Changes in Middle Latency Auditory-Evoked Potentials of the Rat Exposed to Styrene

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(Received January 7, 2001; Accepted January 22, 2001)

Effects of styrene exposure on middle latency auditory-evoked potentials (MAEPs), which reflect the central auditory function, were investigated. As significant delay in latencies of No, Po, Na, and Pa components and increases in the peak to peak intervals of MAEPs, but not amplitudes, were observed in a dose–dependent manner. The present and our previous studies demonstrate that electrophysiological alterations occur before neuronal cell damage induced by styrene exposure. The present results suggested that styrene has a marked effect on the processing of auditory information related to MAEPs.

Key words —— styrene, middle latency auditory-evoked potential, auditory function, toxicity

INTRODUCTION

The styrene monomer is an aromatic industrial solvent. It is used for example, to make polystyrene, resins and rubbers. Because styrene has high affinity for lipid-rich tissue, like brain tissue, the nervous system seems to be sensitive to styrene toxicity.[1–3] The effects of styrene on both central and peripheral nervous systems have been demonstrated in many previous studies[4–6] Jegaden *et al*. reported neurobehavioral effects of styrene exposure at work.[7] The symptoms noted were memory disturbance, nausea, dizziness, irritability, *etc*. Neurophysiological effects of styrene were also studied in humans as well as in animals.[8–10] In addition, an investigation by Mutti *et al*. showed that styrene exposure to rabbits resulted in a marked dose–dependent decrease in the striatal dopamine (DA) concentration.[11,12] Moreover, a similar effect was also described when phenylglyoxylic acid (PGA) and phenylglycine (PG), important metabolites of styrene, were administered.[13] These results suggested that the DA metabolism may be a target for the neurotoxic effect of styrene and its metabolites. In addition, another reactive metabolite, 7, 8 oxide styrene was documented by genetic toxicology and conjugation with glutathione to lead to damage to the cellular structure.[14,15]

In contrast, to our knowledge, although evoked potentials, in particular, brainstem auditory-evoked potentials (EAEPs) has been examined in humans and experimental animals exposed to organic solvents, including styrene in previous studies,[16,17] no report has pointed out any changes in the central nervous system caused by styrene exposure using middle latency auditory-evoked potentials (MAEPs). Analysis of MAEPs as a new and relatively noninvasive technique for evaluating the function of the central nervous system has been widely used in clinical and experimental studies.[18,19] It has been used for humans as well as in a variety of non-human species, including the monkey, cat, guinea pig and rat.[20,21] Thus, MAEPs were chosen in this present study to evaluate the effects of styrene on central nervous system in the rat.

The purpose of the present study was to investigate the neurophysiological changes in the central nervous system using middle latency auditory-evoked potentials.

MATERIALS AND METHODS

Animals —— Eighteen male Wistar rats (6 rats for each group) were used in the investigation of MAEPs. The initial body weights of rats ranged from 200 to 250 g. Animals were housed on individual

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plastic cage for one week prior to surgical operation for implantation of an electrode and maintained at a constant room temperature 22 ± 2°C on a 12-hr light/dark (8 : 30/20 : 30) cycle. Food and tap water were given *ad libitum*.

Surgical procedures and placement of recording electrodes were performed one week prior to treatment with styrene. Animals were given Nembutal injection intraperitoneally (ip. 60 mg/kg) for anesthetization. A supplemental dose was administered when necessary to maintain the anesthesia. Once anesthetized, the superior surface of the head was shaved. A midline scalp incision was made beginning approximately 5 mm posterior to the caudal plane of the eyes and extending to the posterior of the pinna. Connective tissue was separated away from the skull surface and burr holes were then drilled into the skull to accommodate the electrodes.

A recording electrode was placed 2 mm to the right of the central suture at precisely one-half the distance between the bregma and lambda. A screw that was placed 2 mm to the right of the central suture and 3 mm anterior to the bregma served as a ground. Care was taken not to compress or penetrate the dura while inserting the electrode and screw. Dental acrylic was then applied to the skull to secure the electrode and screw. Animals were allowed to recover for one week prior to testing.

Exposure Condition —— For treatment with styrene, one group of the rats was intubated styrene liquid (styrene : olive oil = 1 : 4) on 400 mg/kg, and another on 800 mg/kg, once a day, five days for one week, consecutively for two weeks. The control group was intubated saline using the same procedure. Rats were weighed prior to the start of the study and weekly, thereafter. In addition, rats were checked for signs of toxicity once a day.

