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Evoked Potential Alterations in Methylmercury Chloride Toxicity[1]

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ZENICK, H. *Evoked potential alterations in methylmercury chloride toxicity.* PHARMAC. BIOCHEM. BEHAV. 5(3) 253–255, 1976. — The present study was designed to assess the sensitivity of evoked potential techniques to detect alterations in offspring exposed to methylmercury chloride (MMC) at different developmental periods. Recordings were obtained from the visual cortex (VEP) and lateral geniculate (LGP) in 30-day old offspring. Results revealed decreased VEP latencies for peaks N1, P1, and P2 in offspring from mothers exposed either during gestation, or nursing and in offspring exposed directly to MMC for 9 days after weaning. Although not significant, a similar trend was observed in the LPG. It is suggested that the decreased latencies may be a result of compressed brain development.

Methylmercury Evoked potentials

IN AN earlier paper [12], we reported learning deficits in 30-day-old offspring if (1) mothers were exposed to methylmercury chloride (MMC) during gestation or (2) if offspring were exposed directly to MMC following weaning. These learning deficits still persisted when the animals were retested 21 days later, in the absence of detectable levels of mercury in the brain. Offspring from mothers treated during nursing only did not differ from controls.

In the present study, evoked potentials (EP) were recorded on littermates to determine if this technique would yield a similar clinical classification regarding critical exposure periods. Furthermore, the sensitivity of the evoked potential as a diagnostic tool was assessed.

METHOD

Animals

The animals were derived from the breeding of 35 female Holtzman albino rats (Holtzman, Madison, Wisconsin) with males of the same strain. Vaginal lavages were taken to confirm the presence of sperm and the onset of pregnancy. The mothers were caged individually with ad lib food and water. At weaning, the offspring were earmarked and housed individually.

Groups and Conditions

The treatment protocol has been described in detail elsewhere [12]. Five pregnant rats were assigned to each group, with litter size manipulated at birth to insure that each mother nursed 8 offspring. Water consumption and weight gains were noted daily for mothers and offspring with these figures being used to compute the amount of MMC to be added to the water to achieve the approximate dosage of 2.5 mg Hg/kg/day. Pilot work had revealed this to be a dosage the animals would readily comsume and that did not result in the appearance of clinical symptoms (e.g., weight loss, paraplegia, etc.). Water bottles were situated so that only the mothers could drink during nursing.

Three developmental periods were defined: gestation, nursing, and postweaning. For the gestation (onset of pregnancy to parturition) and nursing (birth to 21 days) intervals, MMC was administered to the animals' mothers. For the postweaning interval (21 days of age to 30 days of age), MMC was administered directly to the offspring. Six groups of animals were used. Group G received MMC only during gestation; Group N received MMC only during nursing; and Group PW received MMC only during the postweaning period. Control animals (Group C) were never exposed to MMC. Since Group G animals could be exposed to MMC from both gestational administration and from any residual carryover to the nursing interval, two cross-fostered groups were used: Group CFN was born to control mothers but nursed by mothers that had received MMC during gestation; Group CFG was exposed to MMC during gestation but nursed by control mothers.

Procedure

Five, 30-day old, male rats/group were utilized for recording (one randomly selected from each of the five mothers that had been assigned to each treatment group). With the animal under alpha-chloralose anesthetic (60 mg/kg, IV; 10 mg/ml in 19% NaCl), visual cortex evoked potentials (VEP) were recorded from a right hemisphere epidural electrode (0.080 stainless steel machine screw).

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Electrode placement was 2 mm lateral to lambda against an indifferent screw 3 mm anterior and 1 mm lateral to bregma. Lateral geniculate potentials (LGP) were recorded bipolarly from parallel stainless steel electrodes with 0.5 mm bared tips and a tip to tip separation of 0.5 mm. These electrodes were stereotaxically aimed at pars dorsalis of the right lateral geniculate according to the coordinates of Sherwood and Timiras [8], namely, A. P., 2.9; L, 3.9. The VEP, LGP, and the 20 μV, 20 msec square wave from a calibration unit were each led through identically set and calibrated differential amplifiers (Textronix 2A61) with high and low −30 db filters at 600 Hz and 6 Hz, respectively. One hundred and twenty responses were averaged with a TII Computer of Average Transients (CAT) and plotted with a X-Y Plotter (BBN Plotomatic 600). An American Electronics 104A stimulator triggered the CAT, calibration unit, and a Grass PS-2 photostimulator at 0.3 Hz. The strobe head of the photostimulator was placed in a sound-attenuated box centered 21.59 cm from the rat's eyes. On completion of recording, the brain was immediately removed and stored in 10% formalin. Ten micron sections were stained with hematoxylin and eosin for verification of electrode placement.

RESULTS AND DISCUSSION

Water consumption, weight and weight gains did not differ among mothers or among their offspring, nor were there differences in litter size.

Both the VEP and LGP showed early-late, negative-positive complexes similar to those previously described for rats [3, 5, 7]. Four peaks of these complexes were chosen for analyses and were labelled N1, P1, N2, and P2 as shown on the representative VEP and LGP in Fig. 1.

Both VEPs and LGPs showed marked inter-animal, within-group variability in amplitude, to an extent that precluded inferential analyses. Analyses of variance were run on the VEP and LGP group latencies for N1 and on the difference score for P1 latency minus N1 latency (P1 − N1), N2 − P1, and P2 − N2 latencies. The ANOVAs were run on these difference scores rather than the actual latencies of these peaks in order to partial out the latency effect of an early component that might carry over and contribute to the latency of the later waves.

