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# The association between infant visual evoked potentials and the Bayley Scales of Infant Development in Cuernavaca, Mexico

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B.S.
The University of Georgia
2007

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# Abstract

The association between infant visual evoked potentials and the Bayley Scales of Infant Development in Cuernavaca, Mexico

By Amanda Stinger

Visual evoked potentials (VEPs) have long been used in research, not only to measure infant visual acuity, but also to detect underlying differences in brain development. Little research has been conducted on how well flash VEPs actually correlate with the widely-used test of infant development, the Bayley Scales of Infant Development, second edition (BSID-II). A secondary analysis was performed on data from a large, double-blind randomized controlled trial supplementing pregnant women from 18-22 weeks gestational age to delivery with 400 mg of daily docosahexaenoic acid (DHA) (n=978 live offspring). Infant flash VEP testing was conducted at 3 and 6 months of age, and BSID-II testing performed at 12 and 18 months of age. Main predictor and outcome data were available for 686, 825, 760, and 732 infants at 3, 6, 12, and 18 months of age, respectively. Unadjusted linear regression was significant for an inverse relationship between N1, P1 and N2 latencies at 3 months and the mental developmental index (MDI) at 12 months and a positive relationship between 6-month P1 amplitude and 12-month MDI (all $p<0.05$). Six month N1 latency was also positively associated with MDI at 18 months ($p<0.05$). Coefficients were unchanged following adjustment for infant sex, birth weight, gestational age, home environment score, breastfeeding status, maternal schooling or intelligence, or SES. Using a latency cutoff of the 75$^{th}$ and 90$^{th}$ percentiles, sensitivity of 3-month VEP latencies to predict MDI at 12 months ranged from 30.8-36.3% and 15.4-16.5%, respectively; specificity ranged from 75.7-76.8% and 90.5-91.4%. Area under the receiver operating characteristic (ROC) curve for the 3-month VEP variables to predict 12-month MDI was 0.55-0.57 ($p>0.05$) and similar for prediction of 18-month MDI. The data suggest there is not a strong linear relationship between 3- and 6-month flash VEPs and the BSID-II at 12 and 18 months. The ROC analysis suggests the prognostic ability of flash VEPs to predict BSID-II scores is poor at any threshold.

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*Background*

Visual evoked potentials (VEPs) have long been used to assess visual processing, especially among infants and young children who cannot communicate visual symptoms or cooperate with standard visual testing. The test is relatively simple to administer, non-invasive, and can reveal a great deal of information about the visual processing pathways (1). In flash VEPs, a light stimulus is presented to the infant's eyes, and a cortical response is measured by electrodes placed on the child's scalp. The normal neonatal maturation of flash VEPs has been described previously by several authors (2-5), but briefly, an unspecific cortical response develops prenatally, characterized by long latency and duration, followed by a specific cortical response around 4 weeks of life, characterized by shorter latencies and succeeding positive peaks (4).

In research studies, particularly nutritional supplementation trials, VEPs have been used to detect subtle differences in visual acuity, usually as an indirect measure of differences in underlying brain maturation (6). Docosahexaenoic acid (DHA) supplementation trials often include VEPs as an outcome measure because of the necessity of DHA in fetal cognitive development; it is found in high amounts in the non-myelin membranes of the retina and brain (7). Birch and colleagues found a modest association between sweep VEPs at 4 months and later infant development (8); however, most research studies have not specifically examined the assumed relationship between VEPs and brain maturation.

In the clinical setting, VEPs have been used to project later neurodevelopmental outcomes with varying reliability in preterm and term infants (9-13). In previous research, the predictive value of VEPs tends to be especially useful in the evaluation of high-risk full-term infants, with sensitivities and specificities from around 90-100%; however, most studies conducted VEPs within the first week of life (14). There is little-to-no research examining the predictive value of VEPs performed at three and six months of age in a healthy cohort of infants. Furthermore, it is unknown how flash VEP

measures correlate with a specific developmental index, such as the Bayley Scales of Infant Development (BSID).

Prior research reveals that an infant's sex influences both VEPs and BSID scores, with shorter peak latencies and higher BSID scores observed in females (15-18). Higher infant birth weight and longer gestation are also positively associated with both VEPs and BSID scores (5, 12, 16, 17). In a study by Lundqvist-Persson and colleagues, duration of breastfeeding correlated with the MDI at 3 months in preterm infants (19). Additionally, longer duration of breastfeeding was associated with improved sweep VEP acuity at 52 weeks of life in a study by Morale, et al. (20). Other covariates that have been shown to be related solely to BSID scores include: maternal education, maternal IQ, head circumference at birth, socioeconomic status and home environment (16, 19, 21, 22).

This analysis aims to determine whether VEPs conducted at 3 and 6 months of age are correlated with later infant development, measured by the BSID-II at 12 and 18 months of age. The potential to identify abnormal infant development at a younger age could lead to earlier therapeutic interventions in this population. Furthermore, because VEPs are used in numerous areas of research, particularly in DHA supplementation trials (21, 23-26), this thesis seeks to further validate and elucidate their use in antenatal and pediatric research.

*Methods*

**Overview**

The author performed a secondary analysis on data collected from a randomized controlled trial examining the effect of prenatal DHA supplements on infant development. The trial randomly assigned pregnant women at 18-22 weeks gestation to receive a daily supplement of 400 mg of DHA or placebo until parturition. The intervention was completed in July 2007, and a total of 1,094 pregnant women were recruited. The offspring of these women (n=978) are being followed at regular intervals through 5 years of age, and numerous outcomes are collected on both the mothers and offspring. The goals of this secondary analysis are to assess 1) the relationship between VEPs conducted at 3 and 6 months of age and infant development at 12 and 18 months of age, and 2) the ability to use flash VEPs as a prognostic indicator of infant development.

**Study Population and Setting**

Between February 2005 and February 2007, pregnant women were recruited during routine prenatal care visits at the Mexican Institute of Social Security (Instituto Mexicano de Seguro Social [IMSS]) General Hospital I and three associated health clinics, all located in Cuernavaca, Mexico. Inclusion criteria included: gestation week 18-22, age 18-35 years, planned delivery at the IMSS General Hospital and to remain in the area for the next 2 years, and planned predominate breastfeeding for at least 3 months. Women were excluded from the study if they were considered a high risk pregnancy, had any lipid metabolism/absorption conditions, regularly took DHA or fish oil supplements, or used certain chronic medications (i.e. an antiepileptic). The study was approved by the Emory University Institutional Review Board and the biosafety and ethics committees of the Instituto Nacional de Salud Pública (INSP). After a thorough explanation of study details, written informed consent was obtained from each woman, and participants were allowed to withdraw from the study at any time without consequence. An external data safety committee monitored the trial for adverse events.

**Intervention**

Women were randomly assigned to receive 2 capsules of 200 mg of DHA or placebo daily from weeks 18 through 22 of gestation through delivery. Fieldworkers delivered the capsules weekly to the woman's home or workplace, and compliance was monitored by counting any remaining capsules and through interviews of the participants. All participants and members of the study team were blinded to treatment assignment throughout the intervention.

**Outcomes**

In the first phase of the trial, mothers were scheduled to bring their infants to the study headquarters in the IMSS General Hospital I at 1, 3, 6, 9, 12 and 18 months for measurement of growth and neurodevelopment outcomes (Figure 1). The outcomes relevant to this analysis are described in more detail below.

VEPs and brainstem auditory evoked potentials were performed at the 3- and 6-month postpartum visits, and the BSID-II was conducted at the 6-, 12- and 18-month postpartum visits. Prenatally and at birth, a number of other outcomes were recorded, as seen in Figure 1. However, in relation to this analysis, maternal anthropometry, maternal IQ, maternal education, and socioeconomic status were collected at the time of randomization. Social workers conducted home visits to collect information about the home environment at 6 and 12 months of age.

*Anthropometry*

Birth measurements were collected by hospital staff and retrieved from medical records. A study nurse measured infant weight in kilograms and length and head circumference in centimeters at birth and at each of the postpartum visits. A pediatric weighing scale with precision to 10 grams was used, and the scale was calibrated twice daily with a known reference weight. Infants were weighed without clothing at birth and with only minimal clothing at subsequent visits (i.e. undershirt, underpants or dry diaper). Recumbent length was measured using a baby-board (UNICEF,

Copenhagen, Denmark) and recorded to the nearest 0.1 cm. A nurse also collected the height (to the nearest 0.5 cm) and weight (to the nearest 0.1 kg) for the mothers at the time of recruitment.

