Environmental Health Perspectives
Vol. 89, pp. 21-25, 1990

Effects of Lead on Neurophysiological and Performance Measures: Animal and Human Data

by Hellmuth Lilienthal,* Gerhard Winneke,* and Torsten Ewert†

This paper reports lead-induced changes in neuropsychological measures and behavioral performance measures in monkeys and children. Monkeys were pre- and postnatally exposed to lead via the diet. Blood lead levels at the time of testing were 9.3, 40.3, and 55.7 μg/dL in controls, and animals exposed to 350 ppm or 600 ppm lead acetate, respectively. Flash-evoked and brainstem auditory-evoked potentials were recorded in adult animals. Results indicate latency increases in both measures as well as amplitude decreases in the flash-evoked response. Delayed reaction time and serial choice reaction were determined as measures of behavioral performance in lead-exposed school-age children from two lead smelter areas. In addition, pattern-reversal-evoked potentials and nerve conduction velocity were investigated. Neither nerve conduction velocity nor latency of the pattern-reversal-evoked potential were consistently influenced by lead. Of the behavioral measures, serial choice reaction performance revealed a consistent lead-related deficit, which became more pronounced with increasing task difficulty.

Introduction

Cross-sectional studies relating low-level lead exposure to cognitive and/or attainment deficits in children have often failed to produce consistent results at blood lead levels not exceeding 25 μg/dL or equivalent tooth lead concentrations (*1*). Part of this inconsistency is due both to the known difficulties of cross-sectional as opposed to prospective studies and to the difficult problems of confounding. This latter aspect is particularly relevant for psychometric intelligence as an end point: IQ is known to be influenced by complex interactions of social, hereditary, and home environment factors. Apart from the difficulties of assessing these factors, their relative contribution most likely varies between studies, thus producing varied outcome with respect to the influence of low-level lead exposure.

It is, therefore, highly desirable to check the adequacy of relevant outcome variables that are presumably more resistant to the effects of confounding. Electrophysiological measures as well as performance measures are promising candidates in this respect. The present paper covers findings from animal and human studies dealing with the effects of lead on sensory-evoked potentials and on sensory nerve conduction velocity, as well as with human findings on lead-induced disruption of serial choice reaction performance.

Animal Data

Visual and auditory functions as assessed by means of visual-evoked potentials (VEP) and brainstem auditory-evoked potentials (BAEP) were measured in rhesus monkeys (*M. mulatta*) pre- and postnatally exposed to dietary lead. The exposure regimen has been described elsewhere (*2*). Briefly, female monkeys received either 0, 350 (low lead group) or 600 ppm (high lead group) lead acetate in their diet. Exposure started 50 days prior to mating and was continued until separation when the offspring were 5 months old; the offspring continued to be fed the same diet as their mothers until testing. Five animals were born in the low lead group (three males, two females), six in the high lead group (four males, two females), and six animals served as controls (3 males, 3 females).

After having participated in neurobehavioral studies on learning set formation (*2*), sensory-evoked potentials were measured beginning at age 7 years. Average blood lead levels at that age, measured by means of atomic absorption spectrophotometry (AAS), were 9.3 μg/dL (controls), 40.3 μg/dL (low lead), and 55.7 μg/dL (high lead).

Visual Functions

Methods and results related to visual evoked potentials (VEP) have been published elsewhere (*3*).

*Department of Psychophysiology, Medizinisches Institut für Umwelthygiene an der Universität Düsseldorf, Federal Republic of Germany.
†Department of Neurology, Knappschaftskrankenhaus Würselen, Federal Republic of Germany.
Address reprint requests to H. Lilienthal, Abt. Psychophysiologie, Medizinisches Institut fur Umwelthygiene an der Universität Düsseldorf, Auf'm Hennekamp 50, D-4000 Düsseldorf, Federal Republic of Germany.

