, 250 × 250 mm field, flip angle = 120° of view, 6 min 35 sec scanning time).

### *T1w/T2w Ratios*

T1w/T2w ratio mapping was performed to generate images with contrast related to myelin content (Ganzetti et al., 2014; Glasser & Essen, 2011). This workflow was conducted using the MRtool tool box (Ganzetti et al., 2014, 2015, 2018) in SPM8 (Wellcome Trust Centre for Neuroimaging, London, UK) with Matlab 2023a (Mathworks).

Initially T2w images were co-registered to T1w, followed by separate intensity normalization and segmented into tissues classes with “New Segment” in SPM (inc. gray matter white matter & CSF). Intensity normalization corrects for bias introduced due to slow wave variation and individual cranial structure. Further intensity scaling for both T1w & T2w are calculated by using masked regions outside of the brain to ensure intensity scaling is comparable between subjects (Ganzetti et al., 2014). Following this calibration, images were transformed into standard Montreal Neurological Institute (MNI) space for further statistical analysis. Each participant’s image was visually inspected for quality assurance. Region of Interest (ROI) masks were generated from the Glasser (2016) atlas and registered to standard space. T1w/T2w ratio estimates were calculated for ROIs, specifically *a priori* regions for statistical analysis (i.e., V1, MT/V5).

## Statistical Analyses

All analyses were performed using R Statistical Software (v4.2.1; R Core Team, 2022) in the RStudio interface (v2022.7.0.548; RStudio Team, 2015), and multiple regression analyses were performed using the *stats* package in R (R Core Team, 2022). For each analysis, overly influential data points in each regression model were tested for using the *MASS* package in R (Venables & Ripley; 2002) by evaluating studentized residuals with *a priori* cutoff bounds of -3 and 3 residual deviations. Any detected outliers were removed from the models, which were then re-run. R scripts for regression analyses are available on the Open Science Framework.

***Visual-Evoked Potential (VEP) Analyses***

Given the presence of several extreme scores in the distributions of PAE variables and the potential for these scores to overly-influence the subsequent regression models, we elected to winsorize drinking scores using the *DescTools* package in R (Signorelli, 2023). Within each visit timepoint, for those endorsing alcohol at any point in their pregnancy, scores above the upper 95% quantile of number of drinks endorsed per week in the first, second, and third trimester as well as number of binge episodes per week in the first trimester were winsorized separately (See Figures S1-S4 for frequency distributions of the winsorized number of drinks per week by trimester and of winsorized binge episodes in the first trimester and Table S1 for descriptive statistics of winsorized alcohol endorsement values). Specifically, these extreme scores were given the same value as the score associated with the 95% quantile for that trimester and retained in analyses. This strategy facilitated preserving model parsimony (modeling multiple trimesters simultaneously) and sample size and statistical power (without needing to remove all participants with extreme values in any of the trimesters modeled).

Maternal age at infant birth, maternal education, maternal income, and EDPS depression scores were considered as potential confounding covariates of drinking scores. In both the Visit 1 (n=108) and Visit 2 samples (n=102), maternal age at infant birth, maternal education, maternal income, and EDPS depression scores were not significantly related to winsorized drinking variables for any trimester used as model predictors (i.e., number of drinks

per week in each trimester and number of binge episodes per week in trimester 1; all $p$>.05).

To correct for the potential influence of earlier component latency differences on later component latencies, corrected latencies were calculated and used in all VEP latency analyses of alcohol exposure. Specifically, the P1 latency was corrected for the N1 latency (P1 - N1 latency), and the N2 latency was corrected for the P1 latency (N2 - P1 latency).

Three ordinary-least squares multiple regression models were run for each visit timepoint, one for each VEP component latency as the outcome (i.e. N1, P1, N2 latencies). Models were run including number of drinks per week for each trimester (3 predictors total, one per trimester). Infant age at data collection and number of retained usable VEP trials were included as a priori covariates in all models. To test whether nonlinear age-related effects should also be included in regression models during each visit age range, model comparisons were performed on models with a) linear age effects, b) linear and natural log(age) effects, and c) linear and quadratic age effects using the *AICcmodavg* and *stats* package in R (Mazerolle, 2023; R Core Team, 2022). Only the Visit 1 P1 latency model indicated a statistically significantly better fit with the inclusion of a nonlinear age term (Visit 1 P1: $F(1,3)=27.83$, $p=0.0000008$; all other tests: $p > 0.05$), so Visit 1 P1 results are reported including both age and natural log(age) as predictors in the model (though note the pattern of results with respect to drinking predictors remains the same with and without the nonlinear age term).

Multivariate outliers in each regression model were evaluated using the *MASS* package in R (Venables & Ripley; 2002). We considered data points to be overly influential by evaluating studentized residuals with *a priori* cutoff bounds of -3 and 3 residual deviations. Multivariate outliers were removed and analyses were rerun if any such outliers were detected for each model. No more than 3 participants were removed from each model of number of drinks per week using this criterion. Note that one participant with Visit 1 data was missing a continuous measure of alcohol exposure in the first trimester and was therefore removed from relevant models for missingness.

To control for the number of model comparisons per question (3 models, one for each VEP component), Bonferroni correction was applied to each set of models (alpha=0.05, corrected *p* threshold per model=.016). Significant predictors were examined only in models that survived correction for multiple comparisons.

To examine the effects of binge-drinking behavior, three additional ordinary-least squares regression models were run for each visit timepoint, one for each VEP component latency as the outcome (i.e. N1, P1, N2 latencies). Models were run including number of binge episodes per week for the first trimester as a predictor. Infant age at data collection and number of retained usable VEP trials were included as *a priori* covariates in all models. Nonlinear age terms were tested for potential inclusion as above, and again, only the Visit 1 P1 regression model indicated significantly better fit with the inclusion of a nonlinear age term (P1: $F(1,3)=27.86$, $p=0.0000007$; all other tests: $p>0.05$), so Visit 1 P1 results are reported including both age and natural log(age) as predictors (though note the pattern of results with respect to drinking predictors remains the same with and without the nonlinear age term). No more than 3 participants were removed from each model of number of binge episodes per week using the criterion for outliers described above. To control for the number of model comparisons per question at each visit (3 models, one for each VEP component), Bonferroni correction was applied to each set of models (alpha=0.05, corrected *p* threshold per model=.016). Significant predictors were examined only in models that survived correction for multiple comparisons.

Over the two sets of analyses of interest, (1) effects of drinks per week over three trimesters and (2) effects of binge episodes per week in the first trimester examined at each of the two visit timepoints, a total of 12 regression models were run.

***Exploratory Analyses of T1w/T2w Ratios in Relation to VEP Latencies***

To explore a possible postnatal influence on VEP latencies, we looked at the effect of myelination in the visual cortex on VEP latencies in a cross-sectional subsample of participants. To create this subsample, we collated all participants who had usable VEP data and T1w/T2w

ratios at the same time point (i.e., both EEG and MRI data at Visit 1 or Visit 2). We selected myelination in the primary visual cortex (V1) and in the middle temporal visual area (i.e., MT or V5) as our brain regions of interest (ROI) based on prior work with adults (Barnikol et al., 2006). For both regions of interest, we averaged estimates of myelination lateralized across the left and right hemispheres to create an average value for each ROI. Here we examined VEP latencies without correction as multiple components are derived from the visual regions tested (e.g. correcting the latencies for later components would remove the shared variance of interest for this analysis). We ran a multiple regression looking at the impact of myelination in both of these regions of interest (as indexed by the T1w/T2w ratio) on each component VEP latency (running six models total) with infant age at EEG collection, number of retained VEP trials, and infant age at MRI collection as covariates. No more than 2 participants were removed from each model of myelination using the criterion for outliers described above. No multiple comparison correction was applied given the exploratory nature of the analyses, though notably the significant effect observed would survive such correction. Due to the smaller sample sizes of this exploratory analysis (only 19.15% of infants had prenatal alcohol exposure), we did not have power to explore the impact of alcohol exposure on this relation directly.

## Results

### *Effects of Number of Drinks per Week on Visual Evoked Potential (VEP) Latencies*

**At Visit 1 (Ages 8.286 - 24.571 weeks).** In the Visit 1 subsample (age in weeks: *M*=15.798, *SD*=3.719, range=8.286-24.571, total *n* before removing model outliers=108), the ordinary-least squares regression model examining the effect of number of drinks per week in each trimester, controlling for infant age at Visit 1 EEG collection, the natural log(age at Visit 1), and number of retained VEP trials, significantly explained variance in the corrected P1 latency (*n*=104, model $R^2$=.56, $F(6, 97)=20.89$, $p<.001$) and survived correction for multiple comparisons across models (corrected threshold $p=.016$). Within the model, number of drinks

per week in the first trimester (controlling for number of drinks in the second and third trimesters) significantly predicted corrected P1 latency ($b$=1.16, $p$=.039, 95% CI [0.06, 2.26]), such that those with increased alcohol exposure had longer corrected P1 latencies (i.e. P1 - N1 latency; see Figure 6 for residual plot). In this model, infant age at Visit 1 EEG collection and the natural log(age) also significantly predicted corrected P1 latency over this age range (age: $b$=7.36, $p$<.001, 95% CI [3.61, 11.11]; natural log(age): $b$=-153.50, $p$<.001, 95% CI [-212.39, -94.61]), such that increasing age was associated with shorter P1 latencies.

Neither the N1 nor the N2 VEP components showed statistically significant effects of drinks per week in any trimester over this age range. The model examining effects on the N1 latency was not statistically significant ($n$=105, model $R^2$=.08, $F$(5, 99)=1.81, $p$=.117). The model examining effects on the corrected N2 latency was statistically significant ($n$=106, model $R^2$=.25, $F$(5, 100)=6.60, $p$<.001) and survived correction for multiple comparisons (corrected threshold $p$=.016). However, number of drinks per week in any trimester did not significantly predict corrected N2 latency (all trimesters $p$>0.05). See Table 6 for a regression table of number of drinks per week in each trimester predicting each VEP component at Visit 1.

**At Visit 2 (Ages 26.000 - 51.857 weeks).** In the Visit 2 subsample (age in weeks: M=36.489, SD=6.337, range=26.000-51.857, total $n$ before removing model outliers=102), there were no statistically significant effects of drinks per week in any trimester on any VEP component latency (N1, P1, N2). Specifically, the model examining the effect of number of drinks per week in each trimester significantly explained variance in the N1 latency, controlling for infant age at Visit 2 EEG collection and number of retained VEP trials, but did not survive correction for multiple comparisons across models ($n$=102, model $R^2$=.11, $F$(5, 96)=2.46, $p$=.038; corrected threshold $p$=.016). Neither P1 nor N2 latency models were statistically significant (P1: $n$=100, model $R^2$=.03, $F$(5, 94)=0.64, $p$=.671; N2: $n$=101, model $R^2$=.02, $F$(5, 95)=0.34, $p$=.889). See Table 7 for a regression table of number of drinks per week in each trimester predicting each VEP component at Visit 2.

### *Effects of Binge Drinking Behaviors on Visual Evoked Potential (VEP) Latencies*

When considering the effect of drinking pattern (i.e., binge drinking behaviors), sufficient variability to model effects existed only for trimester 1 (trimester 1 mothers reporting binge drinking *n*=14) but not subsequent trimesters (trimester 2 mothers reporting binge drinking *n*=6, trimester 3 mothers reporting binge drinking *n*=2).