*Perinatal data*

Adverse outcomes during birth and APGAR scores were retrieved from hospital records and verified through interview in a 10% sub-sample. The following adverse events during the birth were recorded: signs of prenatal fetal distress, tachycardia, bradycardia, arrhythmias, or meconium at delivery. Additionally, congenital anomalies such as trisomy, hydrocephalus, spina bifida, enzyme abnormalities, and "other anomaly" were noted.

*Visual Evoked Potentials*

A trained nurse and neurologist at the IMSS General Hospital assessed infant flash VEPs at 3 and 6 months of age. The day before testing, parents were instructed to bathe the infant with mild, fragrance-free soap, without utilizing any shampoos, gels or lotions. The infant was to sleep only from 11:00 PM until 3:00 AM on the morning of the test; parents were encouraged to keep the child awake until the nurse indicated. The last feeding was to be at least 3 hours prior to the appointment, and parents were asked to bring a prepared bottle to give immediately prior to testing. The infant was placed supine on a bed, or if uncooperative, in the mother's arms.

VEPs were recorded using the Cadwell Sierra Wave instrument (Cadwell Laboratories, Inc., Kennewick, WA, USA) from the active electrode ($O_z$) placed 1-2 centimeters above the occiput, with the reference electrode ($F_z$) positioned in the center of the forehead. The ground electrode ($C_z$) was fixed to the vertex, found by measuring the infants head from the right to the left tragus and placing the electrode at the midpoint distance on the scalp. Upon arrival to the test center, these three points were thoroughly cleaned with NuPrep™ soap. While registration data was collected, the parent or guardian was asked to give the prepared bottle and allow the infant to sleep. Once asleep, standard 60" gold cup electrodes were affixed using Ten20™ conductive paste in the appropriate locations.

Without dark adaptation or pupil dilation, VEPs were elicited by light-emitting diode (LED) stimulating goggles placed over the infant's eyes. Each eye was stimulated independently at a rate of 1.1 Hz (1.1/second) for a total of 100 stimuli. Two trials of 100 stimuli each were conducted to ensure reliability and reproducibility.

Latencies of N1, P1 and N2 (measured in milliseconds) and the amplitude of P1 (measured in microvolts) were recorded independently from the right and left eyes, and the average of the two was taken as the final result. If VEPs were unobtainable or missing from either eye, the single value was recorded as final, as research has shown little interocular asymmetry (27). The nurse also recorded the length of time the infant slept immediately prior to the VEP trial and the time the infant slept during the procedure.

*Bayley Scales of Infant Development*

The Bayley Scales of Infant Development (BSID), first developed by Dr. Nancy Bayley in 1969 (28), measures motor (fine and gross), cognitive, and behavioral development from two to thirty months of age. The second edition (BSID-II), released in 1993, renormalized the scale and expanded the age range from one to forty-two months of age (29). The BSID consists of three scales: the Mental Scale, the Psychomotor Scale, and the Behavior Rating Scale. The Mental Scale evaluates several aspects of cognitive development including memory, habituation, problem solving, early number concepts, generalization, classification, vocalizations and language. The Psychomotor Scale tests both gross and fine motor movements including those associated with rolling, crawling, sitting, standing and walking as well as imitation of hand movements and use of writing utensils. The Behavior Rating Scale aids in interpretation of the Mental and Psychomotor Scales by assessing the child's behavior during the testing.

Children receive credit for each item on the Mental and Psychomotor Scales, and the raw score is converted into a standardized score, the Mental Developmental Index (MDI) and Psychomotor Developmental Index (PDI), respectively. The mean of each index is 100 with a

standard deviation of 15 and range of 50-150. An index score on both the MDI and PDI between 85 and 114 is considered "Within Normal Limits." A score equal to or greater than 115 is defined as "Accelerated Performance," between 70 and 84 is "Mildly Delayed Performance," and 69 or below is "Significantly Delayed Performance." The test can be administered in 25-40 minutes by trained professionals. For children aged 6-18 months, the reliability of the BSID-II is expected to range between 0.84 and 0.92, and the interrater reliability is 0.96 and 0.75 for the mental and motor scales, respectively (29).

One of five trained psychologists conducted the BSID-II on infants aged 12 and 18 months at the study headquarters. The index scores were calculated by adding the total number of items for which the child receives credit and utilizing the conversion scale provided in the BSID-II manual. As described in the manual, scoring and test questions were adjusted based on gestational age and age at the time of testing.

**Measurement of Potential Confounders**

*Breastfeeding*

Adherence to breastfeeding was assessed by maternal report at the 1- and 3-month postpartum visits. Mothers were asked to answer "yes" or "no" to the following questions in Spanish: 1) Do you breastfeed your child? and 2) Do you breastfeed every time the child is hungry? Numerous other questions related to the child's alimentation were also asked but are not relevant to this analysis.

*Maternal Factors*

At the time of recruitment, a social worker administered a sociodemographic and obstetric history questionnaire that has been validated for use in pregnant women from low to medium socioeconomic status at the National Institute of Perinatology in Mexico. The first section of the assessment included questions regarding marital status, place of birth, years of schooling, occupation,

and household income and composition. A psychologist also administered the Raven's Progressive Matrices test, a 60-question test (five parts with 12 questions each) that assesses non-verbal intellectual functioning through completion of abstract patterns (30). The Standard Progressive Matrices form was used, and for each matrix answered correctly, she received a point.

*Home Environment*

Social workers conducted home visits to collect information about the home environment at 6 and 12 months of age. The Infant/Toddler HOME inventory was used, a widely-used measure composed of 45 questions divided into six subscales: parental responsivity, acceptance of child, organization of the environment, provision of appropriate materials, parental involvement, and variety of stimulation (31). The scoring is out of 45 based on the number of items for which the family received credit. An abbreviated version of this inventory was utilized at the 18-month visit; therefore, the 18-month results were not examined in this analysis.

**Statistical analysis**

Descriptive statistics were obtained and examined for the main predictor VEP variables, the outcome BSID-II scores, and all the potential covariates. A subsample including all the infants that had data on both VEP at 3 and 6 months and the BSID-II at 12 and 18 months was used to determine the baseline characteristics of the sample. I examined differences among BSID-II groups using one-way ANOVA for the continuous variables and $X^2$ and Fisher's exact tests for the categorical variables. If the p-value was significant, Tukey's method for pairwise comparisons was used to delineate differences among the continuous variables, and the MULTINOM module in SAS with the Bonferroni adjustment for multiple comparisons was utilized for categorical variables. A selection analysis was performed to ensure this subsample was similar to the overall sample population.

Unadjusted linear regression models were formulated between each predictor variable (VEP measures at 3 and 6 months) and each outcome variable (MDI and PDI at 12 and 18 months). The models were assessed for potential confounding and interaction by infant sex, birth weight, gestational age, presence of congenital anomalies and complications at delivery, breastfeeding status, maternal schooling and intelligence, home environment score, and socioeconomic status (SES); however, data were stratified by infant's sex (defined *a priori* based on prior research) whether the interaction term was significant or not. Lastly, the sensitivity and specificity of the 3-month VEP measures for poor performance on the MDI were computed, using cutoff values of the 75$^{th}$ and 90$^{th}$ percentile for VEP latencies. The area under the receiver operating characteristic (ROC) curve was used to further examine the ability of the VEP measures to predict developmental delay on the 12-month and 18-month mental scale. Statistical significance was defined as $p \leq 0.05$ and analyses were performed using SAS 9.3 (SAS Institute, Cary, NC).

*Results*

Of the 1,836 women screened for inclusion in the trial, 1,094 women were randomly assigned to receive the DHA intervention or placebo (Figure 2). Eighty-nine percent of the women completed treatment, resulting in 978 live infants. Main predictor and outcome data were available for 686, 825, 760, and 732 infants at 3, 6, 12, and 18 months of age, respectively.

