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Evoked Potential Alterations Following Prenatal Methyl Mercury Exposure[1]

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DYER, R. S., C. U. ECCLES AND Z. ANNAU. *Evoked potential alterations following prenatal methyl mercury exposure.* PHARMAC. BIOCHEM. BEHAV. 8(2) 137–141, 1978. – Pregnant hooded rats were administered either 5 mg/kg CH_3 Hg or 0 mg/kg CH_3 Hg by gastric intubation on day seven of gestation. Female offspring were implanted with recording electrodes 60 days after birth and had their cortically recorded visual evoked potentials studied at four different flash intensities. Mercury exposed animals had higher P1–N1 and N1–P2 amplitudes and shorter P2 and N2 latencies than controls. The data provides evidence that a single ingestion of CH_3 Hg by pregnant rats is sufficient to produce long term alterations in CNS activity.

Prenatal Methyl mercury Visual evoked potentials Hooded rats Females Toxicity

METHYL mercury (CH_3 Hg) is known to be a potent neurotoxin which in sufficient quantities produces a variety of symptoms, including peripheral neuropathies [4], visual deficits [6] and cerebellar degeneration [12]. In addition to these effects of frank methyl mercury intoxication, more subtle effects have been observed to result from low level exposure. Indeed, exposure of pregnant mice to doses of CH_3 Hg which produce no obvious impairment in the mothers are clearly capable of producing CNS damage to the offspring [9,13].

Recently electrophysiological methods have been used to detect CNS damage induced by exposures to organic Hg [11,15]. It was reported that in anesthetized rats, the cortically recorded somatosensory evoked potential waveform began to change after 18 daily IP injections of 2 mg/kg methoxy-ethyl-mercury chloride [11]. The changes reported were characterized by gradually increasing latencies of late peaks in the evoked potential.

When flash evoked potentials were recorded from the cortex of anesthetized 30 day old male offspring of albino rats exposed to 2.5 mg/kg/day CH_3 Hg in their drinking water throughout pregnancy, latencies were decreased [15]. These results are puzzling, first because they are quite different from those reported for somatosensory evoked potentials, and secondly because it is difficult to understand how CH_3 Hg could increase conduction velocity. Both experiments suffer from the shortcoming of having failed to adequately measure evoked potential amplitudes, and both were performed upon chloralose anesthetized animals. In the present experiment an attempt was made to improve the sensitivity of the evoked potential technique by making recordings from unanesthetized animals and including amplitude measurements as part of the protocol.

METHOD

Long-Evans hooded rats were obtained from Blue Spruce Farms and housed in the laboratory for two weeks before breeding. Experienced males were then placed with naive females weighing between 270 and 320 g. Twelve hr later vaginal smears were taken, and if found to be sperm positive the females were immediately removed and housed individually. On gestational Day 7, four pregnant rats were given a single dose of 5 mg/kg CH_3 Hg as CH_3 Hg Cl dissolved in corn oil and administered by gastric intubation. This dose was chosen to be representative of those low doses at which behavioral effects had been reported in mice [9]. Six pregnant control rats received corn oil alone.

Litters were reduced to eight on Day 1 post-partum (average pup weight 64.0 g control, 64.8 g methyl mercury), weaned at 22 days of age (average pup weight 45.7 control, 53.3 methyl mercury), and housed by sex in groups of 2–4 until Day 65. On Day 65, the males were removed for subsequent testing and the females (average weight 244 g for controls, 258 for methyl mercury) were anesthetized with 0.3 ml/100 g of Equithesin and implanted with 0–80 x 1/16 in. stainless steel screws for recording the cortical evoked potential. The active screw was placed 5.0 mm posterior to bregma and 3.0 mm lateral to the midline, the reference screw was placed 1.0 mm anterior to

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bregma and 1.0 mm lateral, and a third screw was placed 5.0 mm anterior to bregma for purposes of grounding the animal. The screws were connected by nichrome wires to an Amphenol receptacle, which was then fixed to the skull with dental acrylic. All animals were given 100,000 units of bicillin and placed into individual cages for the remainder of the experiment.