**At Visit 1 (Ages 8.286 - 24.571 weeks).** In the Visit 1 subsample (total *n* before removing model outliers=107), the ordinary-least squares regression model examining the effect of number of binge episodes per week in the first trimester, controlling for infant age at Visit 1 EEG collection, natural log(age at Visit 1) and number of retained VEP trials, significantly explained variance in the corrected P1 latency (*n*=104, model $R^2$=.56, $F(4, 99)=30.99$, $p<.001$) and survived correction for multiple comparisons across models (corrected threshold $p=.016$). Within the model, number of binge episodes in trimester 1 significantly predicted corrected P1 latency (*b*=9.14, *p*=.028, 95% CI [0.98, 17.31]), such that those with increased binge exposure had longer P1 latencies (see Figure 7 for residual plot). In this model, infant age at Visit 1 EEG collection and the natural log(age) also significantly predicted corrected P1 latency over this age range (age: *b*=7.47, *p*<.001, 95% CI [3.72, 11.21]; natural log(age): *b*=-154.65, *p*<.001, 95% CI [-213.41, -95.89]), such that increasing age was associated with shorter P1 latencies.

Neither the N1 nor the N2 VEP components showed statistically significant effects of binge episodes per week over this age range. The model examining effects on the N1 latency was statistically significant but did not survive correction for multiple comparisons (corrected threshold $p=.016$; *n*=105, model $R^2$=.07, $F(3, 101)=2.71$, $p=.049$). The model examining effects on the corrected N2 latency was statistically significant (*n*=106, model $R^2$=.24, $F(3, 102)=10.92$, $p<.001$) and survived correction for multiple comparisons. However, number of binge episodes per week in the first trimester did not significantly predict corrected N2 latency. See Table 8 for a regression table of number of binge episodes per week in the first trimester predicting each VEP component at Visit 1.

**At Visit 2 (Ages 26.000 - 51.857 weeks).** In the Visit 2 subsample (total n before removing model outliers=102), there were no statistically significant effects of binge episodes per week in the first trimester on any VEP component latency (N1, P1, N2). Specifically, the model examining the effect of number of binge episodes per week in the first trimester on the N1 latency, controlling for infant age at Visit 2 EEG collection and number of retained VEP trials, was statistically significant ($n$=101, model $R^2$=.11, $F$(3, 97)=3.94, $p$=.011) and survived correction for multiple comparisons, but the number of binge episodes in the first trimester did not significantly predict N1 latency (p>0.05). The model examining the effect of number of binge episodes per week in the first trimester did not significantly explain variance in the corrected P1 latency ($n$=100, model $R^2$=.02, $F$(3, 96)=0.56, $p$=.645). The model examining the effect of number of binge episodes per week in the first trimester did not significantly explain variance in the corrected N2 latency either ($n$=101, model $R^2$=.01, $F$(3, 97)=0.47, $p$=.702). See Table 9 for a regression table of number of binge episodes per week in the first trimester predicting each VEP component at Visit 2.

Given previous findings from Scher et al. (1998) as well as the dysmyelinating effect of prenatal alcohol exposure and the link between ERP component latency and myelination, our primary outcome of interest was in VEP latencies, to reduce the number of total comparisons performed in the study. However, given potential interest in the effects of prenatal alcohol exposure on VEP amplitudes, we include exploratory analyses of VEP amplitudes in supplementary files (See Tables S2-S5). Notably, we do not find any statistically significant effects of prenatal alcohol exposure (number of drinks per week in each trimester or number of binge episodes per week in the first trimester) on VEP amplitudes (also corrected for previous component amplitude, i.e., corrected P1 amplitude = P1 - N1 amplitude and corrected N2 amplitude = N2 - P1 amplitude) at either timepoint that survive correction for multiple comparisons in these supplemental analyses.

***Exploratory Results of T1w/T2w Ratios in Relation to VEP latencies***

**Myelination in the Primary Visual Cortex (V1).** Exploratory cross-sectional analyses (*n* before removing outliers=47) of whether MRI-estimates of myelin related to individual differences in VEP latencies regardless of prenatal alcohol exposure status revealed that estimates of myelination in the primary visual cortex (V1) as indexed by the T1w/T2w ratio significantly predicted uncorrected P1 latency (*b*=-12.80, *p*=.040, 95% CI [-24.98, -0.61]) such that those with decreased myelination had longer P1 latencies, when controlling for infant age at MRI collection, infant age at EEG collection, and number of retained VEP trials (*n*=45, model $R^2$=.63, *F*(4, 40)=16.82, *p*<.001; see Figure 8 for residual plot). Note uncorrected latencies were used for these analyses as the underlying visual regions contribute to multiple VEP components' latencies simultaneously. V1 myelination indexed by the T1w/T2w ratio did not significantly predict either N1 latency (*n* after removing outliers=46, model $R^2$=.34, *F*(4, 41)=5.39, *p*=.001) or uncorrected N2 latency (*n*=46, model $R^2$=.27, *F*(4, 41)=3.72, *p*=.011). See Table 10 for a regression table of primary visual cortex (V1) T1w/T2w estimates of myelin predicting each VEP component.

**Myelination in the Middle Temporal Visual Area (MT/V5)**. Estimates of myelination in the middle temporal visual area (i.e., MT or V5) did not significantly predict latency at any VEP component it serves (i.e., P1 or N2; Barnikol et al., 2006) when controlling for infant age at MRI collection, infant age at EEG collection, and number of retained VEP trials (*n* before removing outliers=47; P1 model: *n*=45, model $R^2$=.61, *F*(4, 40)=15.35, *p*<.001; N2 model: *n*=46, model $R^2$=.26, *F*(4, 41)=3.55, *p*=.014). See Table 11 for a regression table of middle temporal visual area (MT/V5) T1w/T2w estimates of myelin predicting the P1 and N2 VEP components.

## Discussion

Though a rich literature has linked prenatal alcohol exposure (PAE) to visually-mediated sensory and behavioral differences over childhood (Hunt et al., 1995; Jacobson et al., 1993; Janzen, 1995; Mattson et al., 1996, 1998; Uecker & Nadel, 1996), there is still a paucity of

research exploring neurodevelopmental mechanisms in humans that may drive such behavioral differences (Coles & Li, 2011; Donald et al., 2015; Wozniak & Muetzel, 2011). Here we leveraged a multimodal longitudinal study to address this gap by examining early visual neurodevelopment as a candidate pathway. We observed differential effects of PAE on visual neurodevelopment according to the following predictor characteristics: trimester of exposure, dose, and drinking pattern (i.e., presence of binge episodes). In this longitudinal sample, PAE differentially impacted the EEG-derived Visual-Evoked Potential (VEP) P1 component measured within the first 6 postnatal months of age, but these effects resolved temporally by the end of the first postnatal year. In a separate analysis in the subsample of infants (collapsing across those with and without PAE) with MRI measures modeled to be sensitive to myelin in visual cortical regions known to drive the VEP response, we also established an association between individual differences in myelination and the VEP response latencies over the first postnatal year of life. These findings provide evidence that PAE and postnatal myelin may each alter early visual neurodevelopmental timing over infancy in the following ways.

First, we found that increased PAE resulted in less mature electrophysiological responses to visual stimuli, as indexed by longer VEP component latencies, depending on trimester of exposure, specific VEP component, and drinking pattern. Specifically, we found that increased alcohol exposure in the first trimester resulted in longer latency of the P1 component assessed within the first 6 months of life, replicating and extending a prior finding from Scher and colleagues that first trimester alcohol exposure resulted in longer P1 latencies at one month of age (1998). A similar effect of binge drinking on VEP P1 latencies was found as well, such that increased exposure to binge drinking in the first trimester resulted in increased latencies of the P1 component over the first 6 postnatal months of life. These effects together suggest that the P1 component is particularly sensitive to PAE exposure. These differential effects of trimester and consistent findings across both binge and non-binge-patterned drinking provide further evidence that timing and drinking patterns are important in characterizing the effects of

prenatal alcohol exposure.

The first trimester is a time of rapid visual development for the fetus as the building blocks of visual structures and many connections are established during this period (Finlay et al., 2003; Finlay & Darlington, 1995; Hevner, 2000; Johnson, 2011). Indeed, the fetus's eyes open during the second trimester after much of the neural structural organization is completed in the first trimester (though there is substantial functional and structural revision postnatally as well). Therefore, this period of rapid development and change is likely highly vulnerable to environmental disturbances and teratogenic effects such as the prenatal alcohol exposure demonstrated in this sample and as noted in other samples (e.g., Scher et al 1998).

Further, the pattern of findings across the two longitudinal study visits suggest that these early VEP latency differences may manifest in developmental delays that resolve neurally by the end of the first year. Specifically, analyses of the Visit 1 EEG data over the first 6 postnatal months also revealed significant age-related changes in latency timing such that latencies shortened with older ages. This finding that P1 VEP latencies speed up with age is consistent with prior literature on the development of the VEP over the first years of life (Lippé et al., 2007; Lippé et al., 2009). Our findings that PAE results in longer latencies during this developmental window of change provide one indicator of delayed development. Importantly, the lack of PAE effects on latencies in the longitudinal sample at the second visit further suggest developmental delays in basic visual processing that resolve neurally by the end of the first postnatal year. Although these VEP differences were no longer observable within the longitudinal sample after 6-months of age, this should not be taken to indicate that earlier VEP delays have no developmental consequences. Altered neurodevelopmental timing, especially within sensory cortices, can have critical and lasting consequences for cognition and behavior, and mis-timing of neurodevelopment is implicated in a variety of other conditions and disorders (e.g. Hensch, 2005; Ivanaov, 2021; Makinodan et al., 2012; Marín, 2016; Takesian & Hensch, 2013). Moreover, visually-mediated sensory and behavioral differences following PAE have been

documented from childhood through adulthood (Hunt et al., 1995; Jacobson et al., 1993; Janzen, 1995; Mattson et al., 1996, 1998; Uecker & Nadel, 1996), consistent with longer-term consequences of early neurodevelopmental changes. Whether the currently observed pattern of delayed VEP development contributes to behavioral differences within this sample remains to be tested at later visits within this longitudinal study.

To explore one potential structural mechanism that could contribute to VEP latencies in development (independent of PAE) we also tested associations in a subsample of infants with an MRI-derived estimate of myelin, the T1w/T2w ratio. PAE has been shown repeatedly to impact myelination (Darbinian & Selzer, 2021; Donald et al., 2015; Ghazi Sherbaf et al., 2019; McLachlan et al., 2019; Newville et al., 2017, 2022; Wozniak & Muetzel, 2011). Moreover, while it is largely accepted that the significant accumulation of myelin in the brain at least partially explains the shortening of VEP latencies across infancy, as far as we are aware, this is the first empirical study to test the relation between cortical myelination and VEP latencies in infancy (though one prior study found decreased subcortical myelination, indexed by diffusion tensor imaging, was linked to longer P1 VEP latencies; Dubois et al., 2008). Here we show that decreased myelination, as indexed by T1w/T2w ratios, in the primary visual cortex (V1) was associated with longer P1 component VEP latencies over the first postnatal year. This association is consistent with those previously noted in adult and clinical samples (Heidari et al., 2019; Klistorner & Graham, 2021; Walt et al., 2015). This set of findings suggests that myelination over infancy may be partially responsible for the maturation of VEP component timing during this developmental window, but also indicates other factors contribute to the increasingly efficient and quick neural responses to basic visual stimuli. Future research with larger samples can evaluate the effects of prenatal alcohol exposure on myelination and whether that influence explains the latency changes related to PAE observed here in VEP P1 latency in infancy.

The present study design bolsters interpretation of the neurodevelopmental effects of

prenatal alcohol exposure observed by addressing several key limitations in prior literature examining sequelae of PAE. Specifically, through the use of a non-clinical sample, we were able to capture a wider spectrum of possible prenatal alcohol exposure with increased ecological validity for the broader population drinking during pregnancy. We were also able to examine the effects of timing, dose, and drinking pattern (i.e., binge drinking) by measuring alcohol exposure continuously over each trimester. However, we do note that in the current sample, there were insufficient binge drinking cases to model effects of binge exposure beyond trimester 1. Additionally, we specifically selected our sample for the current study to have prenatal exposure to exclusively alcohol (i.e., no comorbid substance exposure) to better elucidate the effects of PAE specifically. Prior neurodevelopmental and behavioral research in humans has largely been subject to the confounds that come with polysubstance exposure prenatally (Coles & Li, 2011; Donald et al., 2015). Finally, we selected a comparison sample that was matched to the PAE sample on maternal age at infant birth, maternal education, maternal income, and EPDS depression scores to further reduce potential sociodemographic confounds in analyses and results.