To examine differences in baseline characteristics, observations with data missing for the main predictor variables (VEP measures at 3 months, n=292 and 6 months, n=153) and main outcomes (BSID-II at 12 months, n=218 and 18 months, n=246) were excluded from the analysis. Baseline characteristics for both infants and mothers overall and stratified by their MDI-at-18 month standardized score are shown in Table 1 and Table 2. Anthropometric indicators at birth did not differ between groups, and overall mean values were similar to the 50$^{th}$ percentile on the WHO growth charts (32). A large majority of infants were born at full-term (≥37 weeks of gestation); only 9.7% of infants were premature by this definition. Furthermore, the incidence of adverse perinatal events was low and did not differ among groups (Table 1). There were significantly more male infants in the "mild delay" and "significant delay" categories and fewer males in the "accelerated" category compared to male infants considered "within normal limits" (61.8% and 87.5% vs. 50.6%; P<0.001; and 33.3% vs. 50.6%; P<0.001).

The average age of mothers at the time of randomization was 26 years old, and mean BMI was ~26 kg/m$^2$ (Table 2). The average number of completed school years was 11.9, and mean score on the Raven's test was 41.2 (out of 60). Among the categories of infant MDI at 18 months, there were no significant differences in the selected maternal characteristics.

Mean scores for the MDI and PDI at 12 and 18 months were slightly less than the standardized mean of 100 (Table 3). When categorized into MDI score classifications, the PDI at 18 months was significantly different among groups; the "accelerated" group was higher than the other 3 categories and "within normal limits" higher than the "mild delay" group (102.7 accelerated vs.

94.4, 88.7, and 92.0; P<0.001). Examining VEP latencies and amplitudes at both 3 and 6 months did not reveal any significant differences among MDI groups. Overall, average VEP latencies at 6 months were shorter than average VEP latencies at 3 months, and mean amplitude at 6 months was higher than at 3 months (all p<0.001 except N2 latency, p=0.13).

*Unadjusted linear regression*

A selection analysis showed no difference in regression coefficients when comparing the subset of infants with data available for all main predictors and outcomes (n=471) to the full dataset; therefore, all available data were used to increase precision (selection analysis not shown). There was a significant inverse relationship between the latency of N1, P1 and N2 at 3 months and the MDI at 12 months (Table 4). The amplitude of P1 at 6 months, but not at 3 months, showed a small but significant positive association with MDI at 12 months (β=0.11; 95% CI: 0.003, 0.21; Table 4). Visual evoked potential P1 latency at 3 months was borderline significant for a negative relationship with PDI at 12 months (Table 5). At 18 months, for both MDI and PDI, the only significant association was a positive relationship between N1 latency at 6 months and MDI (Table 4 & Table 5).

*Subset analysis*

To further examine whether VEP might be more strongly associated with BSID-II in certain populations, I examined the relationship among particular subsets of the data, based on associations found in prior research. There were no significant interactions by infant's sex or socioeconomic status. Only one interaction was significant by birth weight: MDI at 12 months on N2 latency at 6 months (p=0.02). The interaction term was significant for gestational age in the relationship between MDI at 12 months and P1 amplitude at 3 months (p=0.03); therefore, stratified models were also formulated (Table 6). The regression coefficient was more negative among preterm infants compared to term infants in the relationship between MDI at 12 months and N1 and P1 latency at 3 months; although, only one of the coefficients remained significant (N1: -0.15 preterm vs. -0.04 term;

P1: -0.13 preterm vs. -0.05 term; Table 6). Home environment score at 12 months also significantly interacted in the regression of MDI at 12 months on P1 amplitude and N1 latency at 3 months and in the regression of MDI at 18 months on P1 amplitude at 3 months ($p<0.05$ for both amplitude variables and $p=0.01$ for N1 latency). Stratified models were notable for a more negative regression coefficient among infants in the highest tertile of home environment score compared to the lowest tertile for the regression of MDI at 12 months on N1 and P1 latency at 3 months (-0.16 vs. -0.01 and -0.15 vs. -0.06, respectively; Table 7).

Although the interaction by infant sex was not significant in this analysis, models were stratified by sex to look at overall trend. Among male infants, the regression coefficients for MDI at 12 months on N1 and P1 latencies were slightly more negative compared to female infants (N1 latency: -0.08 for males vs. -0.005 for females; P1 latency: -0.08 for males vs. -0.04 for females; Table 8). In the subset of infants with congenital anomalies (including trisomy, hydrocephalus, spina bifida, enzymatic abnormalities or "other"), significant inverse relationships emerged in the regression of MDI 12 on P1 and N2 latency at 6 months; these variables were not significantly associated in the full dataset (Table 9). Furthermore, among the infants with delivery complications (including signs of fetal distress, tachycardia, bradycardia, or meconium at delivery), a new significant positive association emerged between P1 latency at 6 months and MDI at 18 months (Table 10).

*Adjustment for potential confounders*

Because MDI at 12 months and VEP N1 and P1 latency at 3 months had the strongest correlations, Pearson partial correlations were only calculated for these variables (Table 11). The potential covariates were all based on current literature or on associations noted in this dataset and included: interviewer 83, birth weight, infant's sex, home environment summary score, maternal Raven score, maternal schooling, breastfeeding status, gestational age at birth, and SES score. Interviewer code was necessary to include because interviewer number 83 gave significantly higher scores on the BSID-II than the other interviewers (Mean MDI at 12 months 100.7 vs. 92.7;

P<0.001). There were no notable differences in the partial correlation coefficients compared to the unadjusted correlation (Table 11). When the effect of all other variables was controlled for at once, the adjusted correlation was slightly less than the unadjusted and no longer significant (MDI at 12 months and N1 latency at 3 months:-0.10 vs. -0.08; MDI at 12 months and P1 latency at 3 months: -0.12 vs. -0.09).

*Predictive value*

To my knowledge, there are no set norms for flash VEP values in this Mexican population. To calculate sensitivity and specificity, a prolonged VEP latency was defined as a latency of greater than or equal to the 75$^{th}$ percentile in this sample. At this cutoff level, the sensitivity of any of the latency variables at 3 months (N1, P1 or N2) for predicting delay on the MDI at 12 months (defined by a score less than 85) ranged from 30.8% to 36.3% with specificities ranging from 75.7% to 76.8%. The sensitivity of predicting delay on the MDI at 18 months was slightly lower, ranging from 20.8% to 26.4%, with similar specificities of 75.3% to 75.7%. Using a VEP prolonged cutoff of the 90$^{th}$ percentile revealed lower sensitivities and higher specificities. For MDI at 12 months, sensitivities ranged from 15.4% to 16.5% and specificities from 90.5% to 91.4%. Similarly for predicting MDI at 18 months, sensitivities were from 8.3% to 11.1% with specificities ranging from 90.1% to 90.7%.

The area under the ROC curve for the 3-month VEP latency measures to predict the MDI at 12 months was 0.55 (95% confidence interval: 0.48, 0.61) for N1 latency, 0.55 (95% confidence interval: 0.48, 0.62) for P1 latency, 0.57 (95% confidence interval: 0.50, 0.63) for N2 latency, and 0.56 (95% confidence interval: 0.49, 0.62) for P1 amplitude (Table 12 & Figure 3). Slightly lower values were calculated in the area under the ROC curve to predict the MDI at 18 months: 0.49 (95% confidence interval: 0.41, 0.56) for N1 latency, 0.49 (95% confidence interval 0.42, 0.57) for P1 latency, 0.50 (95% confidence interval: 0.43, 0.58) for N2 latency, and 0.52 (95% confidence interval: 0.45, 0.58). Area under the ROC curve for 6-month VEP measures to predict MDI at 12 and 18 months were similarly close to 0.5 and not significant at $p< 0.05$ (Table 12).

*Discussion*

This was a secondary data analysis of a large, double-blinded randomized controlled trial evaluating the effect of DHA supplementation during pregnancy on infant development in Cuernavaca, Mexico. Overall, the data suggest there is not a strong linear correlation between infant flash VEPs at 3 or 6 months with Bayley Scales of Infant Development scores at 12 or 18 months; however, the tendency toward an inverse association was in accordance with prior expectations. Based on previous studies, it was hypothesized that shorter latencies would be associated with higher BSID-II scores. The relationship between VEPs and BSID-II scores remained weak among certain high-risk subgroups of the population. Furthermore, the ROC analysis suggests that the prognostic ability of flash VEPs at this age group is poor at any threshold.