Methods. VEPs were elicited with flash stimulation at a frequency of 1.5 Hz. One hundred twenty-eight responses were averaged on a DISA 1500 EMG-system (Dantec, Skovlunde, Denmark). The frequency range of the amplifier was 2 Hz to 0.1 kHz. Needle electrodes were placed under the skin over the right striate cortex and on the midline of the frontal cortex for reference. Potentials were recorded at two levels of background illumination: bright (95 lux) versus dark (0.02 lux). During a single session, 5 VEPs were recorded in each condition, and means were taken across two sessions. Pupil dilation was achieved with one drop each of a 0.2% solution of tropicamide and a 2% solution of phenylephrine. A half circular arrangement of mirrors was placed in front of the monkey's head. The flash was behind and above the head pointing to the mirror image of his face.

Results. Individual VEPs from two animals are shown in Figure 1. Amplitude was defined as the overall amplitude of the signal as described in Figure 1. In the dark background condition, there was a dose-dependent decrease of amplitudes (Fig. 2). The difference was significant for controls versus high-lead animals ($p < 0.05$; Mann-Whitney U-test), and approaching significance for controls versus low-lead animals ($p < 0.1$). For the bright background, there was a significant decrease ($p < 0.05$) of amplitudes in the low-lead group as compared to controls, and a borderline decrease for the high-lead group.

VEP latencies of the latest peak in the first negative deflection of the signal were measured as shown in Figure 1. There was a significant increase in latencies for controls versus high-lead animals ($p < 0.05$) under dark background conditions. For the bright background, a dose-dependent increase in latencies occurred. Differences were significant for both lead groups versus controls. VEP latency results are illustrated in Figure 3.

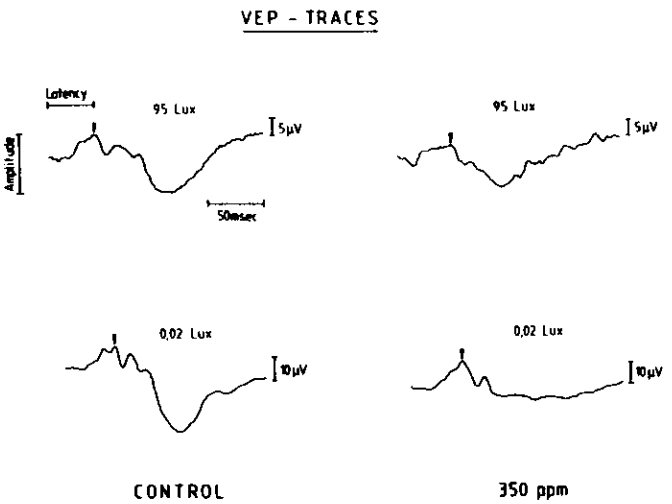

FIGURE 1. Individual VEPs from a control and a lead-treated monkey recorded at different background illuminance levels. The parameters measured, peak latency, and overall amplitude are indicated.

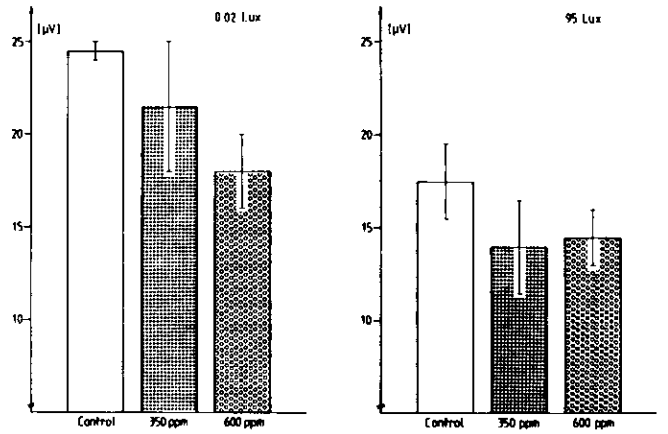

FIGURE 2. Decrease in VEP amplitudes with increasing dietary lead exposure in rhesus monkeys.

Auditory Functions

Otto and co-workers studied auditory function in lead-exposed children (*4–7*). Whereas lead-related increase of hearing thresholds proved replicable (*5–7*), exposure-related BAEP alterations proved less consistent at low blood lead; however, there was a consistent increase in latencies associated with blood lead levels higher than 25 µg/dL.