Progress in future work should consider limitations of the current study. First, this study sample had limited endorsement of drinking in later trimesters (especially when characterizing binge drinking behaviors). The limited variability in drinking endorsement in later pregnancy meant that this study was underpowered to detect effects of alcohol exposure in the third trimester, if any exist. While this sample structure precluded careful examination of the effects of prenatal alcohol exposure in later trimesters on visual neurodevelopment, it also reflects ecologically-valid sampling of the patterns found in the community. Second, future work can include larger sample sizes of infants with both MRI and electrophysiological data to facilitate more sensitive analyses of associations between MRI-derived myelin measures and EEG-derived VEP latencies as well as explicitly test how PAE may impact these associations. The current limited sample size made it difficult to examine or detect potential PAE effects on

associations between the MRI and EEG measures. The limited sample size, and thus limited statistical power to detect a wide range of effects from subtle to large, also made it difficult to make specificity claims about which visual areas' myelination patterns better explain VEP component latencies. That is, some visual regions where no statistical effects were noted in this sample may demonstrate effects in a larger and more sensitive sample. Indeed, the preliminary results from this sample demonstrating an association between MRI-derived myelin and VEP latency should motivate further research in this area. Third, the current sample, though longitudinal, included only two timepoints of data per individual over a large age range for each study visit unlike many longitudinal designs in the first year of life. Thus, while the study was able to test several nonlinear associations between VEP measures and age at the between-participant level (i.e., cross-sectionally), the study was unable to model nonlinear age associations within-participant (i.e., longitudinally). Though this design facilitated study flexibility for families and retention over the first year in this community, future studies may prioritize more visits within the first year to facilitate additional analysis strategies like nonlinear longitudinal growth models. Next, future work can more completely measure comorbid non-alcohol substance exposure over the entire period of pregnancy as opposed to the three months prior to assessment in pregnancy. The current strategy may have missed comorbid substance use occurring outside of the assessment window. As a result, we are not able to statistically exclude potential effects of comorbid substance exposures in early pregnancy, outside of the assessment window. That is, there may be effects of these exposures in the results presented that could not be explicitly attributed to comorbid substance use outside of the assessment window. The current sampling strategy was employed to balance participant burden as this analysis was not part of the primary aims for the ongoing longitudinal study. Finally, although the study is ongoing and in the future can examine whether behavioral consequences emerge from these early infant neurodevelopmental changes, the current set of analyses cannot link the neurodevelopmental measures to any behavioral phenotypes at this time.

Finally, with an eye towards supporting future pregnant people and infants in contexts of prenatal alcohol use and exposure, we empirically probed whether a potential screening tool would relate to self-reported alcohol use in our sample. Further supporting the robustness of the WHO ASSIST, a lifetime risk measure, we found that the alcohol risk score calculated using this tool was related to the self-report of number of drinks per week and of binge episodes during pregnancy throughout all three trimesters for this South African community. Moreover, 100% of the mothers who scored above lower risk on the WHO ASSIST reported drinking during pregnancy, so this tool may be useful to identify individuals who may benefit from counseling and support around drinking during pregnancy. This analysis is one of the first empirical evaluations of the WHO ASSIST as a tool to detect those at higher risk of endorsing alcohol use during pregnancy and further demonstrates its utility and acceptability in an under-resourced community like the Gugulethu community (WHO ASSIST Working Group, 2002).

In conclusion, prenatal exposure to alcohol, specifically in the first trimester, may alter timing of visual neurodevelopment in the first 6 months of life, with possible consequences lasting across the lifespan. Future directions of this work can identify pre- and postnatal resilience factors that may protect against the effects of prenatal alcohol exposure described in this study. Given our exploratory findings that myelin is also separately related to VEP latency in infancy (across those with and without prenatal alcohol exposure), future studies should examine whether there are links between prenatal alcohol exposure, myelination, and VEP latency that could explain the VEP differences we observed here and offer a postnatal intervention target. In these ways, better elucidating the neurodevelopmental sequelae of prenatal alcohol exposure using longitudinal multimodal studies such as the current study will be important in identifying how to best support pregnant people and their infants' development.

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**Table 1**

*Descriptive Statistics of Number of Drinks per Week in each Trimester and Number of Binge Episodes per Week in each Trimester for those Endorsing Alcohol Use at any Point in Pregnancy (Non-Winsorized; n=65)*

| Drinking Variable | *n-endorsing* | *M* | *SD* | Median | Range | |
|---|---|---|---|---|---|---|
| | | | | | min | max |
| Number of Drinks per Week in | | | | | | |
| Trimester 1 | 56 | 2.829 | 5.677 | 0.875 | 0.000 | 30.000 |
| Trimester 2 | 29 | 1.390 | 4.506 | 0.000 | 0.000 | 30.000 |
| Trimester 3 | 13 | 0.210 | 0.503 | 0.000 | 0.000 | 2.400 |
| Number of Binge Episodes per Week in | | | | | | |
| Trimester 1 | 14 | 0.203 | 0.547 | 0.000 | 0.000 | 2.500 |
| Trimester 2 | 6 | 0.077 | 0.333 | 0.000 | 0.000 | 2.500 |
| Trimester 3 | 2 | 0.015 | 0.087 | 0.000 | 0.000 | 0.500 |

*Note*. These are descriptive statistics of the sample with prenatal alcohol exposure, no comorbid non-alcohol substance exposure, and usable EEG data at either time point that is included in the main analyses.

**Table 2**

*Demographic Characteristics*

| | **Total (N=162)** |
|---|---|
| **Maternal Place of Birth** | |
| South Africa | 158 (97.5%) |
| In the African Continent (not South Africa) | 4 (2.5%) |
| **Primary Spoken Language** | |
| Xhosa Language | 155 (95.7%) |
| English Language | 3 (1.9%) |
| Sotho Language | 2 (1.2%) |
| Afrikaans Language | 1 (0.6%) |
| Zulu Language | 1 (0.6%) |
| **Maternal Age at Infant Birth (years)** | |
| Mean (SD) | 28.9 (5.88) |
| Median [Min, Max] | 29.0 [18.0, 43.0] |
| Missing | 1 (0.6%) |
| **Maternal Educational Attainment** [a] | |
| Completed Grade 6 (Standard 4) to Grade 7 (Standard 5) | 3 (1.9%) |
| Completed Grade 8 (Standard 6) to Grade 11 (Standard 9) | 70 (43.2%) |
| Completed Grade 12 (Standard 10; i.e., Completed High School) | 63 (38.9%) |
| Some University/College/Post-Matric Education | 20 (12.3%) |
| Completed University/College/Post-Matric Education | 6 (3.7%) |
| **Maternal Monthly Income (South African Rand - ZAR)** [b] | |
| Less than R1,000 per month | 90 (55.6%) |
| R1000 - R5,000 per month | 53 (32.7%) |

| | |
|---|---|
| R5000 - R10,000 per month | 13 (8.0%) |
| More than R10,000 per month | 1 (0.6%) |
| Unknown | 5 (3.1%) |
| **EPDS Depression Score [c]** | |
| Mean (SD) | 6.85 (5.75) |
| Median [Min, Max] | 6.00 [0, 22.0] |
| **WHO ASSIST Alcohol Risk Score [d]** | |
| Mean (SD) | 1.23 (4.53) |
| Median [Min, Max] | 0 [0, 30.0] |
| **Infant Biological Sex** | |
| Female | 78 (48.1%) |
| Male | 82 (50.6%) |
| Missing | 2 (1.2%) |

[a] The South African Educational System was formerly divided into years called standards, similarly to the way the United States Educational System is divided into grades. The equivalent in terms of standards is provided in parentheses next to each mentioned grade. “University/College/Post-Matric Education” refers to tertiary or post-secondary education as defined by the World Bank.
[b] At the time of writing (05/17/2023), 1 United States Dollar (USD) = 19.24 South African Rand (ZAR).
[c] Depression was measured using the Edinburgh Postnatal Depression Scale (EPDS) at enrollment (Cox et al., 1987).
[d] Alcohol risk score was calculated using the World Health Organization Alcohol, Smoking and Substance Involvement Screening Test (WHO ASSIST) at enrollment (WHO ASSIST Working Group, 2002).

**Table 3**

*HAPPE v3.3 Pre-Processing Script Parameters*

| | |
|---|---|
| **Density** | High (>30 channels) |
| **Resting State or Task** | Task |
| **ERP Analysis** | Yes |
| **Acquisition Layout** | 128 channel EGI HydroCel Geodesic Sensor Net |
| **Channels** | All except E1, E8, E14, E17, E21, E25, E32, E38, E43, E44, E48, E49, E56, E63, E68, E73, E81, E88, E94, E99, E107, E113, E114, E119, E120, E121, E125, E126, E127, E128 |
| **Line Noise** | |
| **Line Noise Frequency** | 50 Hz |
| **Line Noise Reduction Method** | CleanLine - Default |
| **Resample** | Off |
| **Filter** | |
| **Filter - Lowpass Cutoff** | 30 Hz |
| **Filter - Highpass Cutoff** | 0.3 Hz |
| **Filter Type** | EEGLAB's FIR |
| **Bad Channel Detection** | On |
| **Bad Channel Detection Method** | Default |
| **Wavelet Thresholding** | Default |
| **Wavelet Threshold Rule** | Hard |
| **MusclL** | Off |
| **Segmentation** | On |
| **Starting Parameter for Stimulus** | - 0.1 seconds |
| **Ending Parameter for Stimulus** | 0.5 seconds |
| **Task Offset** | 11 milliseconds |
| **Baseline Correction** | On |
| **Baseline Correction Start** | - 100 milliseconds |

| | |
|---|---|
| **Baseline Correction End** | 0 milliseconds |
| **Interpolation** | Off |
| **Segment Rejection** | On |
| **Segment Rejection Method** | Amplitude criteria only |
| **Minimum Segment Rejection Threshold** | - 200 |
| **Maximum Segment Rejection Threshold** | 200 |
| **Segment Rejection based on All Channels or ROI** | ROI |
| **ROI Channels** | E70, E71, E75, E76, E83 |
| **Re-Reference Method** | Average |