A similar analysis examining the relationship of VEPs and BSID-II scores was performed by Birch and colleagues in conjunction with a dietary LCPUFA supplementation trial of infants (8). The authors of this study found a modest inverse relationship between logMAR (log of the minimum angle of resolution) sweep VEP acuities at 4 months and MDI and PDI at 18 months of age ($r=-0.37$ and $r=-0.33$, respectively). Sweep VEP (steady-state VEP) is an entirely different procedure for measuring visual acuity and is often thought to be more reliable than flash VEP (12, 14). In this thesis, there was also a tendency towards an inverse association (better visual acuity is associated with higher BSID-II scores), but the association was substantially lower in magnitude. The significant differences in VEP procedure, however, may preclude direct comparison of these studies.

The potential predictive value of flash VEPs for neurodevelopmental outcome has been studied extensively in preterm infants and in term infants with birth asphyxia. A number of studies have shown flash VEPs conducted in the first week of life to be sensitive and specific for later neurodevelopmental outcome in full-term infants with birth asphyxia (10, 12, 13). Studies on the predictive value in preterm infants, however, have revealed mixed results (14). The definition of an abnormal VEP varies across studies and includes: an absent potential, prolonged latency or missing

components based on normative data, and an unusual waveform. In this analysis, cutoffs of the 75th and 90th percentile for the 3-month VEP latency were used to define an abnormal VEP and to calculate the sensitivity and specificity for predicting MDI scores at 12 and 18 months. Sensitivity was poor, ranging from ~8-16%; however, specificity was better, ranging from ~75-91%.

The difference in predictive capability in this study is likely multifactorial. First, selection bias may have occurred in previous studies because infants were at high risk for developmental delay, severe neurological disease, and death, either due to prematurity or asphyxiation at birth. Flash VEPs were performed within the first three weeks of life, usually within the first 3 days of life. The definition of an abnormal flash VEP was much broader than simply a prolonged latency, and sample sizes were small (ranging from 20-120 infants). Heterogeneity of studies examining the prognostic ability of flash VEPs is one of the reasons consensus on the clinical utility of this test has not been reached.

Significant interactions were found by gestational age, complications at delivery, congenital anomalies, and home environment summary score. Although results were no longer significant due to a lack of power, the regression coefficients are three times higher among preterm infants compared to the full sample. This suggests that flash VEPs may be more useful in preterm infants. The same trend was observed among infants with complications at delivery and congenital anomalies, which is in congruence with previous studies. The results after stratifying by home environment summary score are difficult to explain. In the highest tertile, the relationship was three times more negative than the full sample, which is in contrast to the other findings. If flash VEPs are more predictive in higher-risk infants, then one would expect the lowest tertile to have a stronger relationship between VEPs and BSID-II scores. However, since a poor home environment is an independent predictor of lower MDI scores, perhaps by eliminating the effect of this environment, flash VEPs become more predictive.

The utility of using 3-month flash VEPs to predict neurodevelopmental outcome was further rejected by the ROC analysis. The mean areas under the ROC curve were all close to 0.5 and included 0.5 in the 95% confidence interval. These results indicate that each gain in sensitivity is balanced by an equal loss in specificity, and predictive ability would be similar to flipping a coin. Although the test for heterogeneity was not significant by gender, the area under the ROC curve was slightly higher (~0.6) among males, and the confidence interval no longer included 0.5. This result suggests that VEPs may be more predictive among male infants and deserves further study.

It is interesting that three of the five significant relationships were between the VEP latencies at 3 months and the MDI at 12 months, and only amplitude was significant in the association of 6-month VEP and MDI at 12 months. Perhaps children with VEP deficits at 3 months have "caught up" by the 6-month testing. Similarly, if 3-month VEPs are predicting early cognitive deficits, this delay could be present at 12 months, but improved by 18 months. Three to 18 months is a period of dramatic growth in infant cognition, so changes within a short time period are to be expected.

In this study, other variables such as infant's sex, gestational age, and home environment score appear to be better predictors of BSID-II scores at 12 and 18 months; therefore, is flash VEP a useful additional piece of information? In fact, when these variables are jointly controlled for in the model, VEP measures are no longer significant. These results suggest that in the clinical setting, measuring flash VEPs outside of the neonatal period does not aid in prognosis, but for evaluating small differences in brain maturation in the research setting, VEPs may still be applicable.

In conclusion, there appears to be no relationship between infant flash VEPs conducted at 3 and 6 months of age with later infant development. One plausible explanation for this finding is that flash VEPs are measuring a distinct aspect of development than the BSID-II. Flash VEPs conducted at 3 or 6 months of age in a healthy cohort of infants should not be used to predict infant development at 12 and 18 months of age.

*Strengths and Limitations*

The strengths of this study include a large sample size and excellent follow-up. The sample size of up to 800 infants with flash VEP data is one of the largest datasets known: up to 8 times larger than previous studies (14, 33). The participants came from a low-to-middle income community in a developing nation where VEP norms are not readily available. Relatively few studies have been conducted on flash visual evoked potentials at 3 and 6 months of age, as most VEP studies are performed at birth or within the first month of life; therefore, in the future, the data from this study could be used to develop normative values for this age range and population. Additionally, the BSID-II is one of the most widely-used tools for measuring child development and was performed by psychologists trained in this technique. To my knowledge, no other study examining the correlation of flash VEPs outside the first week of life with the Bayley Scales of Infant Development has been conducted, particularly in a low-risk population.

This thesis analysis was not without limitations. First, VEP is a very specific measure of visual function and the BSID-II is a global measure of infant development; therefore, if there is a true relationship between VEPs and infant development, differences in VEP latencies may not be adequately accounted for in BSID-II scores. Furthermore, in clinical situations, VEP is mainly used for assessing visual acuity, and disorders of the visual pathway, such as delayed visual maturation, amblyopia, lesions of the afferent visual pathway, and cortical blindness (12), and the utility in the general population has not been demonstrated.

One-hundred and eighty-one infants were lost to follow-up for unknown reasons. It is conceivable that infants who had either 3- or 6-month VEP measures and did not return for BSID-II testing had poorer outcomes such as neurodevelopmental disability. Scores from these infants could have improved the predictive ability of VEP; although, loss of follow-up was fairly low (13.7% loss from 3 to 12 months; 8.7% additional loss from 6 to 12 months), and regression coefficients likely would not have been altered by a large margin.

Although BSID-II scores have high interrater reliability (0.96 and 0.75 for the mental and motor scales respectively), one interviewer had a significantly higher average score on both the MDI and PDI at 12 and 18 months. Fortunately, this did not seem to greatly affect the correlation coefficient, as evidenced by partial correlations. Several studies have critiqued the stability of the BSID-II over time (34), and indeed in this study, the correlations between the MDI at 12 and 18 months ($r=0.28$) and PDI at 12 and 18 months ($r=0.30$) are fairly weak. The BSID-II is considered more stable and reliable in children with developmental delay (35), but the children in this study were from a healthy population, with very few risk factors for developmental delay, such as preterm birth, low birth weight and other birth complications. As a result, very few children were classified as delayed (mild or significant delay: 12.3%; significant delay alone: 1.2%). In general, developmental test scores become more reliable as age increases, suggesting that the 18-month measure would be more valid than the 12-month score; however, all significant correlations were found with the MDI at 12 months.

Lastly, flash VEPs were conducted in this randomized controlled trial with the primary aim of measuring differences in visual acuity among infants whose mothers received DHA vs. placebo during pregnancy, not to examine the relationship between VEP and later infant development. If the purpose was solely for this analysis, the procedure for collecting and recording VEPs should have been more detailed and the definition for an abnormal VEP clearly defined before the trial began.

*Future Directions*

VEPs are an important tool in the evaluation of early infant visual acuity and cognition in research studies. It is still unclear what specific area of cognition VEPs are measuring and how this translates into global infant development. This uncertainty arises for several reasons, including: the complexity of infant brain maturation, flash VEP variability among populations, heterogeneity between previous studies, and limited objective testing to assess child development (particularly tests that focus on minute differences in development). The third edition of the Bayley Scales of Infant

Development, released in 2005, divides the MDI into cognitive and language scales, which may help to further delineate what VEP latencies are measuring. A study of a healthy cohort of infants that examines the relationship between flash VEPs and the new version of the Bayley Scales should be conducted. Furthermore, additional studies that that are specifically designed to examine the various VEP techniques and their relationship to assorted measures of infant development are necessary.

