Increased ERG a- and b-Wave Amplitudes in 7- to 10-Year-Old Children Resulting from Prenatal Lead Exposure

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PURPOSE. To determine the dose–response relationship between blood lead concentration ([PbB]) and scotopic ERG amplitude in 7- to 10-year-old children with lifetime lead exposure.

METHODS. Full-field flash scotopic ERGs were recorded over a 4-log-unit range in 45 dark-adapted children with normal visual acuity. [PbB] was measured throughout pregnancy and postnatal development, and the subjects' [PbB] levels were grouped at each age by tertiles.

RESULTS. The median [PbB] during pregnancy was, from lowest to highest tertile, 2.5 to 5.0, 7.5 to 9.0, and 14.0 to 16.5 μg/dL, and after birth was 4.0 to 8.0, 6.0 to 14.5, and 7.5 to 21.0 μg/dL. Only maternal [PbB] at 12 weeks of pregnancy showed a significant dose–response relationship with the ERG measures, so that with increasing [PbB] there were significant increases in leading-edge a-wave amplitude, peak a-wave amplitude, and b-wave amplitude and sensitivity, with no changes in implicit times. Data analyses showed that children whose mothers had [PbB] of 10.5 μg/dL or more at 12 weeks of pregnancy had relatively increased a- and b-waves.

CONCLUSIONS. Lead exposure during the first trimester of pregnancy produces dose-dependent increases in scotopic a- and b-wave amplitudes in 7- to 10-year-old children. The results suggest that the increases in a- and b-wave amplitudes originate from rods; however, the increased b-wave amplitude and sensitivity may also originate in the inner retina. These alterations occurred at maternal [PbB] at or below currently accepted safe levels. These novel findings reveal that the developing retina is a sensitive target for lead and suggest that lead-exposed children be examined for possible future visual system deficits. (*Invest Ophthalmol Vis Sci.* 2002;43:2036–2044)

Lead is a pervasive and potent neurotoxicant that produces persistent adverse effects on the developing nervous system after low-level exposure.[1–3] In the United States, almost 2 million children between the ages of 1 and 5 years have blood lead concentrations ([PbB]) of 10 μg/dL or more, which places them at risk for these and other adverse health effects.[4,5] In less-developed countries the percentages of children with [PbB] of 10 μg/dL or more are even higher.[3,6–8] The long-term adverse effects of low-level developmental lead exposure on cognitive function have been well documented during the past two decades.[1,3] In addition, deleterious effects of low-level lead exposure on the developing auditory system have been reported in children and monkeys.[2,7,9–12] In contrast, few studies have examined the impact of lead exposure on retinal function in children,[2,13] despite documented long-term behavioral, electroretinographic (ERG), biochemical, and structural abnormalities in the retinas of occupationally lead-exposed workers, lead-exposed adult rats, and monkeys and rats after lead exposure during prenatal and/or postnatal development.[2,14–23] This is surprising, because retinal and visual deficits can adversely affect learning and memory as well as the experimental procedures used to assess these cognitive parameters.[6,24–26]

Long-term scotopic (rod-mediated), but not photopic (cone-mediated), retinal deficits occur in lead-exposed workers and in monkeys and rats after lead exposure during development.[2,16–22] ERG studies conducted in these lead-exposed workers and postnatally lead-exposed rats reveal selective rod-mediated decreases in sensitivity, amplitude, and temporal resolution, as well as increased implicit times and dark-adaptation time. In contrast, monkeys exposed to lead during gestation and postnatal development have persistent increases in the amplitude of the scotopic ERG b-wave (i.e., relative supernormality) with no changes in implicit time.[21,22]

The goal of this part of our prospective pediatric lead study was to determine whether there were significant dose–response relationships between the [PbB] of subjects at specific gestational or postnatal ages and the scotopic ERG a- and b-wave measures obtained from 7- to 10-year-old children. Blood lead concentrations were obtained routinely in mothers during pregnancy and in children during development.

MATERIALS AND METHODS

Subjects

All subjects were participants in the Mexico City Prospective Lead Study.[27] Because of limitations in availability and access to subjects, a small group of subjects whose blood lead history during gestation and postnatal development as well as other clinical values that matched those of the larger cohort was used for ERG analysis. At the time of ERG testing, the 45 subjects (21 boys and 24 girls) ranged in age from 84 to 124 months (mean $\pm$ SD, 109.7 $\pm$ 11.3). Data analyses showed that

gender had no effect on any measure, so the data for male and female subjects were combined and are presented together. Both parents and child participated in the informed consent, during which the complete procedure was described. If both parents and child agreed to the testing, the parents signed an informed consent form approved by the Institutional Review Board (IRB) of the National Institute of Perinatology, Mexico City. The IRB assures that studies adhere to the tenets of the Declaration of Helsinki.

Blood Lead, Iron, Zinc, and Vitamin A Measurements

Beginning at the 12th week of pregnancy and then every 8 weeks, blood was obtained from pregnant women. At birth, blood was obtained from the mother and umbilical cord. Children's blood was obtained every 6 months until the ERG testing was conducted. Environmental Science Associates Laboratories, Inc. (New Bedford, MA) determined the [PbB] in duplicate analyses. Full details on quality assurance and control and [PbB] means, SEs, and ranges for the mothers and children have been published.[8,27,28] After ERG testing, blood was obtained from each subject for serum ferritin, zinc, and retinol analysis. Serum zinc, ferritin, and retinol were determined as described.[29,30]

ERG Procedures and Analysis

A pediatric ophthalmologist performed all procedures. All subjects had uncorrected visual acuity of 20/40 or better. No child had clinical or anecdotal evidence of retinal or visual disturbance. For the ERG experiments, the pupils were dilated with 1% cyclopentolate HCl and 2.5% phenylephrine HCl. After pupils were dilated, the subjects were dark adapted by sitting with their parent(s) in a completely dark room for 40 minutes. Thirty-five minutes into the dark-adaptation period and under dim red light (<1 lux; $\lambda \geq 650$ nm), the corneas were anesthetized with 0.5% proparacaine and a sterile contact lens electrode (Jet; LKC Technologies, Inc., Gaithersburg, MD) was placed on the eye with the best visual acuity. No corneal abrasions or postprocedure eye infections were observed or reported.