**Table 4**

*EEG Quality Control Measures*

| | Visit 1 Data (*n*=108) Sample Age: 15.798 weeks (3.719) [8.286, 24.571] | | | Visit 2 Data (N=102) Sample Age: 36.489 weeks (6.337) [26.000, 51.857] | | |
|---|---|---|---|---|---|---|
| | **Alcohol Exposure (*n*=54)** | **No Alcohol Exposure (*n*=54)** | **t-test between groups (α=.05)** | **Alcohol Exposure (*n*=51)** | **No Alcohol Exposure (*n*=51)** | **t-test between groups (α=.05)** |
| **VEP Trial Retention** | | | | | | |
| **Number of Collected VEP Trials** | | | | | | |
| Mean (SD) | 99.9 (0.408) | 100 (0) | *n.s.* | 100 (0) | 100 (0) | *n.s.* |
| Median [Min, Max] | 100 [97.0, 100] | 100 [100, 100] | | 100 [100, 100] | 100 [100, 100] | |
| **Number of Retained VEP Trials** | | | | | | |
| Mean (SD) | 92.9 (16.4) | 92.5 (15.7) | *n.s.* | 95.3 (13.7) | 95.5 (12.8) | *n.s.* |
| Median [Min, Max] | 100 [17.0, 100] | 100 [32.0, 100] | | 100 [21.0, 100] | 100 [29.0, 100] | |
| **Percent Retained VEP Trials** | | | | | | |
| Mean (SD) | 93.0 (16.5) | 92.5 (15.7) | *n.s.* | 95.3 (13.7) | 95.5 (12.8) | *n.s.* |
| Median [Min, Max] | 100 [17.0, 100] | 100 [32.0, 100] | | 100 [21.0, 100] | 100 [29.0, 100] | |
| **Whole Head Channel Retention** | | | | | | |
| **Number of Retained Channels** | | | | | | |
| Mean (SD) | 78.5 (8.83) | 79.1 (9.37) | *n.s.* | 78.7 (10.7) | 81.5 (8.34) | *n.s.* |
| Median [Min, Max] | 79.5 [56.0, 95.0] | 78.5 [55.0, 97.0] | | 81.0 [46.0, 96.0] | 83.0 [59.0, 96.0] | |
| **Percent Retained Channels** | | | | | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| Mean (SD) | 79.3 (8.92) | 79.9 (9.47) | *n.s.* | 78.7 (10.7) | 81.9 (8.18) | *n.s.* |
| Median [Min, Max] | 80.3 [56.6, 96.0] | 79.3 [55.6, 98.0] | | 80.8 [46.5, 94.9] | 82.8 [59.6, 93.9] | |
| **Region of Interest (ROI) Channel Retention** | | | | | | |
| **Number of Retained Channels** | | | | | | |
| Mean (SD) | 4.24 (0.930) | 4.31 (1.01) | *n.s.* | 4.04 (1.06) | 4.47 (0.758) | $t(100)=2.37$ $p=.020$ |
| Median [Min, Max] | 4.50 [2.00, 5.00] | 5.00 [1.00, 5.00] | | 4.00 [1.00, 5.00] | 5.00 [2.00, 5.00] | |
| **Percent Retained Channels** | | | | | | |
| Mean (SD) | 0.848 (0.186) | 0.863 (0.201) | *n.s.* | 0.808 (0.212) | 0.894 (0.152) | $t(100)=2.37$ $p=.020$ |
| Median [Min, Max] | 0.900 [0.400, 1.00] | 1.00 [0.200, 1.00] | | 0.800 [0.200, 1.00] | 1.00 [0.400, 1.00] | |
| **Correlation of Data Pre-v s. Post- Wavelet Thresholding (Pearson's r)** | | | | | | |
| **At 5 Hz** | | | | | | |
| Mean (SD) | 0.529 (0.192) | 0.500 (0.184) | *n.s.* | 0.455 (0.226) | 0.384 (0.251) | *n.s.* |
| Median [Min, Max] | 0.569 [0.0838, 0.901] | 0.514 [0.0389, 0.835] | | 0.484 [0.0497, 0.795] | 0.401 [0.0317, 0.818] | |
| **At 8 Hz** | | | | | | |
| Mean (SD) | 0.450 (0.176) | 0.411 (0.165) | *n.s.* | 0.425 (0.235) | 0.354 (0.222) | *n.s.* |
| Median [Min, Max] | 0.459 [0.0884, 0.844] | 0.421 [0.0689, 0.763] | | 0.440 [0.0240, 0.805] | 0.349 [0.0262, 0.764] | |
| **At 12 Hz** | | | | | | |
| Mean (SD) | 0.428 (0.166) | 0.405 (0.159) | *n.s.* | 0.383 (0.237) | 0.300 (0.193) | *n.s.* |
| Median [Min, Max] | 0.439 [0.0702, 0.798] | 0.442 [0.0377, 0.644] | | 0.400 [0.0179, 0.762] | 0.323 [0.0159, 0.623] | |
| **At 20 Hz** | | | | | | |
| Mean (SD) | 0.492 (0.177) | 0.481 (0.164) | *n.s.* | 0.431 (0.249) | 0.358 (0.220) | *n.s.* |
| Median [Min, Max] | 0.533 [0.100, 0.832] | 0.506 [0.0839, 0.757] | | 0.475 [0.0229, 0.830] | 0.399 [0.0145, 0.737] | |

*Note.* EEG data were pre-processed and VEPs were extracted using HAPPE+ER v3.3 software, an automated open-source EEG processing software validated for infant data (Monachino et al., 2022).

**Table 5**

*HAPPE v3.3 Generate ERP Script Parameters*

| | |
|---|---|
| **Average or Individual Trials** | Average |
| **Channels of Interest** | E70, E71, E75, E76, E83 |
| **Bad Channels Included/Excluded** | Included |
| **Calculating ERP Values** | On |
| **Windows** | Min 40-100 milliseconds<br>Max 75-175 milliseconds<br>Min 100-325 milliseconds |

**Table 6**

*Regression Models of Number of Drinks per Week in each Trimester Predicting Visual-Evoked Potential (VEP) Component Latencies at Visit 1 (n before removing outliers=108)*

| Component | Variable | *b* | *SE* | *p* | 95% CI [LL, | UL] |
|---|---|---|---|---|---|---|
| N1 Latency | Model: $n$=105, model $R^2$=.08, $F$(5, 99)=1.81, $p$=.117 | | | | | |
| | Number of Drinks per Week in | | | | | |
| | Trimester 1 | -0.23 | 0.49 | .646 | [-1.20, | 0.74] |
| | Trimester 2 | -2.21 | 1.39 | .116 | [-4.96, | 0.55] |
| | Trimester 3 | -0.21 | 2.89 | .941 | [-5.96, | 5.53] |
| | Infant Age at EEG Collection | -0.13 | 0.23 | .566 | [-0.60, | 0.33] |
| | Number of Retained EEG Trials | -0.11 | 0.05 | .049 | [-0.21, | 0.00] |
| P1 Latency | Model: $n$=104, model $R^2$=.56, $F$(6, 97)=20.89, $p$<.001 | | | | | |
| | Number of Drinks per Week in | | | | | |
| | Trimester 1 | 1.16 | 0.55 | .039 | [0.06, | 2.26] |
| | Trimester 2 | 0.18 | 1.53 | .906 | [-2.86, | 3.22] |
| | Trimester 3 | -5.04 | 3.23 | .121 | [-11.45, | 1.36] |
| | Infant Age at EEG Collection | 7.36 | 1.89 | <.001 | [3.61, | 11.11] |
| | Natural Log of Infant EEG Age | -153.50 | 29.67 | <.001 | [-212.39, | -94.61] |
| | Number of Retained EEG Trials | 0.09 | 0.06 | .142 | [-0.03, | 0.20] |
| N2 Latency | Model: $n$=106, model $R^2$=.25, $F$(5, 100)=6.60, $p$<.001 | | | | | |
| | Number of Drinks per Week in | | | | | |
| | Trimester 1 | 2.92 | 2.00 | .148 | [-1.05, | 6.90] |
| | Trimester 2 | -2.00 | 5.70 | .726 | [-13.31, | 9.31] |
| | Trimester 3 | 0.83 | 11.86 | .944 | [-22.70, | 24.36] |
| | Infant Age at EEG Collection | -5.17 | 0.93 | <.001 | [-7.02, | -3.32] |
| | Number of Retained EEG Trials | 0.14 | 0.22 | .514 | [-0.29, | 0.57] |

**Table 7**

*Regression Models of Number of Drinks per Week in each Trimester Predicting Visual-Evoked Potential (VEP) Component Latencies at Visit 2 (n before removing outliers=102)*

| Component | Variable | *b* | *SE* | *p* | 95% CI [LL, | UL] |
|---|---|---|---|---|---|---|
| N1 Latency | Model: *n*=102, model $R^2$=.11, *F*(5, 96)=2.46, *p*=.038 | | | | | |
| | Number of Drinks per Week in | | | | | |
| | Trimester 1 | 0.21 | 0.20 | .281 | [-0.18, | 0.60] |
| | Trimester 2 | 0.23 | 0.54 | .669 | [-0.84, | 1.31] |
| | Trimester 3 | 0.51 | 2.10 | .810 | [-3.66, | 4.67] |
| | Infant Age at EEG Collection | -0.28 | 0.09 | .002 | [-0.46, | -0.10] |
| | Number of Retained EEG Trials | 0.01 | 0.04 | .804 | [-0.08, | 0.10] |
| P1 Latency | Model: *n*=100, model $R^2$=.03, *F*(5, 94)=0.64, *p*=.671 | | | | | |
| | Number of Drinks per Week in | | | | | |
| | Trimester 1 | 0.06 | 0.15 | .689 | [-0.24, | 0.37] |
| | Trimester 2 | -0.54 | 0.43 | .207 | [-1.39, | 0.31] |
| | Trimester 3 | -0.14 | 1.66 | .931 | [-3.43, | 3.14] |
| | Infant Age at EEG Collection | -0.06 | 0.07 | .378 | [-0.20, | 0.08] |
| | Number of Retained EEG Trials | -0.02 | 0.03 | .566 | [-0.09, | 0.05] |
| N2 Latency | Model: *n*=101, model $R^2$=.02, *F*(5, 95)=0.34, *p*=.889 | | | | | |
| | Number of Drinks per Week in | | | | | |
| | Trimester 1 | 0.09 | 0.58 | .874 | [-1.06, | 1.24] |
| | Trimester 2 | -1.58 | 1.60 | .326 | [-4.75, | 1.59] |
| | Trimester 3 | -3.57 | 6.92 | .607 | [-17.31, | 10.17] |
| | Infant Age at EEG Collection | -0.17 | 0.27 | .515 | [-0.70, | 0.35] |
| | Number of Retained EEG Trials | 0.06 | 0.13 | .650 | [-0.20, | 0.31] |

**Table 8**

*Regression Models of Number of Binge Episodes per Week in each Trimester Predicting Visual-Evoked Potential (VEP) Component Latencies at Visit 1 (n before removing outliers=108)*

| Component | Variable | *b* | *SE* | *p* | 95% CI [LL, | UL] |
|---|---|---|---|---|---|---|
| N1 Latency | Model: *n*=105, model $R^2$=.07, *F*(3, 101)=2.71, *p*=.049 | | | | | |
| | Number of Binge Episodes per Week in Trimester 1 | -5.93 | 3.34 | .079 | [-12.56, | 0.70] |
| | Infant Age at EEG Collection | -0.09 | 0.23 | .710 | [-0.54, | 0.37] |
| | Number of Retained EEG Trials | -0.11 | 0.05 | .040 | [-0.21, | 0.00] |
| P1 Latency | Model: *n*=104, model $R^2$=.56, *F*(4, 99)=30.99, *p*<.001 | | | | | |
| | Number of Binge Episodes per Week in Trimester 1 | 9.14 | 4.11 | .028 | [0.98, | 17.31] |
| | Infant Age at EEG Collection | 7.47 | 1.89 | <.001 | [3.72, | 11.21] |
| | Natural Log of Infant EEG Age | -154.65 | 29.61 | <.001 | [-213.41, | -95.89] |
| | Number of Retained EEG Trials | 0.08 | 0.06 | .154 | [-0.03, | 0.20] |
| N2 Latency | Model: *n*=106, model $R^2$=.24, *F*(3, 102)=10.92, *p*<.001 | | | | | |
| | Number of Binge Episodes per Week in Trimester 1 | 17.67 | 13.68 | .199 | [-9.46, | 44.79] |
| | Infant Age at EEG Collection | -5.17 | 0.92 | <.001 | [-7.00, | -3.35] |
| | Number of Retained EEG Trials | 0.14 | 0.21 | .516 | [-0.28, | 0.56] |