Despite the fact that flash VEPs are commonly used as an outcome measure in nutritional research, norms have to be established for each population under study. Researchers should take this into account when creating a nutritional study with VEP as the outcome; if one is looking for clinically relevant differences in development, normative values should be available. Furthermore, if a stronger relationship between a VEP method and a developmental outcome measure is found, VEP values could be used to predict developmental outcome at an earlier age, reducing the need for long-term follow-up in research studies.

Because research has demonstrated the importance of early detection and intervention, the American Academy of Pediatrics recommends surveillance for developmental concerns at every well-child visit and the use of a validated screening test at the 9-, 18- and 30-month visits (36). Screening programs aimed at discovering disabilities at an early age have focused on parental questionnaires, often combined with direct observation by a physician or other trained professional; thus, most disabilities are a clinical diagnosis. In contrast, visual evoked potentials (VEPs) are an objective, potentially valuable prognostic tool that can be conducted on neonates from the first day of life. The use of VEPs in a clinical setting has mainly been limited to detecting visual abnormalities. There is great potential for using VEPs as a clinical tool for detecting delay at an earlier age. Further research on the predictive ability of VEPs should focus on application and feasibility in a clinical setting and be conducted on a developmentally normal cohort of infants.

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*Tables*

**Table 1. Selected characteristics of infants overall and stratified by the Mental Development Index (MDI) of the Bayley Scales of Infant Development-II at 18 months of age (n=471)**

| Characteristic | Overall n=471 | MDI at 18 mos[a] Accelerated n=15 | Within Normal Limits n=393 | Mild Delay n=55 | Significant Delay n=8 | P-value[b] |
|---|---|---|---|---|---|---|
| | | | Mean (SD) | | | |
| **Anthropometric Indicators, Birth** | | | | | | |
| Weight, kg | 3.2 (0.4) | 3.4 (0.3) | 3.2 (0.4) | 3.2 (0.5) | 3.2 (0.2) | 0.52 |
| Length, cm | 50.4 (2.3) | 50.9 (1.4) | 50.5 (2.2) | 50.0 (2.8) | 50.4 (0.7) | 0.43 |
| Head circumference, cm (n=427) | 34.3 (1.6) | 34.9 (1.3) | 34.3 (1.6) | 34.3 (1.4) | 34.7 (1.9) | 0.56 |
| **Anthropometric Indicators, 18 months of age (n=468)** | | | | | | |
| Weight, kg | 10.4 (1.2) | 11.1 (1.1) | 10.4 (1.2) | 10.5 (1.1) | 10.2 (0.9) | 0.13 |
| Length, cm | 79.5 (2.6) | 81.1 (2.2) | 79.5 (2.7) | 79.5 (2.58) | 79.0 (2.1) | 0.12 |
| Head circumference, cm | 47.0 (1.4) | 47.5 (1.4) | 47.0 (1.4) | 47.0 (1.3) | 46.7 (1.5) | 0.57 |
| | | | n (%) | | | |
| **Child sex, male** | 245 (52.0) | 5 (33.3) | 199 (50.6) | 34 (61.8) | 7 (87.5) | 0.04[cd] |
| **Gestational age at birth, weeks (n=469)** | 39.1 (1.7) | 39.2 (1.2) | 39.1 (1.7) | 38.5 (2.0) | 39.2 (1.1) | 0.11 |
| **Breastfed at 1 month (n=435)** | 412 (87.5) | 13 (92.9) | 345 (95.3) | 47 (92.2) | 7 (87.5) | 0.28[c] |
| **Breastfed at 3 months (n=470)** | 385 (81.7) | 7 (50.0) | 324 (82.4) | 48 (87.3) | 6 (75.0) | 0.02[cd] |
| **Perinatal data Concerning features (n=470)** | | | | | | |
| **Signs of prenatal fetal distress** | 37 (7.9) | 3 (8.1) | 32 (86.5) | 2 (5.4) | 0 (0.0) | 0.20 |
| **Tachycardia** | 19 (4.0) | 1 (5.3) | 17 (89.5) | 1 (5.3) | 0 (0.0) | 0.65 |
| **Bradycardia** | 7 (1.5) | 0 (0.0) | 6 (85.7) | 1 (14.3) | 0 (0.0) | 0.72 |
| **Arrhythmia** | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | -- |

| | | | | | | |
|---|---|---|---|---|---|---|
| **Meconium** | 36 (7.6) | 1 (2.8) | 31 (86.1) | 3 (8.3) | 1 (2.8) | 0.77 |
| **Congenital anomaly present** | 18 (3.8) | 0 (0.0) | 16 (88.9) | 2 (11.1) | 0 (0.0) | 1.00 |
| **APGAR, 1 min (n=457)** | 8.2 (0.7) | 8.1 (1.2) | 8.2 (0.6) | 8.2 (0.9) | 8.3 (0.5) | 0.85 |
| **APGAR, 5 min (n=457)** | 9.0 (0.3) | 8.7 (0.8) | 9.0 (0.2) | 8.9 (0.5) | 9.0 (0.0) | 0.003[e] |

[a]"Accelerated": MDI>115; "Within Normal Limits": 85≤MDI≤115; "Mild Delay": 70≤MDI<85; and "Significant Delay": MDI<70

[b]P-values were calculated by analysis of variance test of means or by $X^2$ test for equality of proportions.

[c] P-value calculated by Fisher's exact test for equality of proportions.

[d] Significant differences between "Accelerated" and both "Within Normal Limits" and "Mild Delay" and between "Within Normal Limits" and both "Mild Delay" and "Significant Delay" and between "Mild Delay" and "Significant Delay."

[e]Tukey significant differences between "Accelerated" and "Within Normal Limits."

**Table 2. Selected characteristics of women overall and stratified by the child's Mental Development Index (MDI) of the Bayley Scales of Infant Development-II at 18 months of age (n=471)**

| Characteristic | Overall n=471 | MDI[a] Accelerated n=15 | Within Normal Limits n=393 | Mild Delay n=55 | Significant Delay n=8 | P-value[b] |
|---|---|---|---|---|---|---|
| | | | Mean (SD) | | | |
| **Age at randomization, years** | 26.5 (4.7) | 26.1 (5.1) | 26.6 (4.8) | 26.9 (4.3) | 23.7 (5.2) | 0.36 |
| **Weight, kg** | 62.6 (10.8) | 61.2 (11.4) | 62.6 (10.5) | 64.3 (12.2) | 57.2 (13.8) | 0.32 |
| **Height, cm** | 154.0 (5.7) | 154.5 (4.1) | 155.2 (5.8) | 153.6 (0.2) | 153.4 (5.9) | 0.20 |
| **Raven Summary Score** | 41.2 (8.9) | 40.5 (10.4) | 41.5 (8.7) | 39.6 (10.2) | 41.9 (5.9) | 0.51 |
| **Schooling, # of total years (n=470)** | 11.9 (3.5) | 13.1 (2.5) | 12.0 (3.6) | 11.0 (3.4) | 11.4 (4.2) | 0.10 |
| **Home environment summary score at 12 mos (n=354)** | 38.0 (3.7) | 37.6 (5.0) | 38.1 (3.6) | 37.4 (3.6) | 34.8 (6.7) | 0.17 |
| | | | n (%) | | | |
| **SES, lowest tertile** | 163 (34.6) | 5 (33.3) | 133 (33.8) | 22 (40.0) | 3 (37.5) | 0.96[c] |

[a] "Accelerated": MDI>115; "Within Normal Limits": 85≤MDI≤115; "Mild Delay": 70≤MDI<85; and "Significant Delay": MDI<70

[b] P-values were calculated by analysis of variance test of means or by $X^2$ test for equality of proportions.

[c] P-value calculated by Fisher's exact test for equality of proportions.