Standard clinical equipment was used for all scotopic ERG recordings, essentially as described.[31] A sterile, disposable contact lens ERG electrode (Jet; LKC Technologies, Inc.), soaked in methylcellulose, was placed on the cornea used to record the ERG. Reference and ground electrodes were placed on the forehead and earlobe, respectively. The signals from the electrodes were amplified (AC coupled; 1–1000-Hz bandpass; gain, 2 K), digitized at 2000 Hz, and stored on computer disks for analysis. Measures of amplitude and implicit time were obtained from autoscaled displays of stored responses by manual cursor adjustments. All ERG responses were obtained to full-field flash stimulation using a commercial Ganzfeld (LKC Technologies, Inc.). The light source was a xenon strobe (P22 photostimulator; Grass Instruments, Quincy, MA) that produced a 10-μs flash of white light.

Full-field scotopic flash ERGs were recorded over a 4-log-unit range (−1.37 to +1.85 log scotopic troland/second [scot td/sec]) in dark-adapted subjects and were used to generate the scotopic voltage (V)-log intensity (I) and implicit time (IT)-log I functions. Starting at −1.37 log scot td/sec, a trial of five flashes was used for each neutral-density filter setting. The stimulus increment was 0.5 log scot td/sec in all cases, except one, in which it was 0.7 log scot td/sec. Troland values were computed with the median pupil diameter of 9 mm (range, 8–11 mm). The Pearson χ^2 test and the likelihood ratio test revealed that there were no significant relationships between visual acuity or pupil diameter and lead exposure.

A verbal warning cued the subjects just before the onset of each flash. The interval between single flashes was chosen so that it did not affect the sensitivity to the subsequent flash. After the series of five flashes, there was a 5-minute interval until the next trial started. Waveforms contaminated with artifacts, due to blink or eye movements, were rejected. The remaining waveforms were averaged (two

to five waveforms per intensity, with the majority using four waveforms), and this average was used for further data analysis.

The a-wave amplitude was measured at a fixed time of 12 ms after the flash and also from baseline to peak of its response. The amplitude at 12 ms was used to directly determine the effects of lead exposure on rod photocurrent, because the earliest part of the ERG response to flash in humans and experimental animals is provided primarily by the photocurrent of rods.[31–33] The b-wave amplitude was measured from baseline to peak of its response in the absence of an a-wave or from the trough of the a-wave to the peak of the b-wave. The time interval from the flash onset to the a- or b-wave peaks was used as a measure of implicit time. The a-wave log V-log I, b-wave V-log I, and IT-log I data were fit by iterative procedures that minimized the mean square error to 0.1% or less. To determine and compare the relationship between the rod response amplitude and flash intensity for each lead tertile group, the mean a-wave slopes (in microvolts per millisecond) were measured between 10 and 12 ms and determined from log–log plots of a-wave slope versus log I, as described.[34]

Statistical Analysis

Blood lead concentrations for each subject at each age (time of sampling) were grouped by tertiles. The first tertile had the lowest blood lead concentration (hereafter termed the relative control group [RCon], because subjects had [PbB] considered within the normal range[1–5,8] and ERGs similar to those in several control studies[32,34–36] as will be described in detail later). The subjects in the second tertile had low [PbB][1–5,8] (hereafter termed the low-lead group [LL]), whereas the subjects in the third tertile had moderate [PbB][1–5,8] (hereafter termed the moderate lead group [ML]). We performed separate repeated-measures (5 × 3) analyses of variance (ANOVAs): with a- and b-wave amplitudes, a- and b-wave implicit times, and a-wave slope as dependent variables; with log flash intensity as the within-subjects measure (five highest flash intensities); and with blood lead tertile at each age as the between-subjects measure (three levels). Orthogonal polynomial contrasts for the log flash intensity variable were used. The number of subjects with at least two artifact-free ERG responses for each of the five highest flash intensities was 45. Because of technical problems with the equipment, there were fewer subjects at the two lowest flash intensities ($n = 10$ and 11). These data were tested with a simple ANOVA, with the three blood lead tertiles as a single factor. The Tukey honest significant difference method was used to test the differences among levels of [PbB], because it corrects for multiple comparisons. Diagnostic tests for the ANOVA indicated the use of Huynh-Feldt corrected degrees of freedom to adjust some F-tests for the within-subjects factor. Pearson correlation coefficients for natural log-transformed [PbB] at each age and the ERG measures were determined. Diagnostic tests and residual analysis indicated that none of the assumptions of the repeated measure ANOVAs were violated. Stata (StataCorp, Austin, TX) and SPSS (SPSS Inc., Chicago, IL) programs were used for data analysis. Correlation coefficients (r) were obtained from best-fit functions. Differences between groups were regarded as significant if two-sided probabilities were less than or equal to 0.05.