**Table 9**

*Regression Models of Number of Binge Episodes per Week in each Trimester Predicting Visual-Evoked Potential (VEP) Component Latencies at Visit 2 (n before removing outliers=102)*

| Component | Variable | *b* | *SE* | *p* | 95% CI [LL, | UL] |
|---|---|---|---|---|---|---|
| N1 Latency | Model: *n*=101, model $R^2$=.11, *F*(3, 97)=3.94, *p*=.011 | | | | | |
| | Number of Binge Episodes per Week in Trimester 1 | 1.20 | 3.72 | .747 | [-6.17, | 8.58] |
| | Infant Age at EEG Collection | -0.29 | 0.09 | <.001 | [-0.46, | -0.12] |
| | Number of Retained EEG Trials | 0.01 | 0.04 | .754 | [-0.07, | 0.09] |
| P1 Latency | Model: *n*=100, model $R^2$=.02, *F*(3, 96)=0.56, *p*=.645 | | | | | |
| | Number of Binge Episodes per Week in Trimester 1 | -0.92 | 3.04 | .763 | [-6.96, | 5.12] |
| | Infant Age at EEG Collection | -0.06 | 0.07 | .414 | [-0.20, | 0.08] |
| | Number of Retained EEG Trials | -0.02 | 0.03 | .485 | [-0.09, | 0.04] |
| N2 Latency | Model: *n*=101, model $R^2$=.01, *F*(3, 97)=0.47, *p*=.702 | | | | | |
| | Number of Binge Episodes per Week in Trimester 1 | -11.38 | 11.31 | .317 | [-33.84, | 11.07] |
| | Infant Age at EEG Collection | -0.16 | 0.26 | .553 | [-0.68, | 0.36] |
| | Number of Retained EEG Trials | 0.05 | 0.13 | .694 | [-0.20, | 0.30] |

**Table 10**

*Regression Models of Primary Visual Cortex (V1) T1w/T2w Ratios (Myelin Estimates) Predicting Visual-Evoked Potential (VEP) Component Latencies (n before removing outliers=47)*

| Component | Variable | *b* | *SE* | *p* | 95% CI [LL, | UL] |
|---|---|---|---|---|---|---|
| N1 Latency | Model: $n$=46, model $R^2$=.34, $F$(4, 41)=5.39, $p$=.001 | | | | | |
| | Primary Visual Cortex (V1) T1w/T2w Ratio | -3.59 | 6.29 | .571 | [-16.30, | 9.11] |
| | Infant Age at EEG Collection | -0.25 | 0.24 | .308 | [-0.74, | 0.24] |
| | Infant Age at MRI Collection | -0.25 | 0.26 | .328 | [-0.77, | 0.27] |
| | Number of Retained EEG Trials | -0.12 | 0.08 | .160 | [-0.28, | 0.05] |
| P1 Latency | Model: $n$=45, model $R^2$=.63, $F$(4, 40)=16.82, $p$<.001 | | | | | |
| | Primary Visual Cortex (V1) T1w/T2w Ratio | -12.80 | 6.03 | .040 | [-24.98, | -0.61] |
| | Infant Age at EEG Collection | -0.18 | 0.24 | .444 | [-0.66, | 0.30] |
| | Infant Age at MRI Collection | -0.71 | 0.25 | .007 | [-1.22, | -0.20] |
| | Number of Retained EEG Trials | -0.06 | 0.08 | .445 | [-0.22, | 0.10] |
| N2 Latency | Model: $n$=46, model $R^2$=.27, $F$(4, 41)=3.72, $p$=.011 | | | | | |
| | Primary Visual Cortex (V1) T1w/T2w Ratio | -49.48 | 39.56 | .218 | [-129.38, | 30.43] |
| | Infant Age at EEG Collection | -1.58 | 1.51 | .304 | [-4.63, | 1.48] |
| | Infant Age at MRI Collection | -1.06 | 1.61 | .514 | [-4.32, | 2.20] |
| | Number of Retained EEG Trials | -0.23 | 0.52 | .658 | [-1.27, | 0.81] |

**Table 11**

*Regression Models of Middle Temporal Visual Area (MT/V5) T1w/T2w Ratios (Myelin Estimates)*

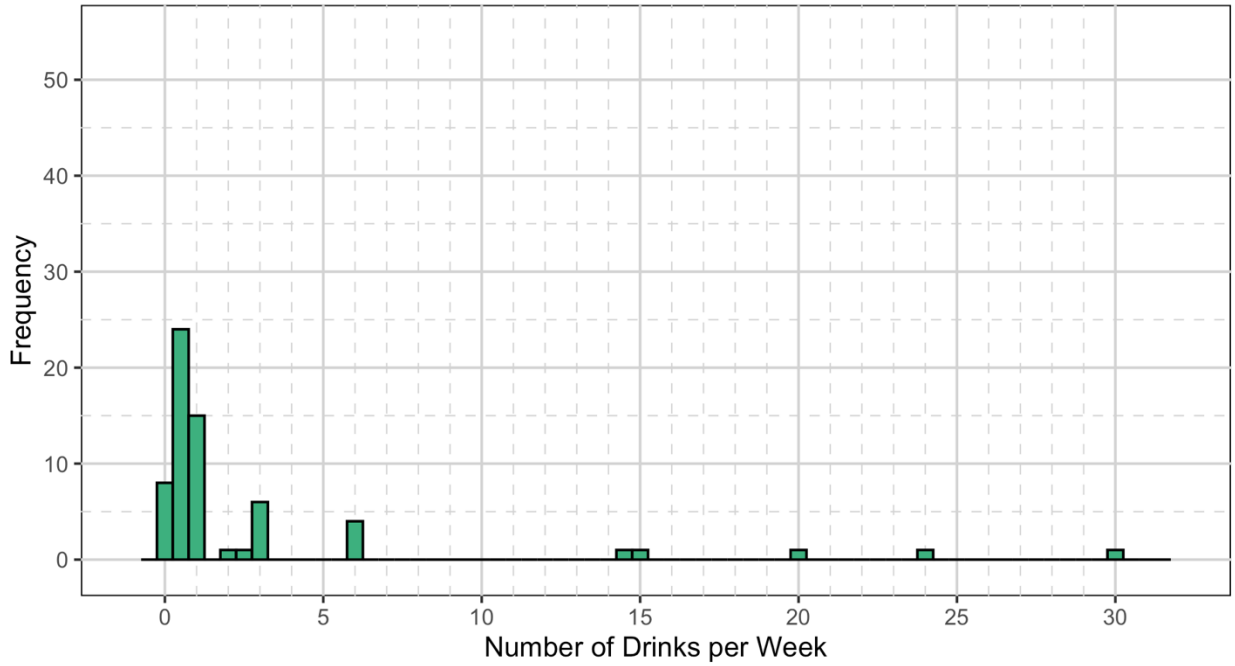
*Predicting Relevant Visual-Evoked Potential (VEP) Component Latencies (n before removing outliers=47)*

| Component | Variable | *b* | *SE* | *p* | 95% CI [LL, | UL] |
|---|---|---|---|---|---|---|
| P1 Latency | Model: $n$=45, model $R^2$=.61, $F$(4, 40)=15.35, $p$<.001 | | | | | |
| | Middle Temporal Visual Area (MT/V5) T1w/T2w Ratio | -10.97 | 7.63 | .158 | [-26.38, | 4.44] |
| | Infant Age at EEG Collection | -0.19 | 0.24 | .440 | [-0.69, | 0.30] |
| | Infant Age at MRI Collection | -0.70 | 0.26 | .010 | [-1.22, | -0.18] |
| | Number of Retained EEG Trials | -0.06 | 0.08 | .436 | [-0.23, | 0.10] |
| N2 Latency | Model: $n$=46, model $R^2$=.26, $F$(4, 41)=3.55, $p$=.014 | | | | | |
| | Middle Temporal Visual Area (MT/V5) T1w/T2w Ratio | -50.14 | 48.76 | .310 | [-148.61, | 48.33] |
| | Infant Age at EEG Collection | -1.57 | 1.53 | .309 | [-4.65, | 1.51] |
| | Infant Age at MRI Collection | -1.02 | 1.62 | .532 | [-4.30, | 2.26] |
| | Number of Retained EEG Trials | -0.25 | 0.52 | .637 | [-1.30, | 0.81] |