Successful implants were performed upon 18 control and 13 CH_3 Hg exposed rats from six and four litters, respectively, and recordings were made following a two week recovery period. The procedure for recording has been described in detail elsewhere [3]. Briefly, pupils were dilated with Cyclogyl 30 min before the session began. Five min before the session the animals were connected to the recording apparatus and placed in a recording chamber which had reflecting surfaces on three walls and a Grass strobe mounted outside the fourth. The light was flashed at 0.4 Hz about 300 times before any recordings were made.

During the recording session, the potentials evoked from 100 flashes were averaged at each of four different flash intensities. The flashes, presented at 0.4 Hz, were 10 μsec duration with intensities that corresponded to settings 1, 2, 4 and 16 on the Grass strobe unit, or roughly 9.4×10^4, 1.0×10^5, 3.8×10^5, and 1.5×10^6 candlepower. All flashes at a given intensity were completed before the intensity was changed, and the different intensities were presented in counterbalanced order. This procedure was repeated at least one day later, the two values obtained from each parameter measured were averaged, and the resulting value was used in statistical analyses.

Recordings were made by connecting the animals via low noise shielded cable to Tektronix 122 preamplifiers with high and low frequency cutoffs set at 10 Hz and 0.2 Hz, respectively. Amplified signals were led to an oscilloscope for monitoring and to a PDP–8 computer for averaging. The poststimulus analysis epoch was 240 msec, each msec representing 1 bin of a 240 point plot display.

Averaged waveforms were displayed on an oscilloscope, and a cursor controlled by the teletype and one analog channel was moved along the averaged waveform by the experimenter. Upon signal the latency in msec and amplitude in μV of the bin corresponding to the cursor location was printed. In addition, Polaroid pictures were taken of the averaged waveforms.

The latencies and peak-to-peak amplitudes of the five major peaks of the cortical evoked potential were measured using common designations [6]. The waveform notation is illustrated in Fig. 3C. Two factor repeated measures unweighted means analyses of variance were performed on the resulting 10 sets of data. The design of the experiment was thus a 2 x 4, with the 2 groups being control female and CH_3 Hg female, and the four treatments being the four different light intensities. Statistical significance was judged to be met with probabilities ≤ 0.05.

RESULTS

Mean litter sizes for the CH_3 Hg and control groups were 11.5 (± 1.13 SEM) and 12.8 (± 0.73 SEM), respectively. The effects of methyl mercury exposure and light intensity on amplitude of the P1–N1 and N1–P2 components of the evoked potential are shown in Fig. 1. Exposure to methyl mercury significantly increased P1–N1 amplitudes. Increasing light intensity also increased P1–N1 amplitudes, and evaluation of the significant group x intensity interaction