RESULTS

Blood Lead, Iron, Zinc, and Vitamin A Analysis

For all subjects participating in the ERG study, a history of [PbB] in pregnant mothers and their offspring was obtained. Table 1 presents the ranges and medians of prenatal and postnatal [PbB] by age in each tertile. The range of [PbB] within each tertile varied across ages. During pregnancy, the median [PbB] in the first tertile (RCon), second tertile (LL), and third tertile (ML) was 2.5 to 5.0, 7.5 to 9.0, and 14.0 to 16.5 μg/dL, respectively, and the range of [PbB] in the three tertiles was 1.0 to 8.0, 6.0 to 10.5, and 9.5 to 37.5 μg/dL, respectively (Table 1). After birth, the median [PbB] in RCon and LL was 4.0 to 8.0 and 6.0 to 14.5 μg/dL, respectively, and the [PbB] range in RCon and LL was 1.5 to 10.0 and 6.0 to 17.5 μg/dL, respec-

TABLE 1. Range and Medians of Blood Lead Tertiles in Subjects with Complete ERG Data

	First Pb Tertile (RCon)			Second Pb Tertile (LL)			Third Pb Tertile (ML)		
Age at Blood Lead Analysis	Median	Range	*n*	Median	Range	*n*	Median	Range	*n*
12 weeks of pregnancy	4.0	2.0–5.5	11	8.5	6.0–10.0	14	14.0	10.5–32.5	14
20 weeks of pregnancy	5.0	1.0–5.5	9	8.0	6.5–10.0	11	14.5	10.5–16.0	9
28 weeks of pregnancy	2.5	1.0–4.5	10	7.5	6.5–7.5	11	15.5	9.5–25.5	10
36 weeks of pregnancy	3.5	1.0–4.5	11	8.0	6.0–8.5	10	15.5	9.5–32.5	11
Mother at delivery	5.0	2.0–6.0	7	9.0	7.0–9.0	7	16.5	10.0–31.5	7
Umbilical cord	4.5	1.0–8.0	8	10.0	8.5–10.5	7	14.0	11.0–37.5	7
6 months	5.5	1.5–7.5	11	9.5	8.0–10.5	11	19.5	11.0–39.0	11
12 months	7.5	2.0–9.0	12	12.0	9.5–15.5	13	21.0	16.0–36.5	12
18 months	8.0	6.0–10.0	12	14.5	10.5–17.5	11	21.0	18.5–59.5	12
24 months	7.0	5.0–9.0	13	13.0	10.0–17.0	14	20.5	17.5–44.0	13
30 months	7.0	3.0–8.5	13	11.0	9.0–13.0	14	21.0	13.5–42.5	14
36 months	6.5	4.0–9.0	14	11.0	9.0–12.5	14	18.0	13.0–30.0	13
42 months	5.0	2.5–7.5	12	9.0	8.5–11.0	10	13.5	12.0–32.5	13
48 months	7.0	5.0–8.0	14	10.0	8.0–14.0	14	17.0	14.5–26.0	13
54 months	6.0	4.5–7.0	12	11.0	8.5–13.0	12	16.5	13.5–27.5	12
60 months	6.0	3.5–7.0	12	9.0	8.0–11.0	12	15.0	12.5–19.0	13
66 months	6.0	4.0–6.0	11	9.0	6.5–9.5	12	12.0	11.0–17.5	11
72 months	5.0	3.0–6.5	11	8.5	7.0–9.5	15	12.0	10.0–21.0	12
78 months	5.0	3.0–5.5	10	6.5	6.0–9.0	14	12.0	9.5–31.5	12
84 months	4.5	2.0–6.0	13	7.5	6.5–8.0	13	11.0	9.0–40.0	13
85–124 months	4.0	2.0–4.5	13	6.0	5.0–6.5	15	7.5	7.0–16.0	17

Tertiles have unequal sizes because of ties in blood lead levels. Blood lead is expressed in micrograms per deciliter. Conversion: 10 μg/dL = 0.483 μmol/L.

tively. After birth, the median [PbB] in ML generally was between 11.0 to 21.0 μg/dL, including an elevated plateau period from 12 to 36 months of age. The range of [PbB] in ML fluctuated from a low of 7.0 to a high of 59.5 μg/dL. The higher maximum values generally occurred during the first 30 months of age. At the time of ERG testing, the median [PbB] for RCon, LL, and ML was 4.0, 6.0, and 7.5, respectively, and the ranges were 2.0 to 4.5, 5.0 to 6.5, and 7.0 to 16.0 μg/dL, respectively (Table 1). Thus, as a group, the subjects in RCon and LL at each age were exposed to low levels of lead throughout gestation and development, whereas those in ML were exposed to a wider range of low-to-moderate levels of lead. Because some mothers and children were not able to contribute a blood sample at a particular age, the number of subjects at each age shown in Table 1 varied. Overall, the group variation in [PbB] with age is similar to the one found in the larger cohort from which this sample was drawn[8,27,28] and to other large groups.[4,37,38]

Because lead exposure was not under experimental control, subjects changed their ranks from one tertile group to another during pregnancy and after delivery. For example, from 12 to 36 weeks of pregnancy, 36% of subjects remained in the same lead tertile, 13% went from ML to RCon, 7% went from RCon to ML, and the remainder were situated jointly within the second or third tertile. A comparison of the changes in lead tertile ranking between 12 weeks of pregnancy and 84 months postnatal age shows that 39% remained unchanged, 3% changed from ML to RCon, 20% changed from RCon to ML, and the remainder was found jointly within the second or third tertile. Overall, the [PbB] of the group of subjects followed published patterns throughout pregnancy and childhood.[8,27,28]

Serum retinol, zinc, and ferritin levels, measured from blood samples collected at the time of ERG testing, were not significantly different among the three tertiles. The mean ± SD and range (in micromolar) for serum retinol, zinc, and ferritin were 1.3 ± 0.34 and 0.59 to 2.40, 12.9 ± 3.14 and 7.04 to 18.67, and 6.7 ± 3.49 and 1.25 to 15.04 μg/dL, respectively. The serum retinol, zinc, and ferritin levels were generally within normal limits[29,30] and were not significantly related to any of the ERG parameters.