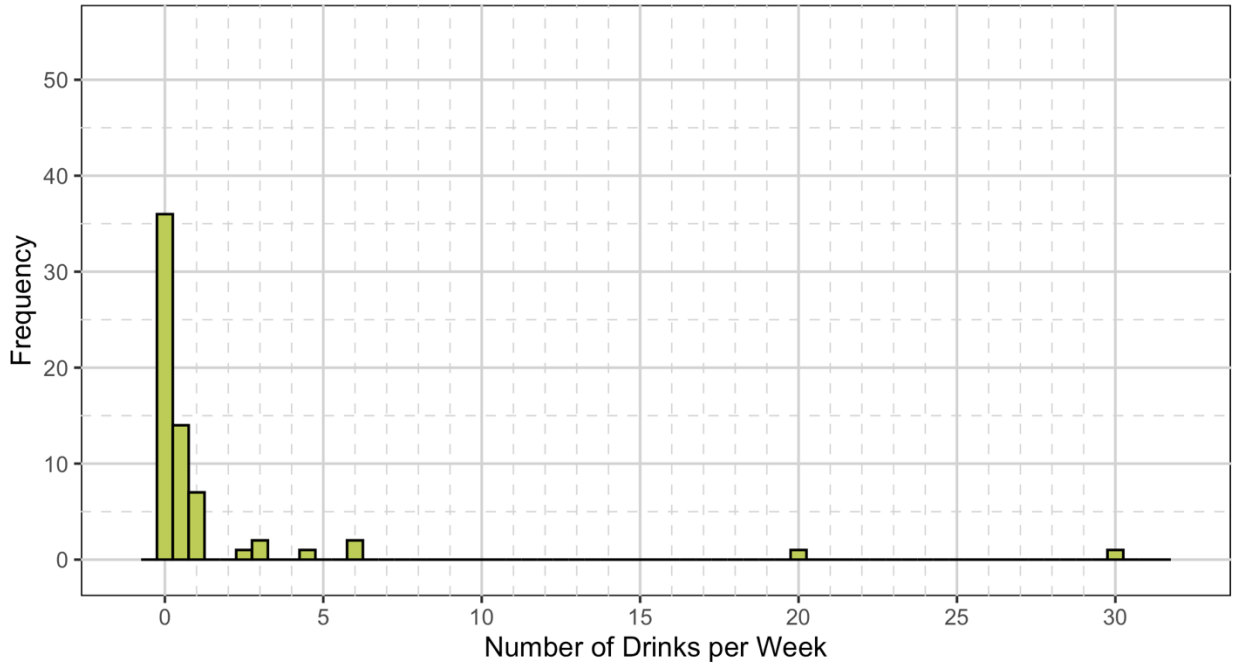
**Figure 1**

*Number of Drinks per Week by Trimester (Non-Winsorized)*

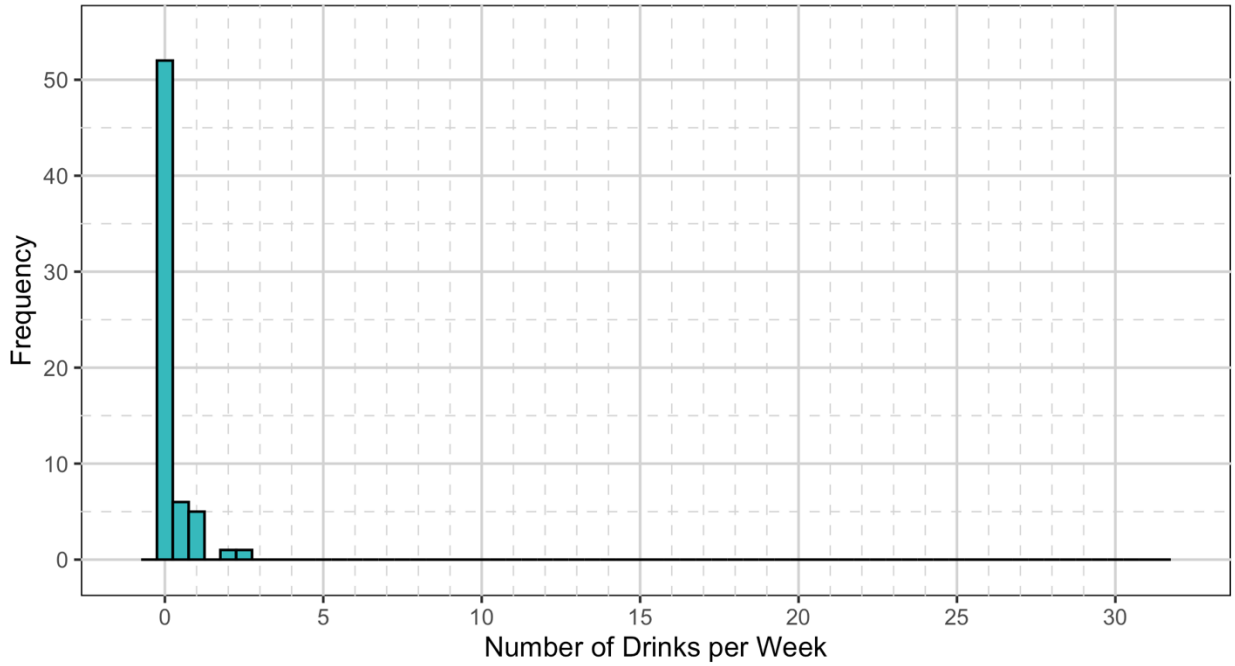
(a) Trimester 1




(b) Trimester 2



(c) Trimester 3

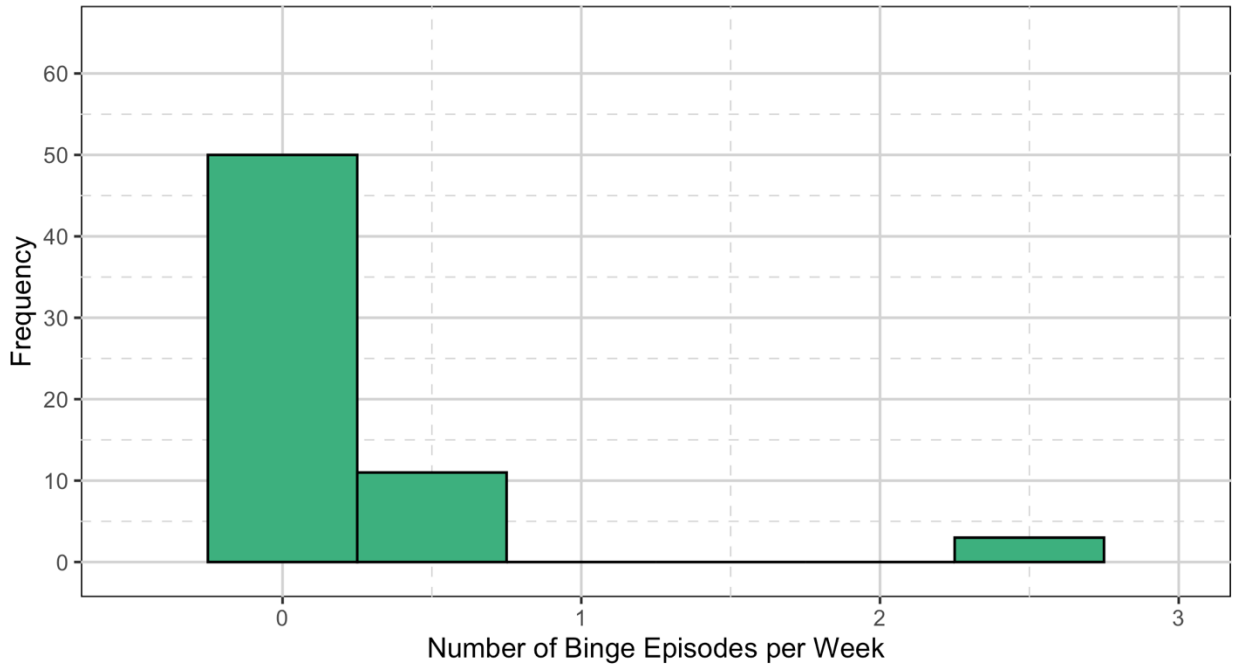



*Note*. These are frequency distributions of the sample (n=65) with prenatal alcohol exposure, no comorbid non-alcohol substance exposure, and usable EEG data at either time point that is included in the main analyses.

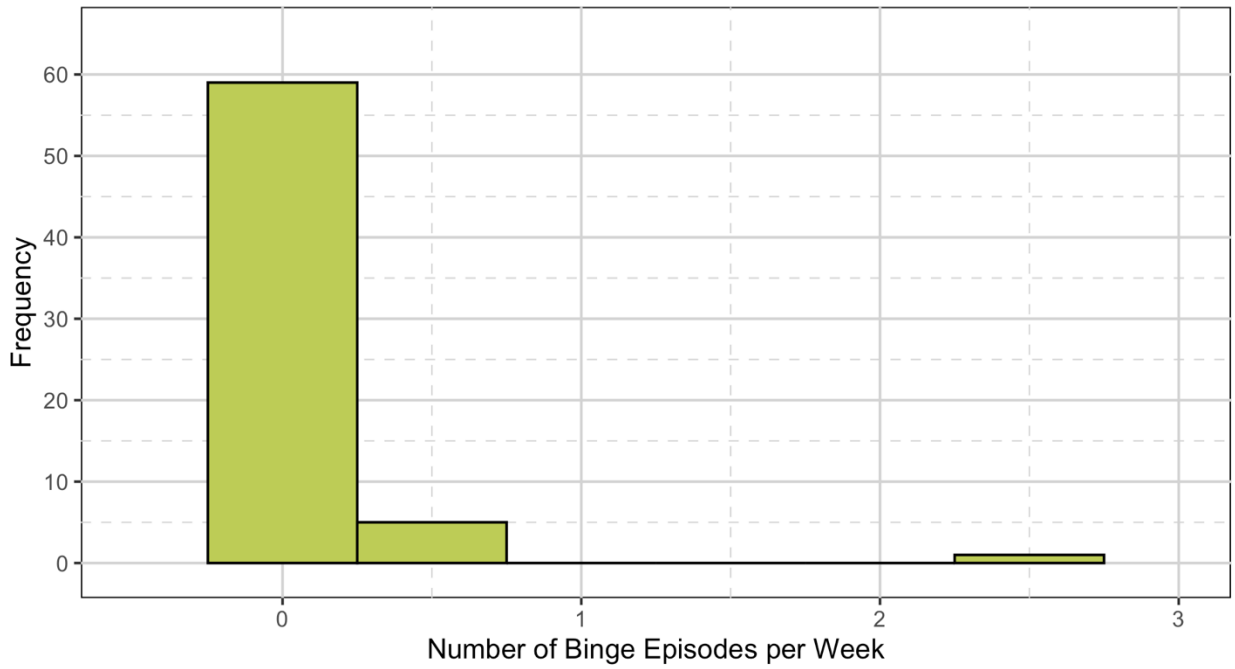
**Figure 2**

*Number of Binge Episodes per Week by Trimester (Non-Winsorized)*

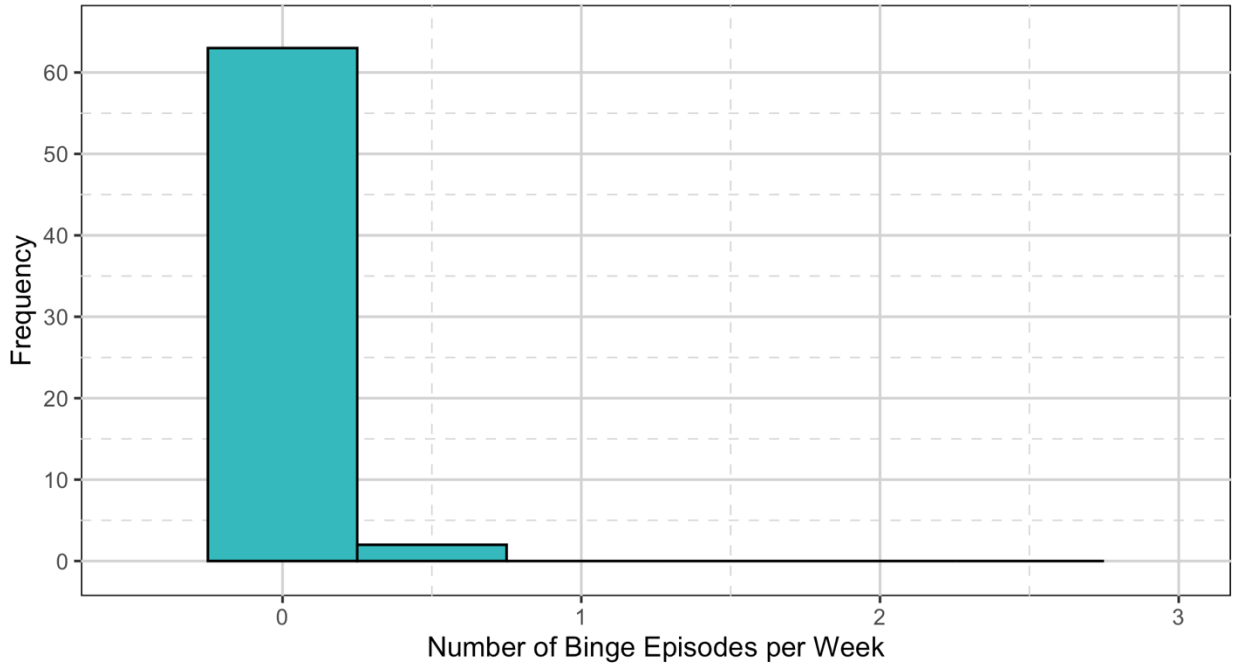
(a) Trimester 1




(b) Trimester 2



(c) Trimester 3



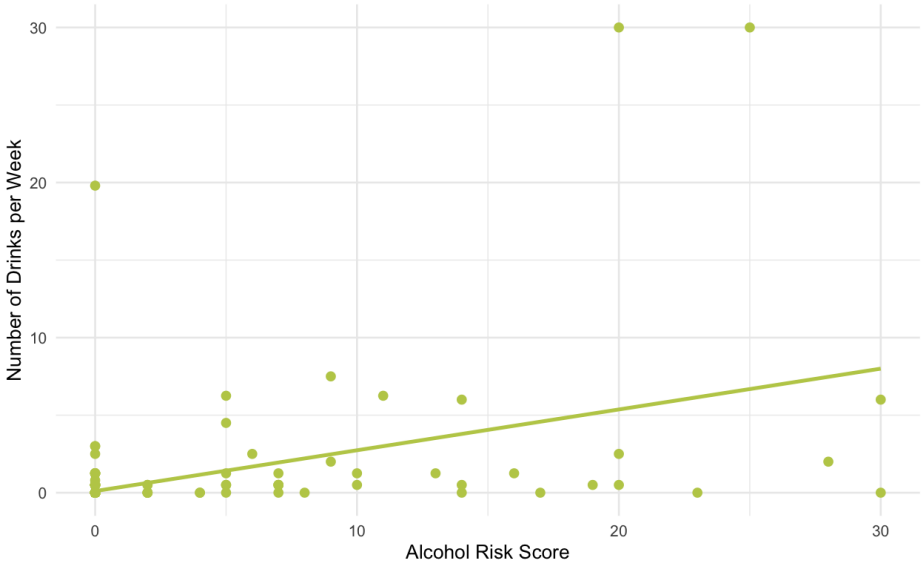

*Note.* These are frequency distributions of the sample (n=65) with prenatal alcohol exposure, no comorbid non-alcohol substance exposure, and usable EEG data at either time point that is included in the main analyses.