**Table 3. Main predictor and outcome data for infants overall and stratified by the Mental Development Index (MDI) of the Bayley Scales of Infant Development-II at 18 months of age (n=471)**

| Characteristic | MDI[a] | | | | | |
|---|---|---|---|---|---|---|
| | **Overall** n=471 | **Accelerated** n=15 | **Within Normal Limits** n=393 | **Mild Delay** n=55 | **Significant Delay** n=8 | P-value[b] |
| | Mean (SD) | | | | | |
| **Mental Development Index Score, 12m** | 94.9 (9.3) | 98.0 (13.7) | 95.3 (9.0) | 92.8 (9.7) | 88.0 (10.0) | 0.02[c] |
| **Psychomotor Development Index Score, 12m (n=469)** | 90.3 (8.5) | 92.2 (10.2) | 90.3 (8.4) | 89.4 (8.9) | 89.5 (4.5) | 0.69 |
| **Mental Development Index Score, 18m** | 94.6 (10.3) | 115-121 | 85-113 | 77-83 | 55-69 | -- |
| **Psychomotor Development Index Score, 18m** | 93.9 (9.1) | 102.7 (4.9) | 94.4 (8.9) | 88.7 (9.8) | 92.0 (4.1) | <.001[d] |
| **Visual Evoked Potentials, 3 months** | | | | | | |
| Latency, N1 | 94.3 (17.2) | 88.7 (16.3) | 94.4 (16.4) | 95.5 (22.4) | 94.1 (18.13) | 0.61 |
| Latency, P1 | 126.4 (18.0) | 123.5 (19.8) | 126.3 (17.6) | 127.3 (20.5) | 128.3 (19.1) | 0.89 |
| Amplitude, P1 | 8.1 (5.89) | 7.7 (5.5) | 8.2 (6.0) | 7.3 (4.2) | 11.5 (8.4) | 0.29 |
| Latency, N2 | 156.0 (23.8) | 155.0 (26.5) | 156.0 (23.5) | 154.8 (25.7) | 164.4 (22.9) | 0.76 |
| **Visual Evoked Potentials, 6 months** | | | | | | |
| Latency, N1 | 90.7 (14.8) | 87.9 (11.0) | 91.4 (14.9) | 87.6 (14.5) | 86.4 (19.0) | 0.22 |
| Latency, P1 | 122.9 (14.0) | 123.3 (15.23) | 123.2 (13.9) | 120.5 (14.4) | 125.4 (13.3) | 0.56 |
| Amplitude, P1 | 11.3 (6.7) | 10.0 (5.1) | 11.2 (6.8) | 12.2 (6.3) | 13.0 (6.1) | 0.58 |
| Latency, N2 | 155.1 (19.3) | 156.4 (20.2) | 155.4 (19.3) | 152.5 (20.1) | 155.9 (15.4) | 0.77 |

[a]"Accelerated": MDI>115; "Within Normal Limits": 85≤MDI≤115; "Mild Delay": 70≤MDI<85; and "Significant Delay": MDI<70

[b] P-values were calculated by analysis of variance test of means.

[c] No significant differences using Tukey's method of all-pairwise comparisons due to violation of the homogeneity of variances assumption.

[d]Tukey significant differences between "Accelerated" and each other category and between "Within Normal Limits" and "Mild Delay."

**Table 4. Unadjusted linear regression coefficients and 95% confidence intervals between each potential predictor VEP variable and MDI at 12 and 18 months**

| | MDI at 12 months | | | MDI at 18 months | | |
|---|---|---|---|---|---|---|
| **VEP, 3 months (n=565 at 12 mos, n=545 at 18 mos)** | β | 95% CI | | β | 95% CI | |
| Latency, N1, ms | **-0.05** | **-0.10** | **-0.01** | -0.01 | -0.06 | 0.04 |
| Latency, P1, ms | **-0.06** | **-0.10** | **-0.02** | 0.002 | -0.05 | 0.05 |
| Amplitude, P1, μV | -0.03 | -0.16 | 0.10 | 0.03 | -0.12 | 0.17 |
| Latency, N2. ms | **-0.04** | **-0.07** | **-0.01** | 0.01 | -0.03 | 0.04 |
| **VEP, 6 months (n=702 at 12 mos, n=675 at 18 mos)** | | | | | | |
| Latency, N1, ms | 0.04 | -0.01 | 0.08 | **0.08** | **0.03** | **0.13** |
| Latency, P1, ms | 0.04 | -0.01 | 0.09 | 0.05 | -0.003 | 0.10 |
| Amplitude, P1, μV | **0.11** | **0.003** | **0.21** | -0.01 | -0.12 | 0.09 |
| Latency, N2, ms | 0.03 | -0.005 | 0.07 | 0.01 | -0.03 | 0.05 |

**Bold**= significant at $p<0.05$

Abbreviations: CI, confidence interval; MDI, mental developmental index; ms, milliseconds; VEP, visual evoked potential; μV, microvolt

**Table 5. Unadjusted linear regression coefficients and 95% confidence intervals between each potential predictor VEP variable and PDI at 12 and 18 months**

| | PDI at 12 months | | | PDI at 18 months | | |
|---|---|---|---|---|---|---|
| **VEP, 3 months (n=561 at 12 mos, n=545 at 18 mos)** | β | 95% CI | | β | 95% CI | |
| Latency, N1, ms | -0.03 | -0.08 | 0.01 | -0.002 | -0.05 | 0.05 |
| Latency, P1, ms | **-0.04** | **-0.08** | **-0.001** | 0.02 | -0.02 | 0.07 |
| Amplitude, P1, μV | -0.03 | -0.15 | 0.09 | -0.03 | -0.16 | 0.10 |
| Latency, N2, ms | -0.03 | -0.05 | 0.005 | 0.03 | 0.00 | 0.07 |
| **VEP, 6 months (n=697 at 12 mos, n=675 at 18 mos)** | | | | | | |
| Latency, N1, ms | -0.01 | -0.06 | 0.04 | 0.03 | -0.02 | 0.08 |
| Latency, P1, ms | -0.0004 | -0.05 | 0.05 | 0.02 | -0.03 | 0.07 |
| Amplitude, P1, μV | 0.06 | -0.04 | 0.16 | 0.04 | -0.06 | 0.15 |
| Latency, N2, ms | -0.001 | -0.04 | 0.03 | -0.01 | -0.05 | 0.02 |

**Bold**= significant at $p<0.05$

Abbreviations: CI, confidence interval; PDI, psychomotor developmental index; ms, milliseconds; VEP, visual evoked potential; μV, microvolt

**Table 6. Unadjusted linear regression of MDI at 12 and 18 months on VEP at 3 and 6 months among preterm and term infants.**

| | Preterm infants (gestational age<37 weeks) | | | | | | Term infants (gestational age≥37 weeks) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | MDI 12 (n=50 at 3 mos; n=63 at 6 mos) | | | MDI 18 (n=51 at 3 mos; n=61 at 6 mos) | | | MDI 12 (n=515 at 3 mos; n=639 at 6 mos) | | | MDI 18 (n=494 at 3 mos; n=614 at 6 mos) | | |
| | β | 95% CI | | β | 95% CI | | β | 95% CI | | β | 95% CI | |
| **VEP, 3 months** | | | | | | | | | | | | |
| Latency, N1, ms | -0.15 | -0.33 | 0.02 | 0.07 | -0.10 | 0.23 | -0.04 | -0.09 | 0.01 | -0.01 | -0.07 | 0.04 |
| Latency, P1, ms | -0.13 | -0.28 | 0.01 | 0.05 | -0.09 | 0.18 | **-0.05** | **-0.09** | **-0.001** | 0.002 | -0.05 | 0.06 |
| Amplitude, P1, μV | 0.21 | -0.27 | 0.69 | 0.06 | -0.38 | 0.49 | -0.06 | -0.19 | 0.08 | 0.02 | -0.13 | 0.17 |
| Latency, N2, ms | -0.08 | -0.19 | 0.03 | 0.03 | -0.07 | 0.13 | -0.03 | -0.07 | 0.00 | 0.01 | -0.03 | 0.05 |
| **VEP, 6 months** | | | | | | | | | | | | |
| Latency, N1, ms | -0.02 | -0.18 | 0.14 | **0.16** | **0.04** | **0.27** | 0.04 | -0.01 | 0.09 | **0.07** | **0.02** | **0.12** |
| Latency, P1, ms | 0.02 | -0.13 | 0.17 | 0.06 | -0.08 | 0.19 | 0.04 | -0.02 | 0.09 | 0.04 | -0.01 | 0.10 |
| Amplitude, P1, μV | 0.37 | -0.07 | 0.81 | -0.10 | -0.46 | 0.27 | 0.09 | -0.02 | 0.19 | -0.01 | -0.12 | 0.11 |
| Latency, N2, ms | 0.005 | -0.10 | 0.11 | -0.04 | -0.13 | 0.06 | 0.03 | -0.01 | 0.07 | 0.02 | -0.02 | 0.06 |

**Bold**= significant at $p<0.05$

Abbreviations: CI, confidence interval; MDI, mental developmental index; VEP, visual evoked potential