Relationship between Blood Lead Concentrations and ERG Amplitude Measures

Repeated-measures ANOVA determined whether there was a significant dose–response relationship between [PbB] at a specific gestational or postnatal age, peak [PbB], or average lifetime [PbB] and the scotopic ERG a-wave and/or b-wave amplitudes and implicit times. Of all the blood lead measures, only the maternal [PbB] at 12 weeks of pregnancy showed a significant dose–response relationship with the ERG measures. That is, at none of the other 20 time points, including the age of ERG testing did the results show a significant relationship with any ERG measure. Between-subjects tests with the 12-week gestational [PbB] showed a significant lead effect, so that with increasing maternal [PbB], there was an associated increase in the a-wave amplitude measured at 12 ms after the flash ($F_{2,36}$ = 4.104; P = 0.025), the a-wave amplitude measured at peak implicit time ($F_{2,36}$ = 5.75; P = 0.007), and the b-wave amplitude ($F_{2,36}$ = 5.75; P = 0.007). Post hoc testing of amplitude differences showed that relative to subjects in RCon, who had ERGs similar to those in several control studies[32,34,35] as will be described in detail immediately following, subjects in the ML group had increased a-wave amplitudes measured at 12 ms after the flash (P = 0.019) and at the peak implicit time (P = 0.005), those in the LL group had borderline significantly increased a-wave amplitudes measured at the peak implicit time (P = 0.073), and those in the ML group had increased b-waves (P = 0.005). Moreover, the Pearson correlation coefficients show that only the 12-week gestation data had significant associations between [PbB] and the ERG measures (Table 2). Thus, the combined statistical analyses show that 7- to 10-year-old children whose mothers had [PbB] of 6 μg/dL or more at 12 weeks of pregnancy would be likely to have relatively increased ERG a-waves, and those whose mothers had [PbB] of 10.5 μg/dL or more would have both relatively in-

TABLE 2. Pearson Correlation Coefficients of Blood Lead Concentration at 12 Weeks of Gestation and at Time of ERG Testing with ERG Parameters

Log Flash Intensity (scot td/sec)	Age at Time of Blood Lead Analysis			
	12 Weeks of Gestation		85–124 Months	
	Coefficient	P	Coefficient	P
a-Wave peak amplitude				
−0.36	0.103	0.532	−0.042	0.783
+0.37	0.459	0.003	0.050	0.743
+0.88	0.460	0.003	0.056	0.713
+1.35	0.364	0.023	0.107	0.484
+1.85	0.341	0.033	0.210	0.167
b-Wave amplitude				
−0.36	0.421	0.008	0.118	0.440
+0.37	0.439	0.005	0.107	0.485
+0.88	0.339	0.035	0.219	0.148
+1.35	0.249	0.126	0.019	0.901
+1.85	0.281	0.083	0.043	0.781

Significance values were calculated for two-tailed Pearson correlation test.

creased a- and b-waves. There were no significant dose-related effects of lead on implicit times of the a- or b-wave.

ERG Measures in the Relative Control Subjects

Although all subjects in this study were exposed to some level of lead throughout prenatal and postnatal development, as are almost all children throughout the world,[1–5] the results from subjects in RCon suggest that they are similar to children and adult control subjects in other studies. First, the V-log I and IT-log I functions for the b-wave of RCon (Figs. 1 and 2) are very similar to those of the dark-adapted adult control subjects, obtained with flash intensities from −1.5 to 1.9 log scot td/sec.[32] Second, the half-saturation constant for the b-wave in RCon (mean ± SEM: −0.81 ± 0.04 log scot td/sec; Table 3) is similar to the half-maximum b-wave amplitude obtained in dark-adapted children and adult control subjects using a similar full-field stimulus (mean ± SD: −0.84 ± 0.15 log scot td/sec).[35,36,39] Third, the mean (± SEM) half-saturation constant for the peak a-wave in RCon (1.40 ± 0.13 log scot td/sec; Table 3) is similar to the mean (±SD) half-maximum peak a-wave amplitude obtained in dark-adapted children and adult control subjects with a similar full-field stimulus (1.20 ± 0.19).[39] Fourth, the slope of the double logarithmic plot of stimulus intensity versus a-wave slope at peak implicit time over a 2-log-unit range of stimulus intensities was 0.54 (r = 0.99) in this study. A similar a-wave slope measured at peak implicit time was obtained in normal dark-adapted adult humans (stated as ∼0.5).[34]
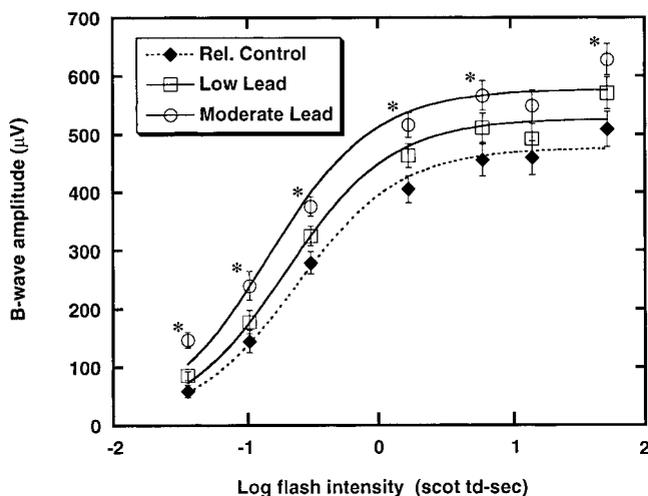