**Figure 3**

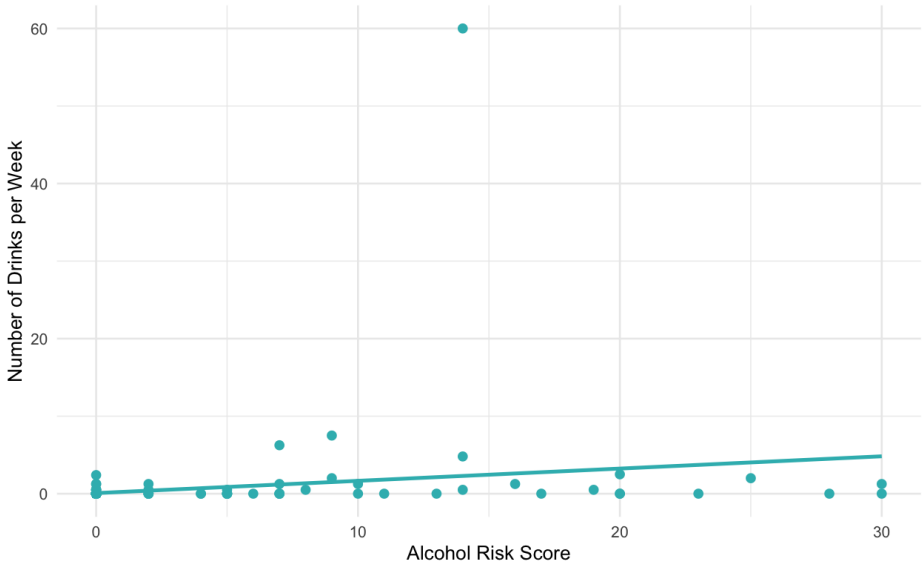
*WHO ASSIST Alcohol Risk Scores and Number of Drinks per Week by Trimester (a-c) and Number of Binge Episodes per Week by Trimester (d-f)*

(a) Number of Drinks per Week in Trimester 1 (r(390)=.31, p<.001)


(b) Number of Drinks per Week in Trimester 2 (r(390)=.45, p<.001)

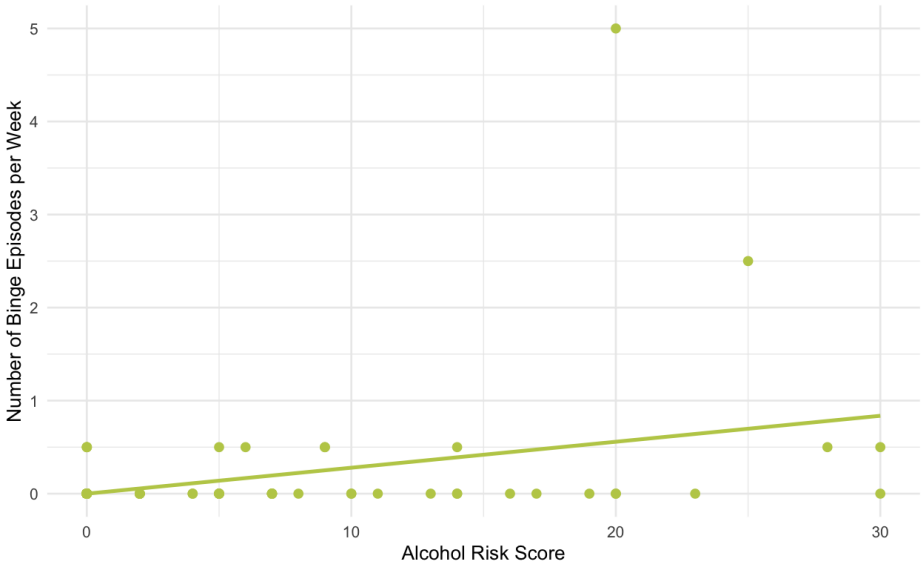


(c) Number of Drinks per Week in Trimester 3 (r(390)=.22, p<.001)

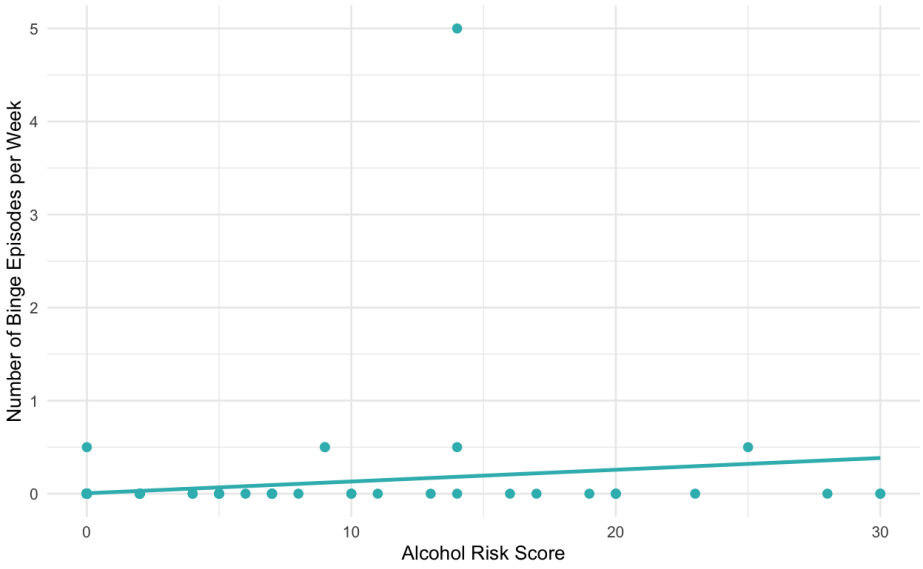



(d) Number of Binge Episodes per Week in Trimester 1 (r(390)=.21, p<.001)

(e) Number of Binge Episodes per Week in Trimester 2 (r(390)=.41, p<.001)




(f) Number of Binge Episodes per Week in Trimester 3 (r(390)=.21, p<.001)

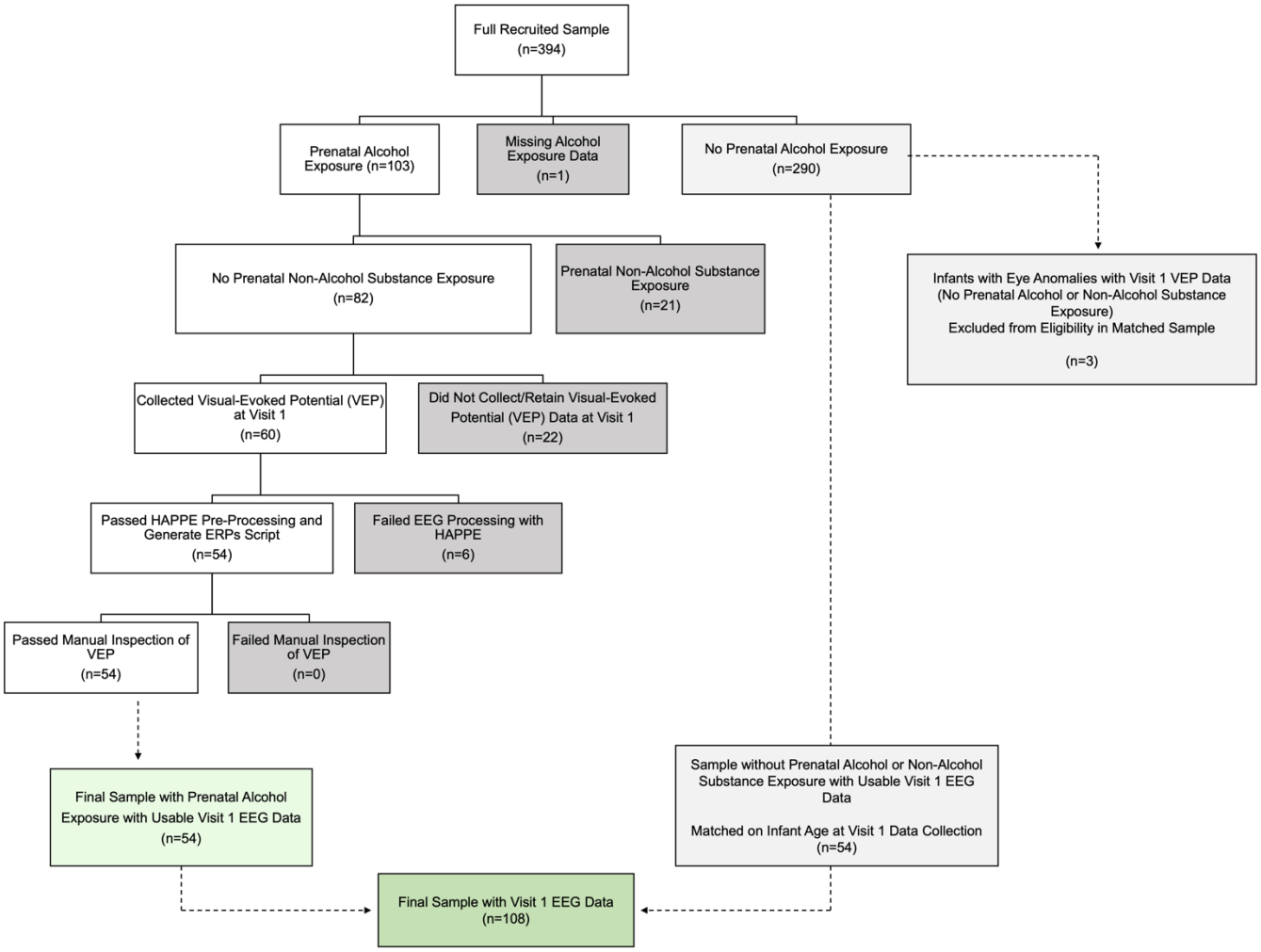



*Note.* Sample size (n=392). Axes vary by trimester to best capture the range of alcohol endorsed in that trimester.

**Figure 4**

*Participants Contributing EEG Data at each Visit*

(a) Visit 1 Participants (n=108)



(b) Visit 2 Participants (n=102)