Significant VEP differences were found for the N1 latency, $F(5,24) = 2.84$, $p \leq 0.05$, and latency difference scores for P1 − N1, $F(5,24) = 3.8$, $p \leq 0.01$, and P2 − N2, $F(5,24) = 10.43$, $p \leq 0.01$, comparisons. The group mean latency for N1, P1 − N1, N2 − P1, and P2 − N2 are presented in Fig. 2. The significant N1 effect was attributed to Groups G and CFG having significantly shorter latencies as compared to the controls, Group C (Duncan's Multiple Range Test, $p \leq 0.05$). The significant P1 − N1 and P2 − N2 latency differences were a result of decreased latencies for the P1 and P2 waves in Groups G, CFG, PW, and N as compared to Groups C or CFN (Duncan's, $p \leq 0.01$).

Similar analyses, applied to the LGP data, did not reveal significant differences.

The evoked potential results distinguish the treatment groups in a manner similar to that seen in the behavioral task [12], in that Groups G, CFG, and PW may be classified apart from Groups C and CFN. The one exception to this is that while Group N was classified as unaffected by behavioral data, the evoked potential analyses relegates Group N to the affected category. That only the gestation exposed groups, G and CFG, had shorter N1 latencies may suggest a greater influence of MMC at this exposure time relative to the other periods examined. It is apparent, however, that exposure during gestation, or nursing (beyond the residual carryover period, Group CFN), or postweaning may be sufficient to induce neurotoxic damage. It is somewhat surprising that Group CFN was not different from the controls; however, three of its waves (N1, P1, and P2) did have shorter latencies than Group C. The residual in the mothers' milk may have been insufficient, the exposure time during nursing too brief, or both, to allow a significant effect to emerge.

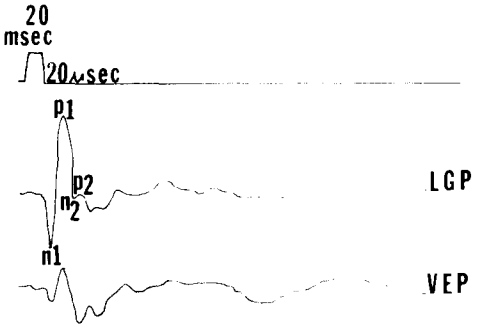

FIG. 1. Representation of peaks selected for analyses of visual cortical evoked potential (VEP) and lateral geniculate potential (LGP).

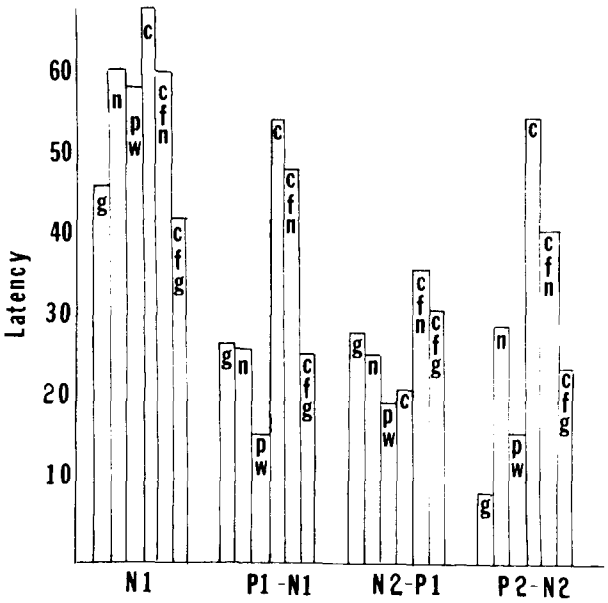

FIG. 2. Group mean latency for N1 and group mean latency difference scores for P1 − N1, N2 − P1, and P2 − N2. Group designation defined in text.

The evoked potential data are in agreement with behavioral and anatomical studies suggesting the visual cortex as one of the sites of MMC action [1, 4, 10]. However, the interpretation of decreased latencies is difficult. The possibility that MMC in the brain may have been directly responsible for the effect is not reasonable in light of biochemical analyses conducted on 30-day old littermates revealing MMC to be present only in the brains of

offspring still exposed at the time of recording, Group PW [12]. Lehotzky and Mezaros [6] recorded somatosensory evoked potentials in 140–160 g rats injected IP with methoxyethylmercury for 6 weeks. They found diminished amplitudes and increased duration of the first positive wave. Since the amplitude in the present study was not analyzed, a comparison of this parameter cannot be drawn; however, the increased duration observed by Lehotzky and Mezaros [6] would appear to be in contradiction to the decreased latencies seen in this study. Unfortunately, differences in age, route, dosage, duration and method are great enough between the two studies to prevent any valid conclusions.

A study by Sobotka *et al.* [9] revealed the earlier appearance of eye opening and clinging ability in pups whose mothers were exposed to MMC during gestation. The authors suggest that this may be a result of MMC compressing brain development into a shorter temporal interval, limiting the behavioral flexibility of the organism. The relationship between this postulated compressed brain development and the decreased latencies seen in this study needs to be explored.

Another explanation that remains to be examined is the possibility of an increased sensitivity of the MMC animals to the anesthetic employed in this study. However, this explanation seems unlikely, since chloralose primarily seems to increase the amplitude of the evoked potential [11].

There is also the suggestion that this mechanism may be peculiar to the heavy metals, since recent work in our lab has indicated a similar reduction in VEP latency for offspring from mothers exposed to lead during gestation [2]. Although this mechanism requires further elucidation, the evoked potential technique may be as sensitive, if not more so, than conventional assessment techniques (as witnessed by the reclassification of Group N as affected).

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