Methods. BAEPs were recorded using monaural stimulation with broadband rarefactions clicks delivered at five different intensities (73, 83, 93, 103 and 113 dB SPL) by TDH 319p headphones (Telephonics Corp., Huntington, NY). At 113 dB SPL, the repetition rate of the clicks varied as follows: 11.1, 33.3, 55.5, 77.7, and 99.9 Hz. In addition, a masking condition was used; responses were amplified and averaged on a Pathfinder II (Nicolet, Madison, WI). The high pass and low pass filters at the amplifier were 15 and 8000 Hz, respectively. This was preferred over the standard method using a narrow band pass between 150 and 3000 Hz, since several studies have shown distortion of BAEP components by analog filtering (*8,9*). For BAEP recording, monkeys were sedated with 8 mg/kg body weight ketamine followed by 1 mg/kg xylazine.

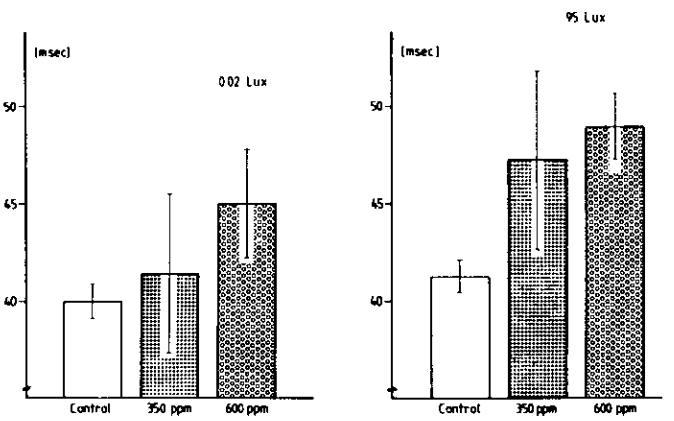

FIGURE 3. VEP latencies are prolonged in lead-exposed monkeys.

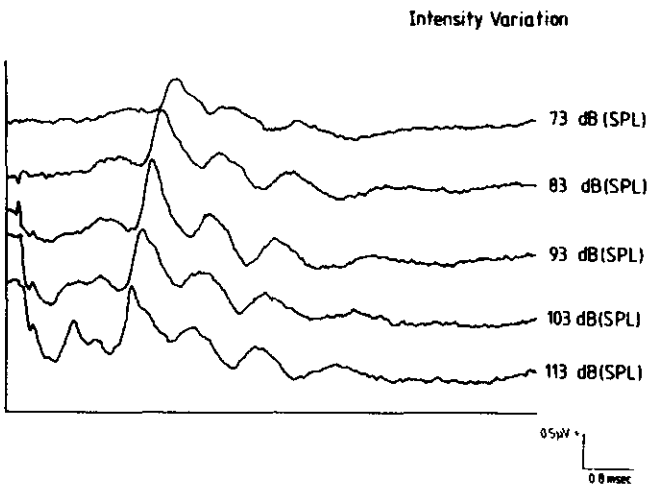

FIGURE 4. BAEPs in monkeys. Latencies increase with increasing click stimulation rate.

Results. In contrast to the human BAEP, in which five early prominent peaks can be detected, only four such peaks are found in monkeys (Fig. 4). This finding is consistent with other investigations of the monkey BAEP. Thus, peaks II, III, and IV in the monkey BAEP represent peaks III, IV, and V in the human record, respectively (*10*). Peaks II and IV were evaluated in our study since they were the most reliable ones. When taking into account all three stimulus conditions, there was a significant overall lead effect according to multivariate analysis of variance for peaks II and IV latencies with repeated measures on the factors conditions and levels [$F(2,13) = 3.84$; $p < 0.05$].