**Table 7. Unadjusted linear regression of MDI at 12 and 18 months on VEP at 3 and 6 months, stratified by home environment score at 12 months.**

| | Home environment score in lowest tertile (score≤36) | | | | | | Home environment score in highest tertile (score>39) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | MDI 12 (n=119 at 3 mos; n=214 at 6 mos) | | | MDI 18 (n=103 at 3 mos; n=186 at 6 mos) | | | MDI 12 (n=157 at 3 mos; n=169 at 6 mos) | | | MDI 18 (n=142 at 3 mos; n=150 at 6 mos) | | |
| | β | 95% CI | | β | 95% CI | | β | 95% CI | | β | 95% CI | |
| **VEP, 3 months** | | | | | | | | | | | | |
| Latency, N1, ms | -0.01 | -0.11 | 0.09 | -0.06 | -0.19 | 0.06 | **-0.16** | **-0.24** | **-0.07** | -0.04 | -0.12 | 0.05 |
| Latency, P1, ms | -0.06 | -0.16 | 0.03 | -0.08 | -0.20 | 0.04 | **-0.15** | **-0.24** | **-0.06** | 0.01 | -0.09 | 0.10 |
| Amplitude, P1, μV | -0.24 | -0.52 | 0.04 | -0.31 | -0.68 | 0.06 | -0.03 | -0.32 | 0.25 | 0.16 | -0.13 | 0.44 |
| Latency, N2, ms | **-0.08** | **-0.15** | **-0.01** | -0.06 | -0.15 | 0.02 | **-0.07** | **-0.14** | **-0.003** | 0.04 | -0.03 | 0.11 |
| **VEP, 6 months** | | | | | | | | | | | | |
| Latency, N1, ms | 0.01 | -0.09 | 0.10 | 0.06 | -0.04 | 0.16 | **0.14** | **0.04** | **0.24** | **0.11** | **0.001** | **0.22** |
| Latency, P1, ms | 0.04 | -0.05 | 0.13 | 0.08 | -0.02 | 0.17 | **0.13** | **0.03** | **0.24** | -0.01 | -0.13 | 0.10 |
| Amplitude, P1, μV | 0.10 | -0.10 | 0.30 | 0.09 | -0.13 | 0.32 | 0.17 | -0.04 | 0.38 | -0.09 | -0.31 | 0.12 |
| Latency, N2, ms | 0.02 | -0.05 | 0.08 | 0.04 | -0.04 | 0.11 | 0.07 | -0.002 | 0.15 | -0.02 | -0.10 | 0.06 |

**Bold**= significant at $p<0.05$

Abbreviations: CI, confidence interval; MDI, mental developmental index; ms, milliseconds; VEP, visual evoked potential; μV, microvolt

**Table 8. Unadjusted linear regression of MDI 12 on VEP at 3 and 6 months stratified by infant's sex.**

| | **Male Infants** n=289 at 3 mos n=362 at 6 mos | | | **Female Infants** n=276 at 3 mos n=340 at 6 mos | | |
|---|---|---|---|---|---|---|
| **VEP, 3 months** | β | 95% CI | | β | 95% CI | |
| Latency, N1, ms | **-0.08** | **-0.14** | **-0.02** | -0.005 | -0.08 | 0.07 |
| Latency, P1, ms | **-0.08** | **-0.14** | **-0.02** | -0.04 | -0.10 | 0.03 |
| Amplitude, P1, μV | -0.08 | -0.27 | 0.12 | -0.03 | -0.20 | 0.15 |
| Latency, N2, ms | -0.04 | -0.08 | 0.005 | -0.04 | -0.09 | 0.01 |
| **VEP, 6 months** | | | | | | |
| Latency, N1, ms | 0.02 | -0.04 | 0.08 | 0.05 | -0.02 | 0.13 |
| Latency, P1, ms | -0.002 | -0.07 | 0.06 | 0.09 | 0.01 | 0.16 |
| Amplitude, P1, μV | 0.02 | -0.12 | 0.16 | 0.18 | 0.03 | 0.33 |
| Latency, N2, ms | 0.01 | -0.04 | 0.05 | 0.06 | 0.01 | 0.11 |

**Bold**= significant at $p<0.05$

Abbreviations: CI, confidence interval; MDI, mental developmental index; ms, milliseconds; VEP, visual evoked potential; μV, microvolt

**Table 9. Regression of MDI at 12 months on VEP at 3 and 6 months among those infants with congenital anomalies.[a]**

| | MDI 12 | | | |
|---|---|---|---|---|
| | β | 95% CI | | P-value for interaction between VEP and congenital anomaly |
| **VEP, 3 months (n=19)** | | | | |
| Latency, N1, ms | -0.19 | -0.46 | 0.07 | 0.15 |
| Latency, P1, ms | -0.25 | -0.49 | 0.00 | 0.05 |
| Amplitude, P1, μV | -0.12 | -0.93 | 0.69 | 0.56 |
| Latency, N2, ms | -0.15 | -0.36 | 0.05 | 0.16 |
| **VEP, 6 months (n=20)** | | | | |
| Latency, N1, ms | -0.08 | -0.43 | 0.27 | 0.57 |
| Latency, P1, ms | **-0.65** | **-1.11** | **-0.18** | **0.003** |
| Amplitude, P1, μV | -0.76 | -2.21 | 0.69 | 0.05 |
| Latency, N2, ms | **-0.32** | **-0.64** | **-0.01** | **0.01** |

**Bold=** significant at $p<0.05$

Abbreviations: CI, confidence interval; MDI, mental developmental index; ms, milliseconds;
VEP, visual evoked potential; μV, microvolt

[a]Congenital anomalies were noted in the birth medical record and include trisomy, hydrocephalus, spina bifida, enzymatic abnormalities, or "other"

**Table 10. Regression of MDI at 12 and 18 months on VEP at 3 and 6 months among those with complications at delivery[a]**

| | MDI 12 n=85 at 3 mos n=101 at 6 mos | | | MDI 18 n=80 at 3 mos n=93 at 6 mos | | |
|---|---|---|---|---|---|---|
| | β | 95% CI | | β | 95% CI | |
| **VEP, 3 months** | | | | | | |
| Latency, N1, ms | -0.07 | -0.19 | 0.06 | 0.12 | -0.02 | 0.26 |
| Latency, P1, ms | -0.08 | -0.18 | 0.02 | 0.10 | -0.01 | 0.21 |
| Amplitude, P1, μV | -0.28 | -0.59 | 0.03 | -0.03 | -0.39 | 0.34 |
| Latency, N2, ms | -0.08 | -0.16 | -0.01 | 0.05 | -0.03 | 0.14 |
| **VEP, 6 months** | | | | | | |
| Latency, N1, ms | -0.09 | -0.24 | 0.06 | **0.19** | **0.01** | **0.36** |
| Latency, P1, ms | -0.02 | -0.16 | 0.13 | **0.21** | **0.04** | **0.38** |
| Amplitude, P1, μV | 0.13 | -0.12 | 0.37 | 0.06 | -0.23 | 0.35 |
| Latency, N2, ms | 0.02 | -0.09 | 0.13 | 0.12 | -0.01 | 0.26 |

**Bold=** $p<0.05$

Abbreviations: CI, confidence interval; MDI, mental developmental index; ms, millisconds; VEP, visual evoked potential; μV, microvolt

[a] Perinatal complications include signs of fetal distress, tachycardia, bradycardia or meconium present at delivery