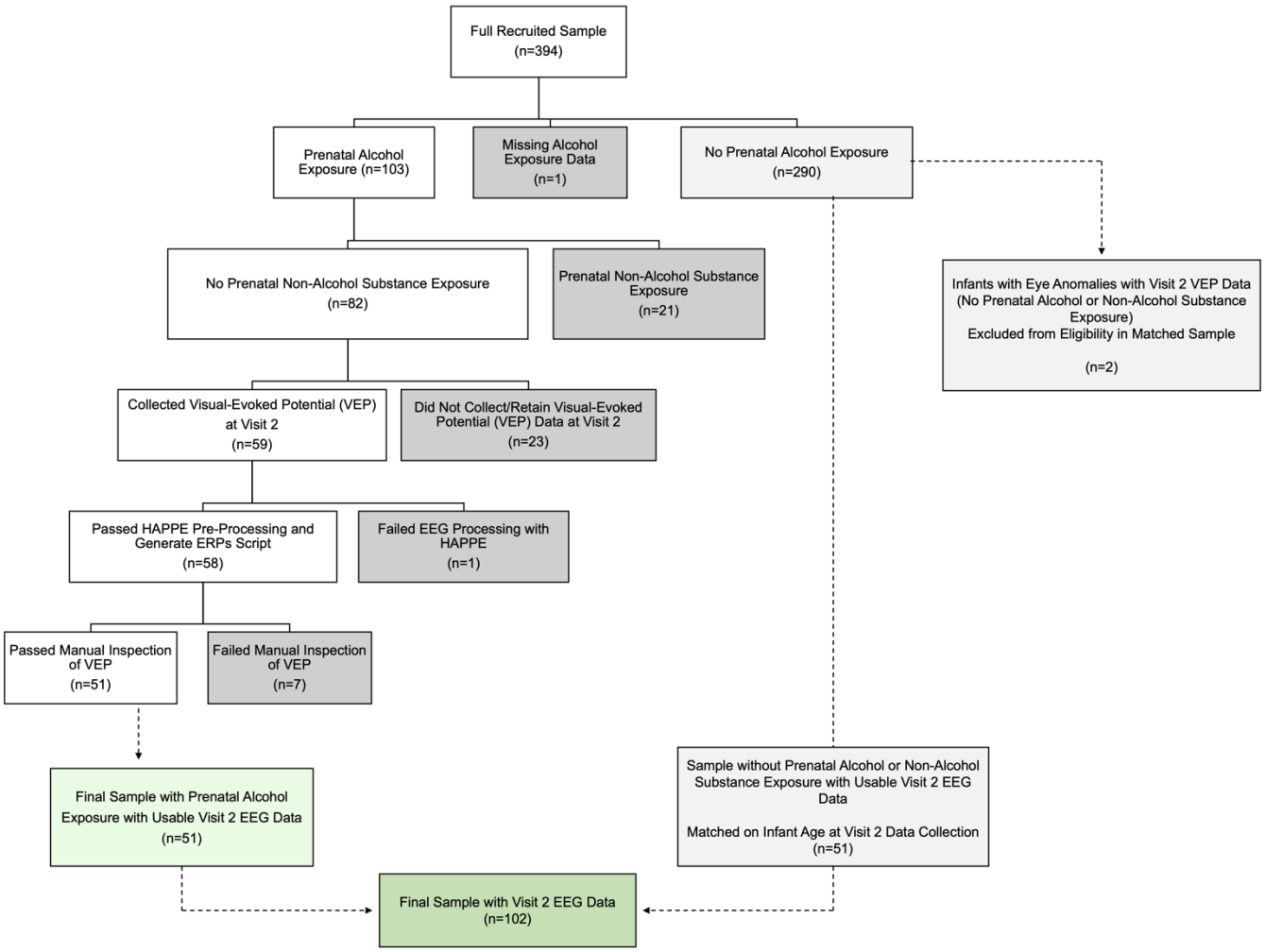


**Figure 5**

*Average Visual-Evoked Potential (VEP) Waveforms at each Visit*

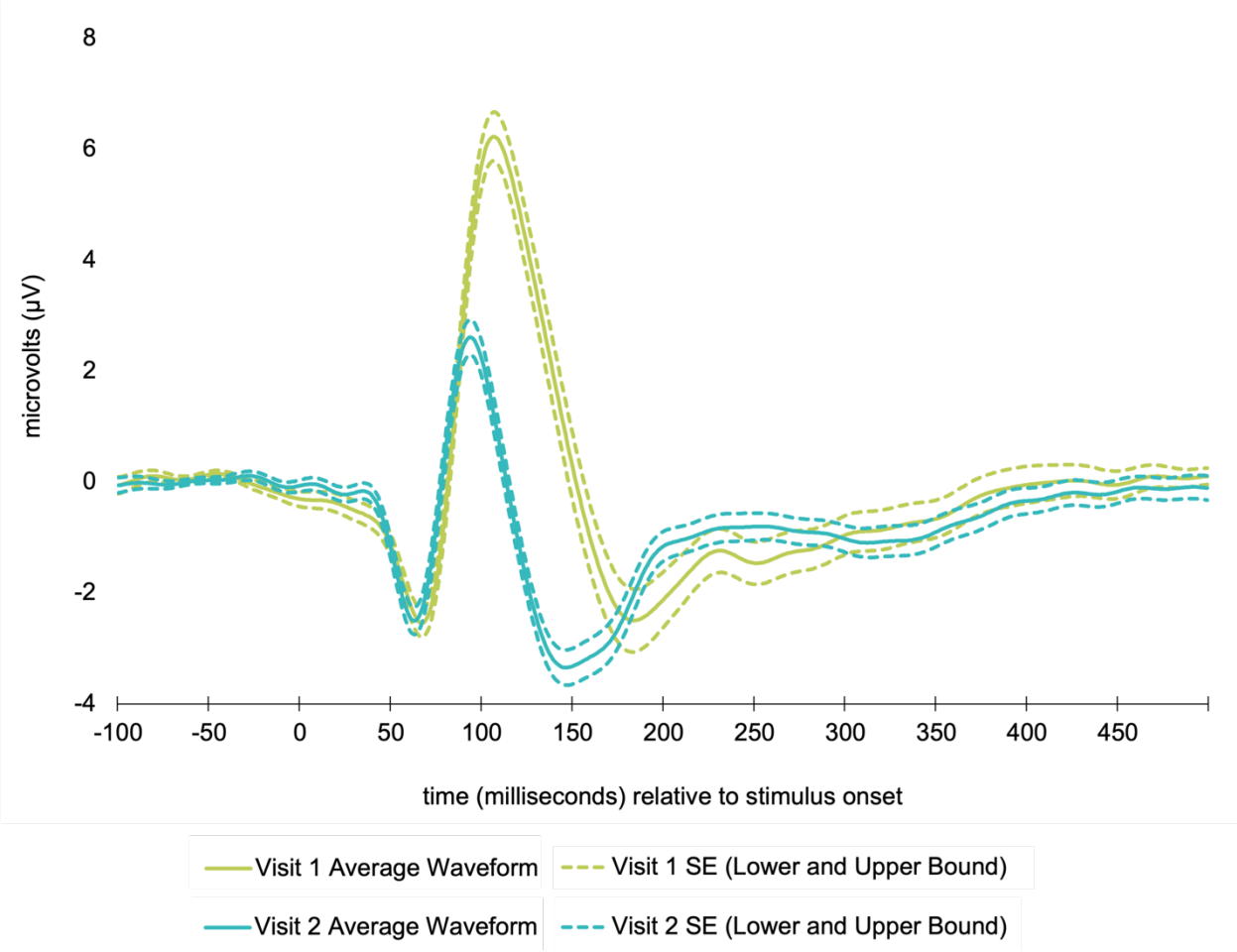


**Figure 6**

*Residual Plot for Number of Drinks per Week in Trimester 1 Predicting Corrected P1 Latency at Visit 1 (n=104)*

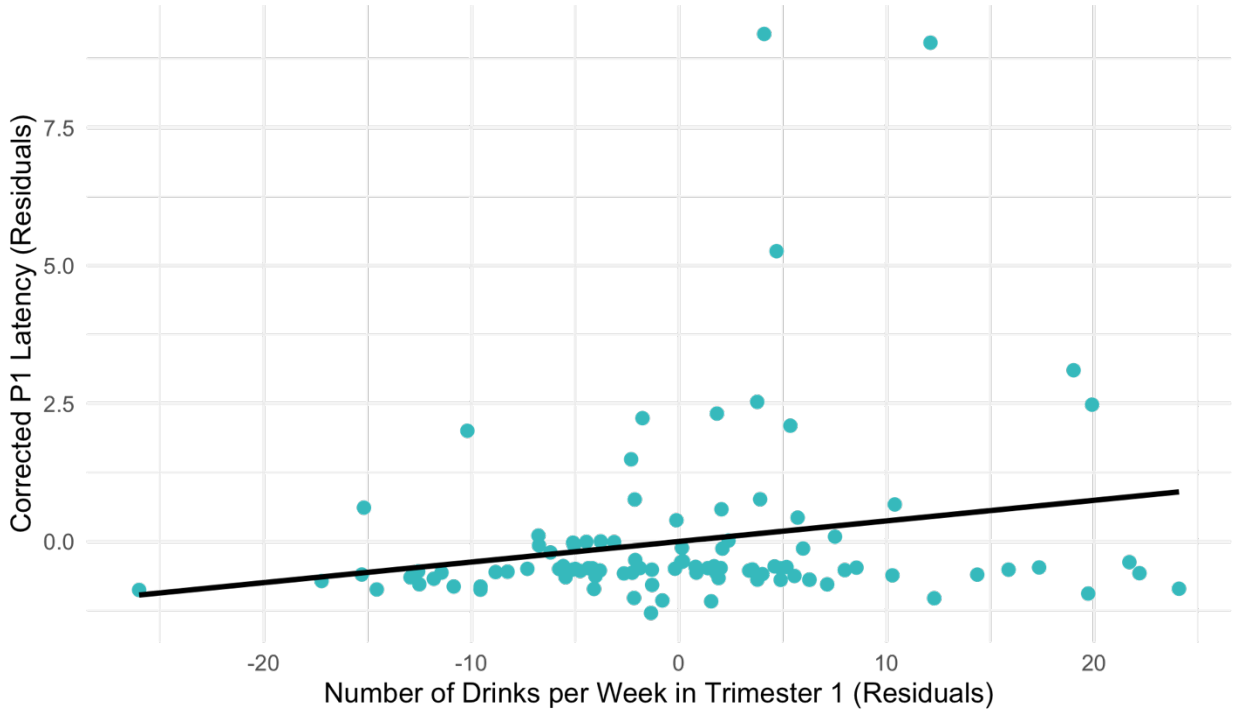


**Figure 7**

*Residual Plot for Number of Binge Episodes per Week in Trimester 1 Predicting Corrected P1 Latency at Visit 1 (n=104)*

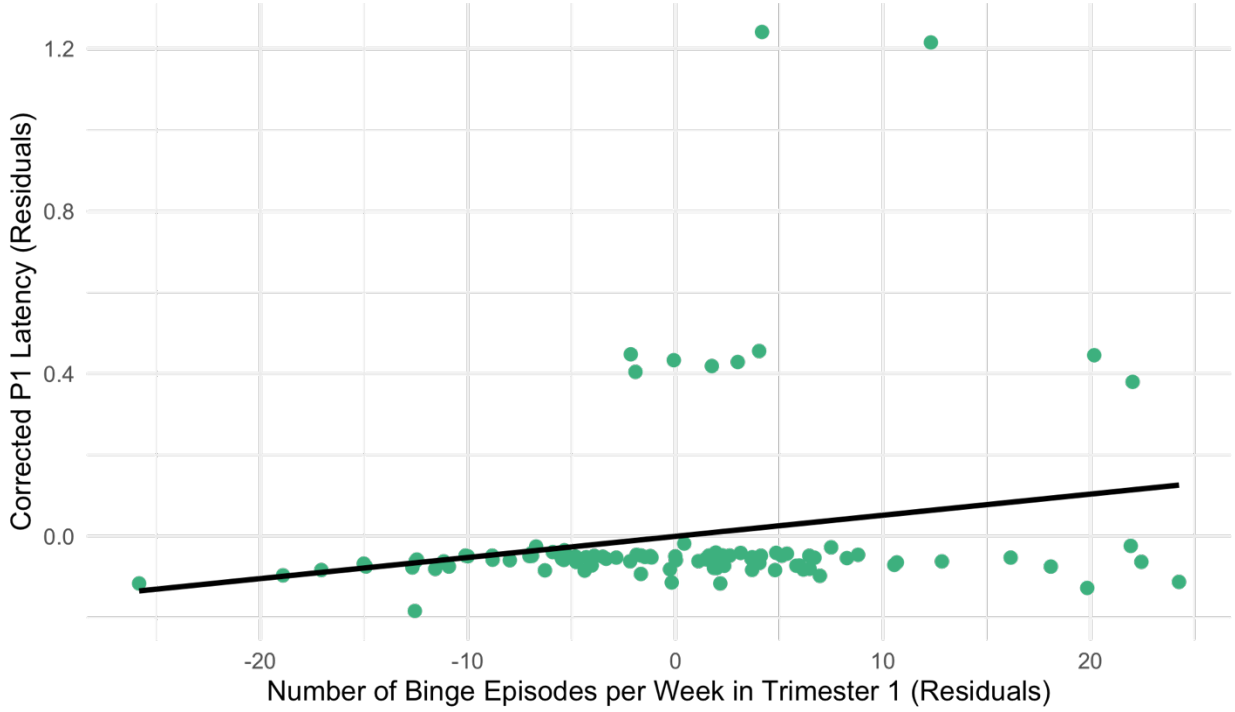


**Figure 8**

*Residual Plot for Primary Visual Cortex (V1) T1w/T2w Ratios (Myelin Estimates) Predicting P1 Latency (n=45)*