Multivariate analysis of peak II and IV latencies revealed significant lead effects at the rate condition [$F(2,13) = 4.08$; $p < 0.05$] and a borderline effect at the intensity condition [$F(2,13) = 3.12$; $p < 0.1$]. Peak II latencies at different click rates are given in Table 1. The high-lead group deviated from the control animals, the degree of deviation increasing with increasing stimulus rate. According to univariate analysis, the lead-induced latency increase was significant for peak II in the high-lead group [$F(2,13) = 5.14$; $p < 0.05$] at rate conditions, but only marginally significant at conditions of intensity variation [$F(2,13) = 3.66$; $p < 0.06$]. Due to the higher variability of peak IV latencies, a significant increase was found only in male subjects of the high-lead group [$F(2,6) = 5.28$; $p < 0.05$ for intensity and $F(2,6) = 4.79$; $p < 0.06$ for rate]. The masking condition failed to reveal lead effects for any of the BAEP components. The slight decrease of peak II latencies for the low-lead group (Table 1) was not significant.

Human Data

Electrophysiological data have been recorded in occupationally exposed adults (*11*) and in environmentally exposed children as well (*5*). In our studies, apart from cognitive functions (psychometric intelligence, visual-motor integration), electrophysiological measures [VEPs and nerve conduction velocities (NCV)] and performance measures [delayed reaction times (DRT), serial choice reaction performance (SCRP)] were obtained in two independent samples of school-age children in two lead smelter areas in West Germany, namely, in the cities of Nordenham ($n = 114$) and Stolberg ($n = 109$). The Nordenham findings have been published (*12,13*), whereas the more recent Stolberg data, collected in 1986, have not been presented before. Average current blood lead levels (PbB) were 8.2 μg/dL (4.4–23.8) in Nordenham, and 7.4 μg/dL (4.2–18.0) in Stolberg; previous PbBs in the Stolberg children, measured 4 years earlier, were 10.7 μg/dL (4.7–30.8).

Lead concentrations in blood were measured in venous blood samples by means of atomic absorption spectroscopy. Statistical analysis was done by stepwise multiple regression analysis, treating age, gender, and social status as exposure/outcome correlates or as confounders.

Clinical Neurophysiology

Methods. VEPs (pattern reversal) were recorded as described elsewhere (*13*) at 2 Hz under conditions of dim background illumination. The distance of the projection screen was 1 m, its angular size 18°, and the checkerboard pattern size about 45 min of arc. Averaging was done over 128 reversals by means of the DISA 1500 EMG-system. Bipolar recordings were obtained from positions Oz-Fz with filters set between 2 and 100 Hz.

Distal sensory NCVs were measured for the radial and median nerves as described elsewhere (*13*). Briefly, orthodromic stimulation was used at a skin temperature held between 36 and 37 °C, which typically necessitated some heating by an infrared lamp. Four to nine nerve action potentials measured under comparable conditions were averaged to yield one compound action potential.

Results. NCVs for radial nerves from both study areas are presented in Figure 5. The abscissa represents midpoints of PbB classes, each range covering 20% of the respective sample. Means and SEMs are given. In the initial Nordenham-sample tested in 1982 (age 6–7 years), contrary to expectation, NCVs increased with increasing PbB in a significant manner for both nerves (medial nerve not shown here). This unexpected finding was, however, not supported in the follow-up study 3 years later: no significant increase was observed.

Table 1. Brainstem auditory-evoked potentials (rate condition): peak II latency.

	Peak II latency, msec[a]				
	Hz				
Group	11.1	33.3	55.5	77.7	99.9
Controls	1.970 ± 0.039	2.134 ± 0.054	2.194 ± 0.059	2.261 ± 0.077	2.350 ± 0.126
350 ppm	1.982 ± 0.065	2.078 ± 0.047	2.138 ± 0.047	2.176 ± 0.048	2.264 ± 0.088
600 ppm	2.067 ± 0.083	2.225 ± 0.125	2.312 ± 0.118	2.395 ± 0.110	2.500 ± 0.094

[a]Values are means ± 95% confidence limit.