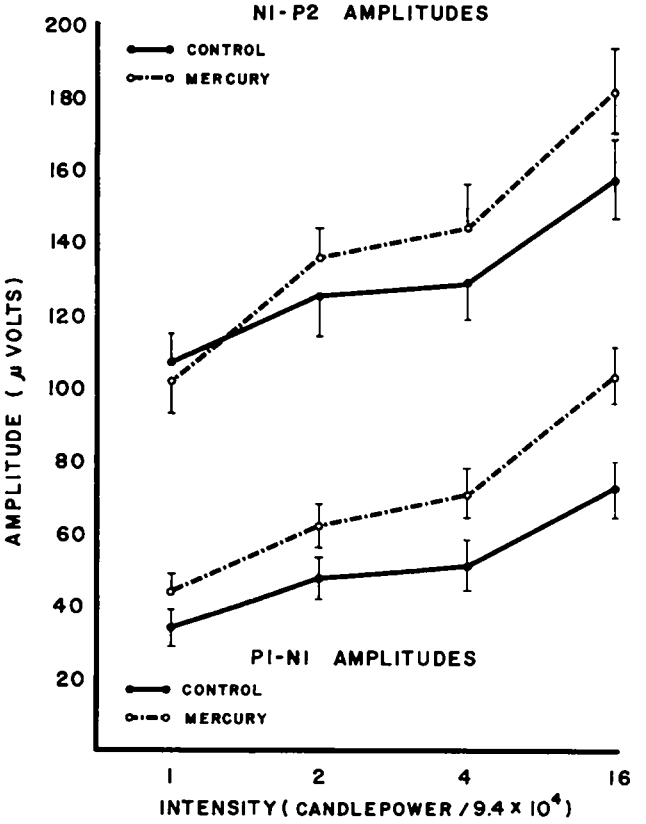

FIG. 1. Effects of prenatal exposure to mercury and changes in light intensity upon P1–N1 and N1–P2 amplitudes of the cortically recorded flash evoked potential. Vertical bars represent the standard error of the mean.

revealed that the methyl mercury exposed animals had a greater increase in amplitude at the highest intensity than did the controls.

There was no overall effect of methyl mercury upon amplitudes of the N1–P2 peak. These amplitudes did increase with increasing stimulus intensity, and a significant group x intensity interaction again revealed that the methyl mercury exposed animals increased their amplitudes more at the highest stimulus intensity than the controls.

Figure 2 shows the effects of methyl mercury exposure and light intensity upon the N2–P3 and P3–N3 amplitudes. Increasing light intensity decreased the amplitudes of N2–P3 components, but exposure to methyl mercury did not significantly affect them, nor was the group x intensity interaction significant. Exposure to methyl mercury did not significantly affect P3–N3 amplitudes but increasing light intensity did significantly increase them.

The effects of mercury and light intensity upon latency of the five peaks are shown in Table 1. Both P2 and N2 latencies were significantly shorter in the Hg exposed group. N1 latencies of both groups became significantly shorter with increasing flash intensities.

DISCUSSION

The results demonstrate that prenatal exposure of rats to a low level of methyl mercury is sufficient to produce long term alterations in the cortically recorded flash evoked

TABLE 1

EFFECTS OF MERCURY AND LIGHT INTENSITY UPON LATENCY
(msec ± SEM)

		Intensity			
		1	2	4	16
N1	Control	32.0 ± 0.57	30.8 ± 0.52	30.7 ± 0.43	28.8 ± 0.34
	Hg	32.1 ± 0.47	30.9 ± 0.46	30.4 ± 0.45	28.5 ± 0.42
P2	Control	47.1 ± 0.79	46.7 ± 0.74	48.2 ± 1.11	47.4 ± 0.79
	Hg	45.2 ± 0.65	45.3 ± 0.31	45.0 ± 0.83	45.2 ± 0.64
N2	Control	64.9 ± 1.18	65.0 ± 1.71	65.3 ± 1.52	67.1 ± 1.85
	Hg	61.3 ± 1.20	59.2 ± 1.29	60.3 ± 1.96	60.7 ± 2.28
P3	Control	86.4 ± 2.69	86.8 ± 1.76	85.6 ± 1.66	87.9 ± 2.58
	Hg	85.6 ± 1.77	80.9 ± 1.63	83.3 ± 1.69	83.5 ± 2.02
N3	Control	164.0 ± 3.90	170.4 ± 5.28	162.3 ± 4.42	164.8 ± 4.27
	Hg	181.5 ± 7.82	175.3 ± 6.26	176.5 ± 5.39	178.3 ± 4.45