FIGURE 1. V-log I functions for the scotopic ERG b-wave for the three lead tertiles as determined by their [PbB] levels at 12 weeks of gestation. The mean half-saturation constant for subjects in the third lead tertile (ML) shifted significantly to the left by 0.18 log units, compared with that for subjects in the first lead tertile (RCon), indicating an increased sensitivity to luminance in ML. The actual (Table 3) and estimated V_{max} in subjects in the second lead tertile (LL) and ML, compared with RCon, were increased 11% to 12% and 21% to 23%, respectively. *b-Wave amplitudes (means ± SEMs) in ML that are significantly different (P < 0.02) from amplitudes at the same luminance intensity in the RCon (ANOVA followed by post hoc analysis with correction for multiple comparisons). The mean half-saturation constant for the RCon (−0.81 log scot td/sec) was similar to that obtained in dark-adapted normal children and adults using a similar scotopic stimulus.[35,36,39]
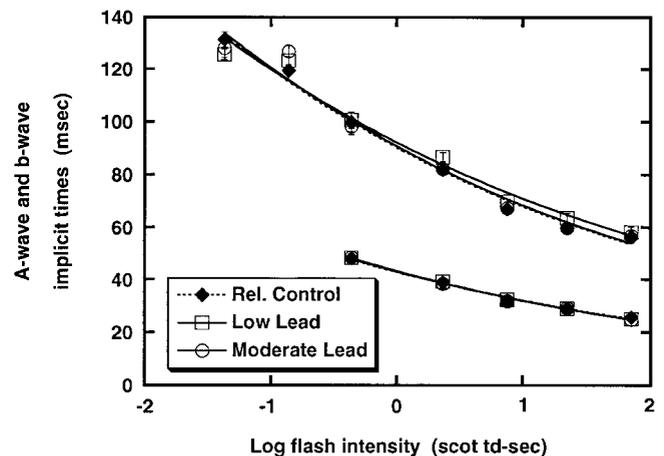

FIGURE 2. IT-log I functions for the scotopic ERG a-wave (*bottom* set of curves) and b-wave (*top* set of curves) for the three lead tertile groups as determined by their [PbB] at 12 weeks of gestation. The a-wave IT-log I data were best fit by a power function. There were no significant dose-related lead effects on implicit times of either the a- or b-wave.

TABLE 3. Comparison of ERG Parameters in School-Aged Children after Low- and Moderate-Level Prenatal and Postnatal Lead Exposure

ERG Parameter	RCon	LL	ML
V_{max} (μV)			
a-Wave at 12 ms latency	70.1 ± 7.1	81.5 ± 6.3	93.6 ± 6.3*
a-Wave at peak latency	220.6 ± 15.3	254.8 ± 13.6	280.6 ± 13.6*
b-Wave	509.3 ± 30.5	570.5 ± 26.9	628.2 ± 27.0*
Half-saturation constants (log scot td/sec)			
a-Wave at 12 ms latency	1.48 ± 0.12	1.49 ± 0.11	1.48 ± 0.10
a-Wave at peak latency	1.40 ± 0.13	1.56 ± 0.12	1.51 ± 0.09
b-Wave	−0.81 ± 0.04	−0.86 ± 0.04	−0.99 ± 0.05*

Data are expressed as the mean ± SEM.
* Significantly different from RCon at $P < 0.05$.

ERG Analysis in Relation to 12-Week Gestational Lead Exposure

Figure 3 shows representative ERGs recorded at three different luminance intensities in a 119-month-old subject in RCon
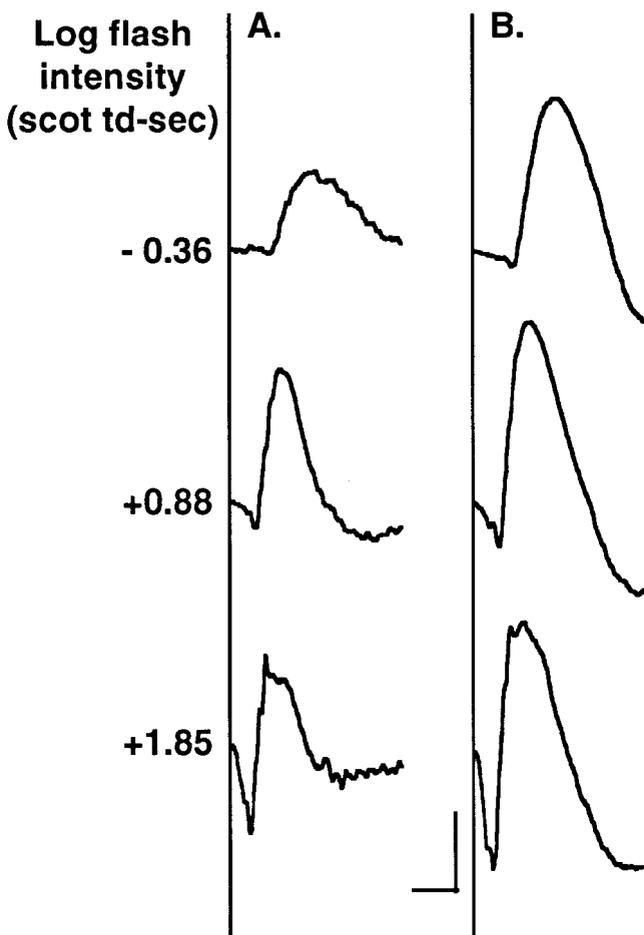

Log flash intensity (scot td-sec)

A.

B.