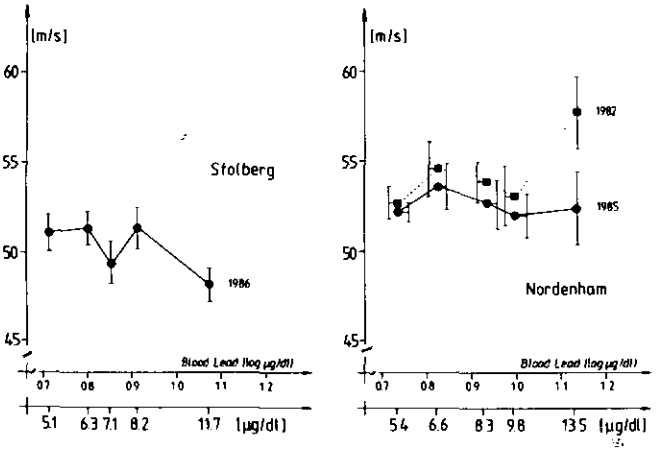

FIGURE 5. Nerve conduction velocity (radical nerve) in lead-exposed children from two study areas (1985 is a follow-up study in a subsample).

Opposite trends occurred in the Stolberg sample (age 7–9 years). NCVs decreased with increasing PbB; for the radial nerve this trend was even significant after correction for confounding ($p < 0.05$), which was not true for median nerve NCVs.

Results for VEP latencies are shown in Figure 6. In the initial Nordenham-study P_2-latencies decreased with increasing PbB ($p < 0.05$). Again, an opposite trend was observed in the Stolberg sample, although this finding did not reach significance. No significant PbB associations were observed for VEP component N_2 in either study.

Reaction Performance

Following Needleman et al. (*14*), DRTs were measured in both studies; in addition, serial choice reaction performance was assessed.

Methods. DRTs were measured at 3 and 10 sec delay across 10 trials as described previously (*12*). Latencies of button-releasing and pressing were recorded. The warning stimulus was a tone, and the reaction stimulus was a light bulb located above the response button.

Serial choice reaction performance was assessed using the Wiener Determinationsgerät (Vienna Reaction Device), requiring that subjects respond to a random series of colored light signals, as well as to occasional tone signals, by pressing appropriate buttons. One hundred eighty successive signals are presented. In both studies the task was run at two age-adjusted signal-rate conditions, namely, a low, easy rate and a high, difficult rate condition. Correct (hits), false (errors), and late responses were recorded as performance measures.

Results. DRTs did not exhibit significant associations with the lead variable in any of the studies. As for serial choice reaction performance, only hits and errors exhibit significant associations with the lead variable in at least one study. In the Nordenham study (*12*), hits decreased with increasing PbB in a significant manner ($p < 0.05$); this effect was most pronounced in the high-rate condition and was also observed in the follow-up study (*14*). In the Stolberg study, however, hits were not significantly associated with lead exposure.

Contrary to correct responses (hits), errors were found to vary directly with current PbB in a significant manner in both studies (Fig. 7). Both in Nordenham and in Stolberg, the error-increase with increasing PbB was more pronounced for the high rate than for the low-rate conditions of the Vienna reaction device. This effect was highly significant ($p < 0.001$) or significant ($p < 0.05$) for the Nordenham and the Stolberg samples, respectively, after correction for confounding.

Summary and Conclusions

Central visual processing, as reflected in VEPs, is clearly affected by lead-exposure in monkeys: Amplitudes are smaller and latencies longer, even at blood lead levels around 40 µg/dL.

Significantly longer latencies were found for peaks II and IV of the monkey BAEP, corresponding to peaks III and

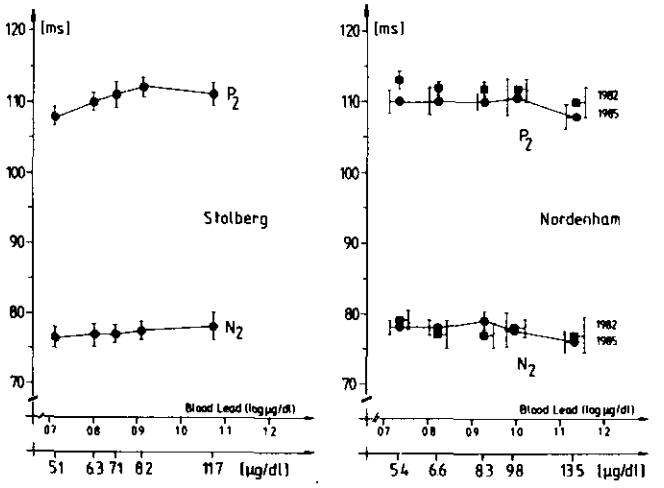

FIGURE 6. VEP latencies in lead-exposed children from two study areas.