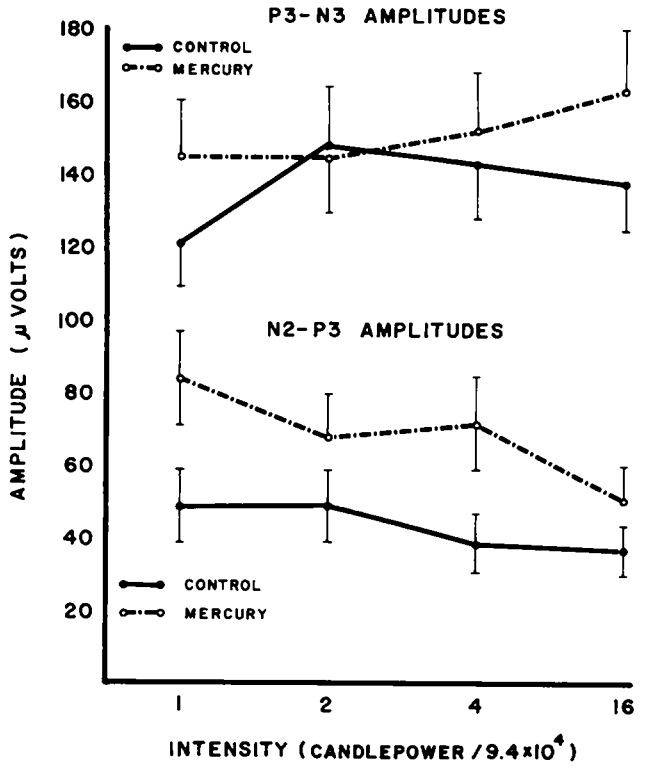

FIG. 2. Effects of prenatal exposure to mercury and changes in light intensity upon N2–P3 and P3–N3 amplitudes of the cortically recorded flash evoked potential. Vertical bars represent the standard error of the mean.

potential. In the present experiment more dramatic effects of mercury were observed with the amplitude data than with the latency data, and therefore future efforts to use the evoked potential technique as a sensitive assay for CNS change should include both types of measurement.

A common interpretation of the cortically recorded flash evoked potential is that the P1–N1 and N1–P2 amplitudes are measures of activity in the retino-striate system, and that the P2–N2 and N2–P3 amplitudes are measures of activity in other pathways [2]. Alterations in electrocortical arousal, at least those which are pharmacologically induced, are presumed to be reflected in the amplitude of the P3–N3 peak [8]. Since exposure to mercury did not alter the P3–N3 amplitudes, one may infer that these animals were not differentially aroused compared to controls. Increased P1–N1 amplitudes indicate functional alterations in the retino-striate system. Although the functional significance of these alterations is not yet apparent, it should be pointed out that similar changes have been observed in rats prenatally exposed to low levels of carbon monoxide [4]. It may also be significant that amplitudes of early components of the somatosensory evoked potential recorded from human females are greater when derived from a population diagnosed as having psychiatric illness than when derived from a presumably normal population, and that these increases are more evident at high stimulus intensities [1].

The results from these different experiments suggest that the pattern of amplitude change demonstrated by the group × intensity interaction in the present study and characterized by an increased amplitude in exposed animals which becomes more evident at higher stimulus intensities, is common to a number of abnormal populations. Since early exposure to lead has been shown to decrease early amplitudes of cortically recorded evoked potentials from rats [5], alternative patterns of change in evoked potential amplitudes are clearly possible. Early exposure to any intoxicant can be expected to produce a pattern of dose related changes, and it is possible that both patterns mentioned above could occur with any agent if the appropriate dose were chosen. Functional correlates of both patterns of change must be determined in order to estimate what sorts of behavioral change to expect when these electrophysiological alterations are observed.

The finding that mercury exposed animals have slightly shorter P2 and N2 latencies than controls partially confirms the report by Zenick [15] while obviating the difficulties attendant to procedures which require subtraction of interpeak latencies. The most simple explanation of decreased latency is increased conduction velocity, but other explanations are equally plausible. Thus, if one considers each peak in the evoked potential to have been produced by a population of inputs with variable conduction velocity, a