Measuring Procedure for MAEPs —— Measurement of MAEP components was performed in 6 rats of each group once, 6 times in total throughout experimental period. This was done prior to treatment with styrene (pre), during treatment with styrene for two weeks, and of the fourth, sixth, and eighth weeks after the beginning of exposure to styrene.

The measurement of MAEPs was performed at the 6 times as mentioned above using Neuropack (MEB 4024, NIHONKOHDEN, JAPAN). Burst tone stimuli were used to generate the evoked potentials. Each burst tone had a rate of 5 Hz and duration of 200 ms (100 ms plateau, 50 ms rise/fall time) with the intensity of 90 dB SPL measured at the center of the wire mesh cage. Evoked potentials were obtained by averaging the responses to 500 stimuli.

The recording chamber consisted of a small wire mesh cage 70 cm wide, 45 cm high and 45 cm depth, located in the center of a shielded soundproof chamber. Two speakers were mounted at the back of the chamber equidistant (110 cm) from the center of the wire cage. MAEPs were recorded from Vx, referenced to the lateral ear and the frontal sinus screw was used as a ground. Amplifier sensitivity was at 20 μV/mm with a band pass of 20 Hz and 1 k Hz filter. The MAEP data were stored on a hard disk and floppy diskettes for subsequent averaging and analysis.

The mean ambient temperature of 22°C and humidity of 60% in the chamber were maintained during testing.

Data Analysis —— The latency of each MAEP component was measured from the time of the onset of the stimulus, without correcting for stimulus transit time. The amplitude of each MAEP component was measured from the point of its onset to the maximum positively or negativity.

The MAEP components in alert rats consist of two negative troughs, labeled the No and Na components, and two positive peaks, the Po and Pa components. The amplitudes and latencies of these four components were measured and the peak-to-peak intervals were computed from these latencies. Statistical comparisons of amplitudes, latencies and peak-to-peak interval among groups were done by two-factor analysis of variance (two-factor ANOVA). Comparisons of significant differences among groups at each point throughout experimental period were performed by using super ANOVA (Abacus Concepts, Inc).

RESULTS

The body weights of rats exposed to styrene at 400 mg/kg and 800 mg/kg, measured once a day, five days per week, did not show any significant difference from those in the control group throughout the experimental period.

Typical middle latency auditory-evoked potentials obtained from one rat each in the control and 800 mg/kg groups at the fourth week after the beginning of treatment with styrene are illustrated in Fig. 1. MAEP components in alert rats consisted typically of two negative troughs, labeled No and Na, with latencies of approximately 8 and 25 msec, and two positive peaks, Po and Pa with latencies of

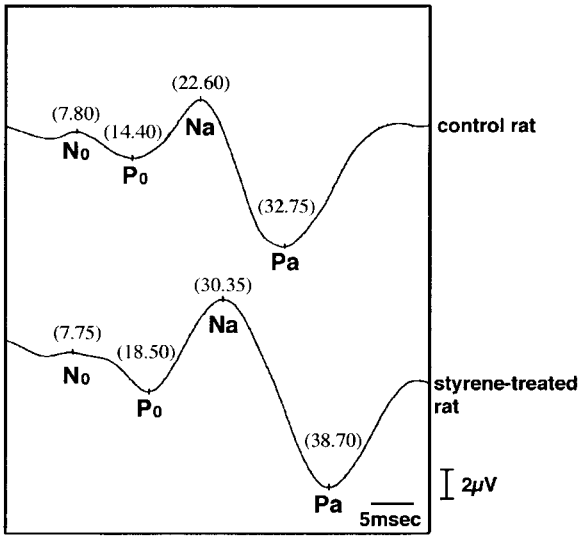

Fig. 1. Typical Graph of MAEP Components in a Control (Upper) and Styrene-Treated Rat (Lower) Respectively
Latencies of each MAEP components for each rat are presented in brackets (ms).

approximately 18 and 35 msec, respectively. In the 800 mg/kg group, latencies of No, Po, Na and Pa components showed delays in varying degrees as compared to those in the control group, while the alteration on amplitude was not significant.

Statistical analyses of latencies, peak-to-peak intervals, and amplitudes obtained from MAEP performance were calculated by two-factor ANOVA. The F values and p values are presented at Table 1.

Latencies of MAEP Components

There was no significant difference in the latencies of MAEP components among groups before treatment with styrene.

Statistical analyses of different styrene exposure doses were done by two-factor ANOVA. Significant differences were found, especially in later components of MAEPs as presented in Table 1.