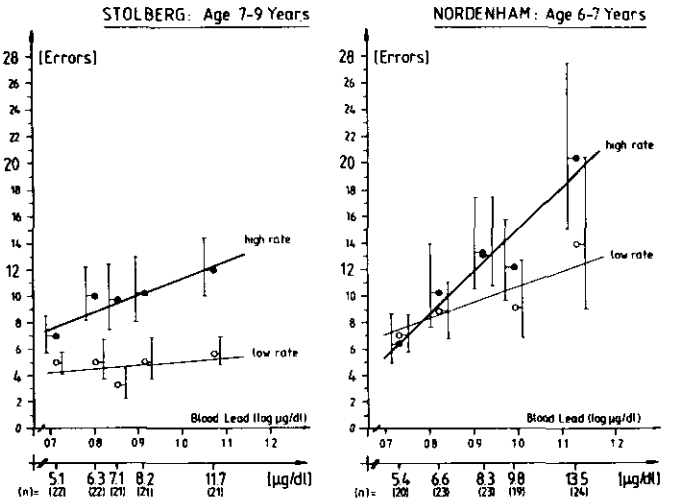

FIGURE 7. Error increase with increasing PbB in a serial choice reaction task run at low and high signal rate conditions. From Winneke et al. (*18*).

V of the human BAEP. In principle, these data support the early findings of Otto et al. (*4*), although the effects in our study seem to have occurred at higher PbBs, than those encountered by Otto and co-workers. Since auditory threshold shifts have been found to occur at much lower PbB (*6*), it is doubtful whether early auditory information processing, as reflected in the BAEP, is sufficient for a full explanation of lead-induced hearing loss.

Although the more recent Stolberg findings on lead-related alterations of sensory nerve conduction velocities and visual- evoked potentials are more consistent with what has been observed in animal studies and in occupational settings, there are no obvious reasons to discard the Nordenham findings. Thus, the results from both studies, taken together, suggest that lead-induced alterations of VEP or NCV in children cannot be expected to occur at PbB levels as low as those encountered in our studies.

Impairment of serial choice reaction performance, as assessed by means of the Vienna reaction device, was consistently associated with low-level childhood lead exposure in both our studies, and performance disruption exhibited an interesting interaction with task difficulty. It should, furthermore, be pointed out that in the Nordenham follow-up study, the same interaction proved significant again (*14*). Similar findings were previously observed in our first Stolberg study based on tooth lead concentrations (*15*) and have recently been reported in Greek children from a lead smelter area as well (*16*). Such performance deficit, bearing resemblance to clinical findings in children with attention deficit disorder, appears to be both a sensitive and robust low-level lead effect in children (*17*).

This study was partly supported by the Commission of the European Communities (CEC), contract No. ENV-733-D(B). The authors wish to thank Arthur Brockhaus (Dusseldorf) and Christian Krause (Berlin) for the lead analyses and Hannelore Krull and Hildegard Schardt for technical assistance.