− 0.36

+0.88

+1.85

FIGURE 3. Representative full-field scotopic flash ERG traces from a 119-month-old subject in the first (RCon), or lowest, lead tertile (**A**) and a 120-month-old subject in the third (ML), or highest, lead tertile (**B**) of the 12-week pregnancy group. Blood lead concentrations of the mothers at 12 weeks of pregnancy, the only time point that showed a significant dose–response relationship with the ERG measures, were 2.0 and 22.0 μg/dL, respectively. Both subjects had [PbB] concentrations of 7 μg/dL at the time of ERG testing. Note that the a- and b-wave amplitudes were significantly increased in the subject in ML compared with the subject in RCon, although the implicit times were not different. The calibration mark is 50 ms (*horizontal*) and 200 μV (*vertical*) for traces of both subjects.

whose mother's [PbB] was 2 μg/dL at 12 weeks of gestation (Fig. 3A) and a 120-month-old subject in ML whose mother's [PbB] was 22 μg/dL at 12 weeks of gestation (Fig. 3B). Both subjects had [PbB] of 7 μg/dL at the time of ERG testing. Note that the a- and b-wave amplitudes in the subject in ML, compared with the one in RCon, were increased at 0.88 and 1.85 log scot td/sec and that the b-wave also was increased at −0.36 log scot td/sec. Note particularly that the a- and b-wave implicit times were not different in either subject.

Log V-log I functions for the scotopic a-wave, determined at 12 ms after the flash and at the peak implicit time, are illustrated in Figures 4A and 4B, respectively. In Figures 1, 2, and 4 the closed symbols and dashed line represent data for RCon and the open symbols and solid lines show data for LL (open squares) and ML (open circles) tertiles as determined by [PbB] at 12 weeks of gestation. The a-wave threshold ($\geq$10 μV criterion response) occurred at −0.36 log scot td/sec for all three lead groups (Fig. 4B), which was approximately 2 log units above the b-wave threshold (data not shown). Reliable amplitude measurements at 12 ms after the flash were obtained at luminance intensities of 0.88 log scot td/sec and above (Fig. 4A). For all three dark-adapted lead groups, the increases in the a-wave amplitude with increasing luminance intensity were best fit by a linear function at 12 ms after the flash ($r = 0.99$) and by a power function at the peak implicit time ($r = 0.99$). The rod a-waves were not fit by a computational model based on transduction biochemistry, because the highest luminance intensity used in these studies was only 1.85 log scot td/sec.[32,33]

Compared with RCon, the a-wave amplitude at 12 ms after the flash was increased in ML, indicating that there was a long-lasting and direct effect of prenatal lead exposure on the rod photocurrent (Fig. 4A). Subjects in ML also had increased a-wave amplitudes measured at the peak implicit time, whereas subjects in LL had borderline significant increases in peak a-wave amplitude compared with those in RCon (Fig. 4B; Table 3). The group data reveal that the a-wave amplitude for LL and ML at peak implicit time increased 36% and 56% at 0.37 log scot td/sec, respectively, and increased approximately 20% and 30% at luminance intensities from 0.88 to 1.85 log scot td/sec, respectively (Table 4). There were no differences in a-wave implicit times between the lead groups (Fig. 2).

ANOVA with repeated measures was used to determine the dose–response effects of maternal [PbB] at 12 weeks of gestation on the slope of the leading edge of the a-wave (measured between 10 and 12 ms) at different flash intensities. Between-subjects testing revealed a lead effect such that, with increasing [PbB], there was a significant increase in the a-wave slope across flash intensities ($F_{2,36} = 5.25$; $P = 0.010$). In all three dark-adapted lead groups, the a-wave slopes were best fit by linear functions ($r = 0.99$) with slopes of 0.18 (RCon), 0.19 (LL), and 0.27 (ML), consistent with the finding that the photoreceptor's output immediately after the flash onset is a linear
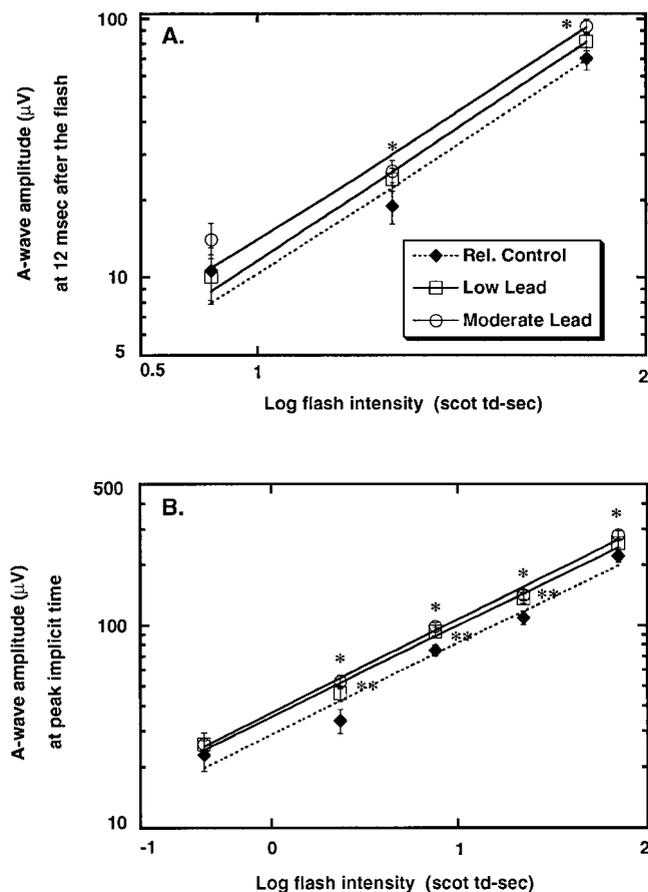
FIGURE 4. Log V-log I functions for the scotopic ERG (**A**) leading edge and (**B**) peak a-wave for the three lead tertiles as determined by their [PbB] levels at 12 weeks of gestation. For all three dark-adapted lead groups, the increases in the a-wave amplitude with increasing luminance intensity were best fit (**A**) by a linear function at 12 ms after the flash ($r = 0.99$) and (**B**) by a power function at the peak implicit time ($r = 0.99$). The equations for the linear fits of the leading edge a-wave in (**A**) are $y = 0.58 + 0.97x$ for RCon, $y = 0.10 + 1.14x$ for LL, and $y = 1.02 + 1.29x$ for ML. The equations for the power function fits for the peak a-wave in (**B**) are $y = 28.69 \cdot x^{0.45}$ for RCon, $y = 35.10 \cdot x^{0.45}$ for LL and $y = 36.82 \cdot x^{0.46}$ for ML. The asterisks located on the a-wave amplitudes (means ± SEMs) in the lead tertiles were different (*$P < 0.02$; **$P < 0.07$) from amplitudes at the same luminance intensity in RCon (ANOVA, followed by post hoc analysis with correction for multiple comparisons). V_{max} and half-saturation constants are presented in Table 3.