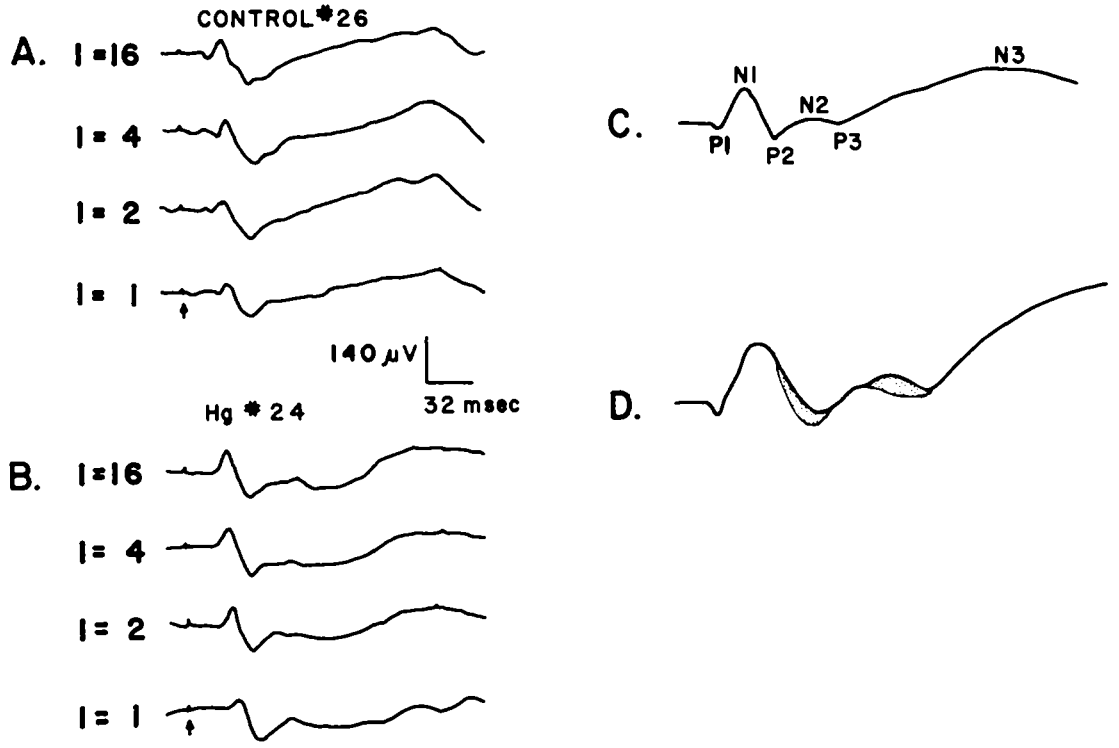

FIG. 3. A and B: Tracings made from the Polaroid pictures taken of the averaged evoked potential in a control rat (A) and exposed rat (B) at the four different flash intensities. Arrow indicates stimulus artifact. C: Schematic representation of the evoked potential showing the peak designations employed in this experiment. D: Diagram illustrating the way in which loss of slowly conducting inputs to the N1 and N2 complexes might change the waveform. Stippled area indicates the portion of the waveform which would theoretically be lost with loss of slowly conducting inputs. See text for further explanation.

process which selectively impairs the slower conduction velocity fibers would have the effect of shifting the peak of activity towards the faster conduction velocity inputs. This concept is illustrated in Fig. 3D, where the stippled segments represent those parts of the wave which might reflect activity in slower conduction velocity axons. Support for such an interpretation comes from reports that small diameter cells are more sensitive to mercury toxicity than larger cells [10], together with the generally acknowledged relationship between small diameter cells and small diameter slowly conducting axons [14].

The results obtained here are quite different from those of Lehotzky and Meszaros [11], who found that long term exposure of adult rats to methoxy-ethyl-mercury decreased conduction velocity. It seems likely that the discrepancy is secondary to the much lower dose and prenatal exposure used in the present experiment.

In conclusion, the present results indicate that a single ingestion by pregnant rats of 5 mg/kg CH_3 Hg is sufficient to produce alterations in the offspring which can be measured in adulthood using the visual evoked potential method. The changes probably reflect impaired processing in the retino-striate system, and may also indicate damage to slowly conducting axons.

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