**Table 11. Pearson partial correlation coefficients between MDI at 12 months and VEP latency at 3 months, controlling for important covariates.**

| | VEP N1 latency | | | VEP P1 Latency | | |
|---|---|---|---|---|---|---|
| **Variable** | Pearson correlation coefficient, r | P-value | n | Pearson correlation coefficient, r | P-value | n |
| VEP latency variable at 3 months and MDI at 12 months, unadjusted | -0.10 | 0.02 | 565 | -0.12 | 0.01 | 565 |
| **Control variable** | Pearson partial correlation coefficient [a], r | | | Pearson partial correlation coefficient, r | | |
| Interviewer code 83 | -0.08 | 0.06 | 565 | -0.11 | 0.01 | 565 |
| Birth weight, kg | -0.10 | 0.02 | 565 | -0.11 | 0.01 | 565 |
| Infant's sex | -0.09 | 0.03 | 565 | -0.11 | 0.01 | 565 |
| Home environment summary score at 12 months | -0.09 | 0.06 | 417 | -0.13 | 0.01 | 417 |
| Breastfed at 1 month | -0.09 | 0.04 | 512 | -0.10 | 0.02 | 512 |
| Breastfed at 3 months | -0.10 | 0.01 | 564 | -0.12 | 0.00 | 564 |
| Gestational age at birth, weeks | -0.09 | 0.03 | 563 | -0.11 | 0.01 | 563 |
| Maternal Raven score | -0.10 | 0.02 | 565 | -0.12 | 0.00 | 565 |
| Maternal schooling, years | -0.09 | 0.03 | 564 | -0.11 | 0.01 | 564 |
| SES score[b] | -0.10 | 0.02 | 565 | -0.12 | 0.01 | 565 |
| **All variables above** | -0.08 | 0.15 | 372 | -0.09 | 0.08 | 372 |

Abbreviations: CI, confidence interval; MDI, mental developmental index; VEP, visual evoked potential

[a]Each variable was controlled for independently using the PARTIAL option in SAS

[b]SES score calculated using Principal Component Analysis, PCA

**Table 12. Analysis of the predictive performance of VEP measures on MDI scores by area under the ROC curve**

| | No. of infants | No. of infants classified as "delayed" | Visual Evoked Potentials at 3 months | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | N1 latency | | | P1 latency | | | N2 latency | | | P1 amplitude | | |
| | | | Area | 95% CI | | Area | 95% CI | | Area | 95% CI | | Area | 95% CI | |
| **MDI at 12 months** | 565 | 91 | 0.55 | 0.48 | 0.61 | 0.55 | 0.48 | 0.62 | 0.57 | 0.50 | 0.63 | 0.56 | 0.49 | 0.62 |
| **MDI at 18 months** | 545 | 72 | 0.49 | 0.41 | 0.56 | 0.49 | 0.42 | 0.57 | 0.50 | 0.43 | 0.58 | 0.52 | 0.45 | 0.58 |
| | | | Visual Evoked Potentials at 6 months | | | | | | | | | | | |
| **MDI at 12 months** | 565 | 91 | 0.56 | 0.49 | 0.62 | 0.54 | 0.48 | 0.60 | 0.53 | 0.47 | 0.59 | 0.51 | 0.46 | 0.57 |
| **MDI at 18 months** | 545 | 72 | 0.57 | 0.50 | 0.64 | 0.54 | 0.48 | 0.61 | 0.53 | 0.46 | 0.60 | 0.56 | 0.50 | 0.62 |

**Bold**= significant at $p<0.05$

Abbreviations: CI, confidence interval; MDI, mental developmental index; ROC, receiver operating characteristic; VEP, visual evoked potential

*Figures*

| **Figure 1. Details of data collection during prenatal and postnatal follow-up** | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | |
| | **Prenatal** | | **Birth** | **Postpartum (mo)** | | | | | |
| | **Time of randomization (18-22 weeks gestation)** | **Intervention** | | **1** | **3** | **6** | **9** | **12** | **18** |
| Sociodemographic characteristics | X | | | | | | | | |
| Obstetric History | X | | | | | | | | |
| Maternal anthropometry | X | | | | X | X | | | |
| Maternal weight | X | X | | | X | | | | |
| Maternal PUFA[a] status | X | | X | X | X | | | | |
| Maternal intelligence | X | | | | | | | | |
| Supplement consumption | | X | | | | | | | |
| Gestational age and birth outcomes | | | X | | | | | | |
| Infant anthropometry | | | X | X | X | X | X | X | X |
| Infant PUFA status | | | X | | X | | | X | X |
| APGAR scores | | | X | | | | | | |
| Auditory Evoked Potentials | | | | X | X | | | | |
| Visual Evoked Potentials | | | | | X | X | | | |
| BSID-II[b] | | | | | | X | | X | X |
| Visual recognition memory and visual attention | | | | | | | | X | X |
| Infant Diet | | | | X | X | X | X | X | X |
| Breast milk PUFA | | | | X | X | | | | |
| Home environment | | | | | | X | | X | X |

[a] PUFA, polyunsaturated fatty acid

[b] BSID-II, Bayley Scales of Infant Development, second edition

**Figure 2.** Details on recruitment of mothers and follow-up of infants in a randomized controlled trial of DHA supplementation in Cuernavaca, Mexico.

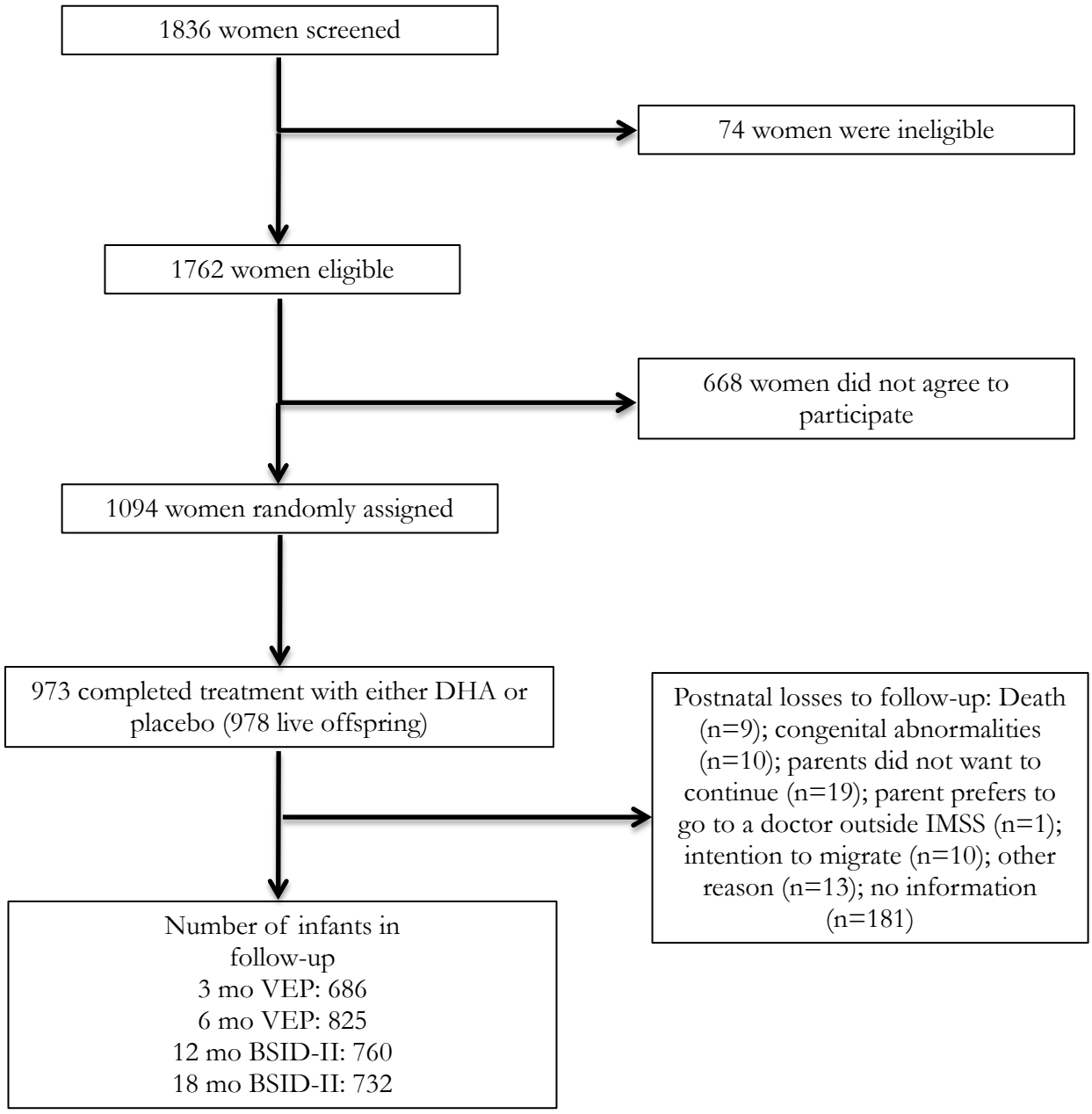

**Figure 3.** ROC curves comparing 3-month VEP measures to predict MDI at 12 months.