function of flash intensity.[31] Post hoc testing revealed that the a-wave increased more rapidly with stimulus intensity in ML than in RCon ($P = 0.049$).

The V-log I functions for the scotopic b-wave are presented in Figure 1. For all three dark-adapted lead groups, the b-wave amplitude increased with increasing luminance intensity and then saturated. Between-subjects testing showed that the b-wave amplitude increased significantly with increasing [PbB] ($F_{2,36} = 5.75$; $P = 0.007$). Post hoc testing indicated that subjects in ML had significantly larger b-wave amplitudes than those in RCon ($P = 0.005$). Although the mean b-wave amplitude values in LL were larger than those in RCon at all luminance intensities (Table 4), the differences did not reach statistical significance ($P = 0.259$). The scotopic b-wave V-log I data were fitted with the Michaelis-Menten equation and the half-saturation constant, a measure of b-wave sensitivity, and maximum amplitude (V_{max}) were estimated (Table 3). The mean (± SEM) half-saturation constant in ML (-0.99 ± 0.05 log scot td/sec) shifted significantly ($P < 0.05$) to the left

compared with that in RCon (-0.81 ± 0.04 log scot td/sec), indicating an increased sensitivity to luminance in ML. The actual V_{max} values are presented in Table 3; the estimated V_{max} values for RCon, LL, and ML were 477, 530 (+11%) and 577 μV (+21%), respectively.

The b-wave amplitude in ML increased significantly above the b-wave in RCon by a constant amount (mean ± SEM, 102.3 ± 4.4 μV). A similar, but smaller, constant increase in the b-wave amplitude was observed in LL compared with RCon (mean ± SEM, 45.0 ± 3.3 μV; Fig. 1; Table 4). There were no significant differences among the groups in the b-wave implicit times (Fig. 2).

DISCUSSION

The major finding in this prospective pediatric ERG study of 7- to 10-year-old children with lifetime lead exposure and normal visual acuity was a surprising one. Only lead exposure during the first trimester of prenatal development was associated with alterations in rod-mediated retinal function. More specifically, rigorous statistical analyses have shown that of the 21 different blood lead sampling periods during pregnancy and throughout postnatal development only the [PbB] at 12 weeks of gestation exhibited a significant dose-dependent relationship with the observed scotopic ERG alterations. The threshold maternal [PbB] for these long-lasting effects is 10.5 μg/dL, but may be as low as 6.0 μg/dL. The retinal changes in LL and ML, compared with RCon, are characterized by an increased a- and b-wave amplitude, as well as increased b-wave sensitivity. These lead-induced increases in ERG amplitude occurred without any changes in implicit times. Taken together, these results suggest that when 12-week-pregnant mothers have [PbB] levels of 10.5 μg/dL or more, their children will have relatively increased ERG scotopic a- and b-waves.

The scotopic a- and b-wave alterations observed in these lead-exposed children appear to be a new form of rod dysfunction. The lead-exposed children are clearly different from patients who have an unusual cone dystrophy characterized by supernormal b-waves.[32,40–43] In contrast to the lead-exposed children, patients with cone dystrophy have subnormal b-waves with increased implicit times to dim flashes, supernormal b-waves with normal implicit times to intense flashes, and decreased visual acuity. Early clinical and experimental studies suggest that this cone dystrophy results from a decrease in rod cGMP PDE activity; however, ERG studies using newer analytical methods indicate that the supernormal b-wave in these patients results from inner retinal alterations.[32]

Accumulating evidence suggests that the period of retinal development during which lead exposure occurs may determine whether the ERG a-waves and/or b-waves will be relatively increased or subnormal. In the current pediatric study and developmental monkey studies,[21,22] mothers were ex-

TABLE 4. Percent of Lead-Induced Increase in ERG a-Wave and b-Wave Amplitudes in the LL and ML Groups Compared with the RCon Group

Log Flash Intensity (scot td/sec)	Peak a-Wave Amplitude		b-Wave Amplitude	
	LL	**ML**	**LL**	**ML**
−1.37			46	154
−0.86			45	65
−0.36	±	±	17	35
0.37	36	56	15	28
0.88	24	32	12	24
1.35	25	30	7	20
1.85	16	27	12	23

posed to lead before and throughout gestation, and their offspring were exposed to lead during postnatal development. The children in LL and ML, compared with those in RCon, exhibited a long-lasting, dose-dependent increase in scotopic a- and b-wave amplitudes, with no change in implicit times, whereas the monkeys exhibited persistent dose-dependent increased scotopic b-waves with no change in implicit times.[21,22] In contrast, rats exposed to lead during neonatal development (birth to weaning) exhibited dose-dependent subnormal scotopic a- and b-waves with increases in implicit times and decreases in rod, but not cone, retinal sensitivity.[15,17,18] The maturity of the rat retina at birth is equivalent to that of a 20-week-old human fetal retina.[44,45] Taken together, these results lead us to hypothesize that the period of lead exposure during development may determine whether the scotopic a- and b-waves will be increased or decreased.