REFERENCES

1. Lansdown, R. Lead, intelligence, attainment and behavior. In: The Lead Debate: The Environment, Toxicology and Child Health (R. Lansdown and W. Yule, Eds.), Croom Helm, London, 1986, pp. 235–270.
2. Lilienthal, H., Winneke, G., Brockhaus, A., and Molik, B. Pre and postnatal lead exposure in monkeys: effects on activity and learning set formation. Neurobehav. Toxicol. Teratol. 8: 265–272 (1986).
3. Lilienthal, H., Lenaerts, C., Winneke, G., and Hennekes, R. Alteration of the visual evoked potential and the electroretinogram in lead-treated monkeys. Neurotoxicol. Teratol. 10: 417–422 (1988).
4. Otto, D., Robinson, G., Baumann, S., Schroeder, S., Mushak, P., Kleinbaum, D., and Boone, L. 5-Year follow-up study of children with low-to-moderate lead absorption: electrophysiological evaluation. Environ. Res. 38: 168–186 (1985).
5. Robinson, G. S., Baumann, S., Kleinbaum, D., Barton, C., Schroeder, S., Mushak, P., and Otto, D. Effects of low to moderate lead exposure on brainstem auditory evoked potentials in children. In: WHO Environmental Health Document, No. 3. Neurobehavioral Methods in Occupational and Environmental Health, WHO Regional Office for Europe, Copenhagen 1985, pp. 177–182.
6. Schwartz, J., and Otto, D. Blood lead, hearing thresholds, and neurobehavioral development in children and youth. Arch. Environ. Health 42: 153–160 (1987).
7. Robinson, G. S., Keith, R. W., Bornschein, R. L., and Otto, D. A. Effects of environmental lead exposure on the developing auditory system as indexed by the brainstem auditory evoked potential and pure tone hearing evaluations in young children. In: Heavy Metals in the Environment. International Conference Proceedings, Vol. 1 (S. E. Lindberg and T. C. Hutchinson, Eds.), CEP Consultants, Edinburgh, 1987, pp. 223–225.
8. Doyle, D. J., and Hyde, M. L. Analogue and digital filtering of auditory brainstem potentials. Scand. Audiol. 10: 81–89 (1981).
9. Janssen, R., Benignus, V. A., Grimes, L. M., and Dyer, R. S. Unrecognized errors due to analog filtering of the brain-stem auditory evoked response. EEG Clin. Neurophysiol. 65: 203–211 (1986).
10. Moller, A. R., and Burgess , J. Neural generators of the brainstem auditory evoked potentials (BAEPs) in the rhesus monkey. EEG Clin. Neurophysiol. 65: 361–372 (1986).
11. Seppalainen, A. M., Tola, S., Hernberg, S., and Kock, B. Subclinical neuropathy at "safe" levels of lead exposure. Arch. Environ. Health 30: 180–183 (1975).
12. Winneke, G., Beginn, U., Ewert, T., Havestadt, C., Kraemer, U., Krause, C., Thron, H. L., and Wagner, H. M. Comparing the effects of perinatal and later childhood lead exposure on neuropsychological outcome. Environ. Res. 38: 155–167 (1985).
13. Ewert, T., Beginn, U., Winneke, G., Hofferberth, B., and Joerg, J. Sensible Neurographie, visuell and somatosensorisch evozierte Potentiale (VEP und SEP) an bleiexponierten Kindern. Nervenarzt 57: 465–471 (1986).
14. Needleman, H. L., Gunnoe, C., Leviton, A., Eed, R., Peresie, H., Maher, C., and Barrett, P. Deficits in psychologic and classroom performance of children with elevated dentine lead levels. N. Engl. J. Med. 300: 689–695 (1979).
15. Winneke, G., Collett, W., Kraemer, U., Brockhaus, A., Ewert, T., and Krause, C. Follow-up studies in lead-exposed children. In: Lead Exposure and Child Development: An International Assessment (M. Smith, L. Grant, and A. Sors, Eds.), Kluwer Academic Publishing Dordrecht, 1989, pp. 260–270.
16. Winneke, G., Kraemer, U., Brockhaus, A., Ewers, U., Kujanek, G., Lechner, H., and Janke, W. Neuropsychological studies in children with elevated tooth-lead concentrations II. Extended study. Int. Arch. Occup. Environ. Health 51: 231–252 (1983).
17. Hatzakis, A., Kokkevi, A., Katsouyanni, K., Maravdias, K., Salaminios, F., Kalandidi, A., Loutselinis, A., Stefanis, K., and Trichopoulos, D. Psychometric intelligence and attentional performance deficits in lead-exposed children. In: Heavy Metals in the Environment. International Conference Proceedings, Vol. 1 (S. E. Lindberg and T. C. Hutchinson, Eds.), CEP Consultants, Edinburgh, 1987, pp. 204–209.
18. Winneke, G., Brockhaus, A., Collet, W., and Kraemer, U. Modulation of lead induced performance deficit in children by varying signal rate in a serial choice reaction task. Neurotoxicol. Teratol. 11: 587–592 (1989).