Statistical analysis of the mean values of No components was performed by two-way ANOVA and significant differences were observed depending upon exposure dose and time as shown in Table 1. Changes in the latencies of No components in both styrene-treated groups as compared to those in the control group are presented in Fig. 2 (No). Significant differences were observed between the 400 mg/kg group and the control group ($p < 0.05$) at the fourth week, and between the 800 mg/kg and the control group at the second and sixth weeks after the beginning of treatment with styrene ($p < 0.05$). Throughout the experimental period, no significant difference in the latencies of No components was found between the 400 mg/kg and 800 mg/kg groups treated with styrene.

Figure 2 (Po) presents the changes in latencies of Po components for the three groups throughout experimental period. Results of comparisons among groups indicated significant differences at the fourth week after treatment with styrene between the control and 400 mg/kg ($p < 0.01$), between the control and 800 mg/kg ($p < 0.01$), and between 400 mg/kg and 800 mg/kg ($p < 0.01$). A significant delay was

Table 1. F Values (p Values) Calculated by Two-Factor ANOVA for MAEP Components

Items	Dose	Time	Dose × Time
Latency			
No	3.161 (0.049)*	2.892 (0.0203)*	1.129 (0.3546)
Po	9.678 (0.0002)**	10.144 (0.0001)**	3.913 (0.0003)**
Na	15.562 (0.0001)**	28.577 (0.0001)**	6.807 (0.0001)**
Pa	27.246 (0.0001)**	17.179 (0.0001)**	4.594 (0.0001)**
Interval			
No-Po	4.777 (0.0116)*	7.614 (0.0001)**	4.817 (0.0001)**
Po-Na	0.030 (0.9709)	1.959 (0.966)	2.934 (0.0043)**
Na-Pa	6.232 (0.0033)**	0.457 (0.8065)	0.805 (0.6243)
Amplitude			
No-Po	2.196 (0.1194)	1.386 (0.2411)	1.225 (0.2925)
Po-Na	1.980 (0.1463)	0.579 (0.7157)	1.065 (0.4021)
Na-Pa	2.096 (0.1312)	1.322 (0.2658)	1.115 (0.3650)

*: $p < 0.05$, **: $p < 0.001$.

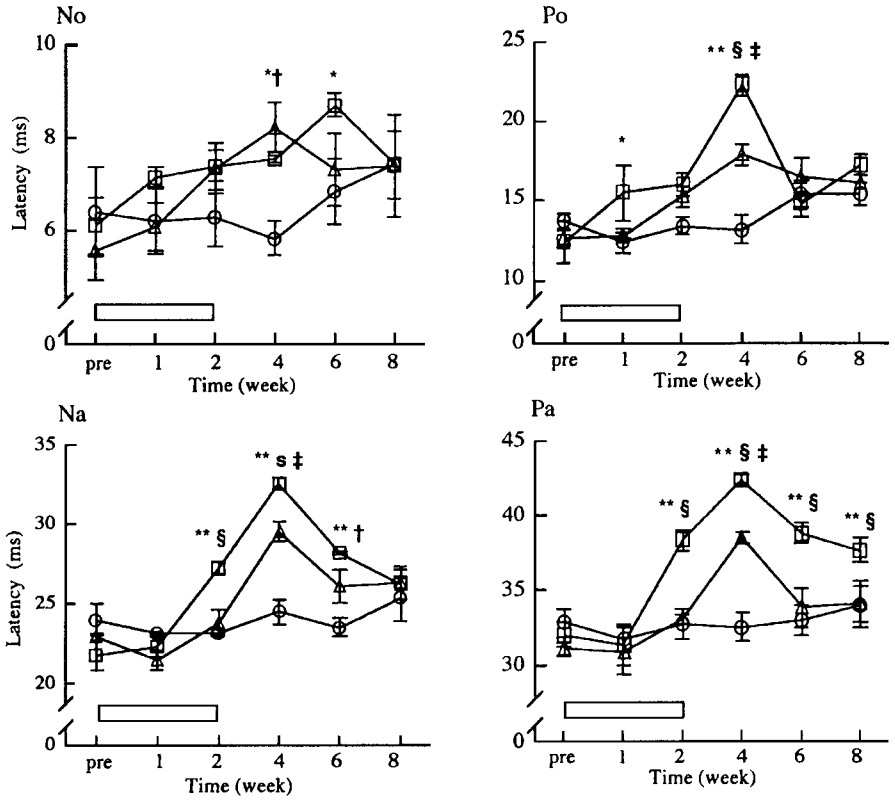

Fig. 2. Changes in the Latencies of No, Po, Na, and Pa of MAEP Components in Rats before (Pre), during (1, 2) and after (4, 6, 8) Treatment with Styrene at 400 mg/kg and 800 mg/kg Compared to Those in Controls