Determining the exact critical developmental period of retinal vulnerability to lead in this study is difficult, given the initial time point of analysis (12 weeks of gestation), the intervals at which the maternal [PbB] was determined (8-week intervals), the estimated 4- to 5-week half-life of lead in blood,[46] and the fact that lead exposure, while variable, was continuous throughout prenatal and postnatal development. For these reasons, the nominal 12-week gestation age—the only point at which the ANOVAs showed a significant positive association between the [PbB] and ERG effects—should be considered as occurring between 8 and 16 weeks of gestation. Thus, the critical developmental period of retinal vulnerability to lead occurred during the first trimester. During this developmental period, all retinal cell mitosis is completed, and the spatiotemporal process of retinal cell migration and differentiation begins.[47–50] Between 8 and 10 weeks of gestation, the posterior half of the retina differentiates into an inner and outer neuroblastic layer. At this stage of development, the outer neuroblastic layer consists of the amacrine and Müller cell somas (distal region), bipolar cells (middle region), and horizontal cells and photoreceptors (outermost region). Between 10 and 12 weeks of gestation, a period of rapid differentiation and development, the outer plexiform layer separates the immature inner nuclear layer from the photoreceptor cell layer and Müller cell nuclei, and the inner plexiform layer separates the ganglion cell layer from the immature inner nuclear layer. Between 12 and 16 weeks of gestation, all retinal cell types, except the horizontal cells, are discernible and the adult retinal pattern is established. Moreover, several synaptic invaginations on photoreceptors, multiple ribbon synapses in the outer and inner plexiform layers, and numerous synaptic vesicles are present.

The mechanisms underlying the lead-induced increases in scotopic a- and b-wave amplitudes in 7- to 10-year-old children, in the absence of changes in implicit times, are unknown. Two candidate mechanisms have emerged from examination of experimental and clinical studies. First, the relatively increased a- and b-wave amplitudes could result from the selective loss of dopaminergic amacrine and interplexiform cells during the first trimester. This hypothesis is consistent with findings that destruction of the dopaminergic retinal cells with the neurotoxin 6-hydroxydopamine results in increased scotopic a- and b-wave amplitudes without affecting implicit times.[51,52] In addition, the retinas of the lead-exposed monkeys with supernormal scotopic b-waves had a loss of tyrosine-hydroxylase–immunofluorescent cells.[21,22,53] Lead is known to produce apoptotic cell death in the outer and inner retina during development[16,19,54] and in tyrosine-hydroxylase–immunoreactive cells in culture.[55]

Second, the relative increase in a-wave amplitude obtained from measurements on the leading edge of the a-wave suggests that the lead-induced increase in a- and b-wave amplitude involves the rod photoreceptors. The composite results from several studies showing that low doses of cGMP PDE inhibitors

increase the amplitude of rod responses and that high doses of cGMP PDE inhibitors produce subnormal rod responses have led us to hypothesize that prenatal lead exposure increases the rod amplitude slightly, but significantly, by decreasing the expression and activity of rod cGMP PDE. For example, the amplitude of the isolated scotopic PIII (photoreceptor) component of the cat ERG was increased in the presence of low doses of nonmethylxanthine cGMP PDE inhibitors such as the benzimidazole derivative AR-L 115BS and the benzylisoquinoline derivative papaverine.[56] Papaverine had no effect on implicit time, whereas AR-L 115BS prolonged implicit time. At higher doses, both drugs decreased the PIII amplitude and AR-L 115BS further prolonged the implicit time.[56] Similar increases in rod responses to low-luminance stimuli were observed in isolated toad rods after exposure to low concentrations of 3-isobutyl-1-methylxanthine (IBMX) and papaverine.[57,58] This increase in rod amplitude was preceded by a dose-dependent depolarization of the rod dark membrane potential. With increasing concentrations of IBMX, the rod amplitude decreased and the time-to-peak of the rod photoresponse increased.[57,58]

In contrast, postnatally lead-exposed rats with subnormal scotopic a-waves and increased implicit times have moderately inhibited retinal cGMP PDE activity[15,17]: the same effects observed after moderate to high doses of IBMX.[59] In these rats, lead appears to produce its ERG effects by directly inhibiting rod cGMP PDE.[60,61] The composite results from the above investigations suggest that the extent of cGMP PDE inhibition as well as the mechanism of inhibition (i.e., decreased expression or direct pharmacologic inhibition) may determine whether the rod response to low luminance stimuli is increased or decreased. Ongoing studies in experimental animals will test these hypotheses.

This prospective study shows that low to moderate lead exposure during the first trimester of pregnancy produces relative increases in the amplitude of the scotopic a- and b-waves that are detectable 8 to 11 years after the initial insult. Similar long-term changes in the brain stem auditory-evoked response were observed in a larger cohort of these lead-exposed children.[12] Moreover, the current results are reminiscent of the positive linear relation between [PbB] in prenatally and postnatally lead-exposed children and the P1N1 amplitude of the pattern-evoked potential elicited with a slowly reversing full-field stimulus under scotopic luminance conditions.[62] Taken together, these findings suggest that the ERG may serve as an excellent biomarker for the long-term alterations observed in the nervous system of prenatally lead-exposed children. Determination of the precise molecular basis for these scotopic ERG effects could provide clues to sensory and cognitive deficits observed in prenatally lead-exposed children with [PbB] currently considered safe.[1,3,5] The present ERG alterations may be associated with functional visual system abnormalities, as observed in postnatally lead-exposed humans and animals.[2,14,18,20,23] This suggests that the retina is a sensitive target for lead poisoning and that prenatally lead-exposed children should be followed longitudinally and examined for possible retinal or visual system alterations.

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