Statistical analysis was calculated by two-factor ANOVA. open circle: control, triangle: 400 mg/kg group, square: 800 mg/kg group. Values are presented as mean ± S.E. Dark rectangle shows the period of exposure to styrene for two weeks, †: $p < 0.05$, ‡: $p < 0.01$, between control and 400 mg/kg group. *: $p < 0.05$, **: $p < 0.01$, between control and 800 mg/kg. s: $p < 0.05$, §: $p < 0.01$, between 400 mg/kg and 800 mg/kg.

also found at the first week after the beginning of styrene administration for the 800 mg/kg group compared to the control group. Moreover, at the end of the second week of exposure to styrene, the latency of the Po component in the 800 mg/kg group showed a delay tendency ($p < 0.0615$) but no significant difference was observed as compared to those in other groups.

Statistical analysis by two-factor ANOVA showed significant differences for styrene exposure doses and time as presented in Table 1. Changes in the latencies of Na components are shown in Fig. 2 (Na). A greater delay was found in the 800 mg/kg group than in both the control and 400 mg/kg groups. Significant differences were observed between the control and 800 mg/kg at the second, fourth, and sixth weeks ($p < 0.01$), between the control and 400 mg/kg at the fourth week ($p < 0.01$) and sixth week ($p < 0.05$), and between 400 mg/kg and 800 mg/kg at the second week ($p < 0.01$), and fourth week ($p < 0.05$).

Effects of subchronic treatment with styrene on MAEPs were also observed as a severe increase in latency of the Pa component. Statistical analysis of the styrene exposure doses and times were performed and significant differences were found as presented in Table 1. Changes in the latencies of Pa components are illustrated in Fig. 2 (Pa). Increased latencies of Pa components, especially in rats treated with 800 mg/kg styrene clearly occurred from the second week during administration of styrene and did not return to the pre-experimental level even at the end of eighth week after the beginning of exposure to styrene. Comparisons between groups showed significant differences for the 800 mg/kg compared to both 400 mg/kg and control group at the second, fourth, sixth, and eighth weeks ($p < 0.01$). A significant difference was also observed between the styrene-treated group on 400 mg/kg and the control ($p < 0.01$) at the fourth week.

Peak to Peak Intervals of MAEPs Components

Statistical analyses were performed by two-factor ANOVA and the results are presented in Table 1. Significant differences were found depending upon styrene exposure dose and time. Changes in peak-

to-peak intervals of No-Po, Po-Sa, and Na-Pa of MAEP components for the three groups of experimental rats were compared. Significant differences in peak-to-peak intervals of No-Po were observed for the 800 mg/kg styrene- treated group compared to both the control ($p < 0.01$) and 400 mg/kg styrene-treated groups ($p < 0.05$) at the fourth week as shown in Fig. 3 (No-Po). Furthermore, the interval of Po-Na in the 800 mg/kg styrene-treated group was clearly prolonged compared to both 400 mg/kg and the control group at the sixth weeks ($p < 0.01$) as presented in Fig. 3 (Po-lia). The Na-Pa interval in the 800 mg/kg styrene-treated group was also delayed and significant differences were observed compared to those in both 400 mg/kg group and the control at the sixth and eighth weeks ($p < 0.05$), respectively, as shown in Fig. 3 (Na-Pa).

Amplitudes of MAEP Components

Statistical analyses of amplitudes of MAEP components were performed and the resulting of F values and p values are presented in Table 1. No significant difference was observed throughout experimental period.

Time Dependent Response of MAEP Component Latency

Time-dependent changes in the latency of each MAEP component in the control, both 400 mg/kg and 800 mg/kg groups are presented in Fig. 4. Significant changes in latency of MAEP components in the control were not observed at any time in the experimental period. Significant delays of latency of No components were found in the 400 mg/kg group at the fourth week ($p < 0.05$), and in the 800 mg/kg group at the sixth week compared to each of them before exposure to styrene as presented in Fig. 4 (No). Latencies of Po components in both styrene-treated groups were delayed significantly at the fourth week compared to each of them before exposure to styrene as presented in Fig. 4 (Po). Significant changes in latencies of Na components were also found in the 400 mg/kg and 800 mg/kg at the fourth week ($p < 0.01$); in addition, delays of Na components in 800 mg/kg group were observed at the second, sixth and eighth weeks ($p < 0.05$) as presented in Fig. 4 (Na). Changes in latency of Pa components in each group are also shown in Fig. 4 (Pa). Significant delays were observed at the fourth week in the 400 mg/kg group, and from the second to the eighth week in the 800 mg/kg group compared to before exposure to styrene.

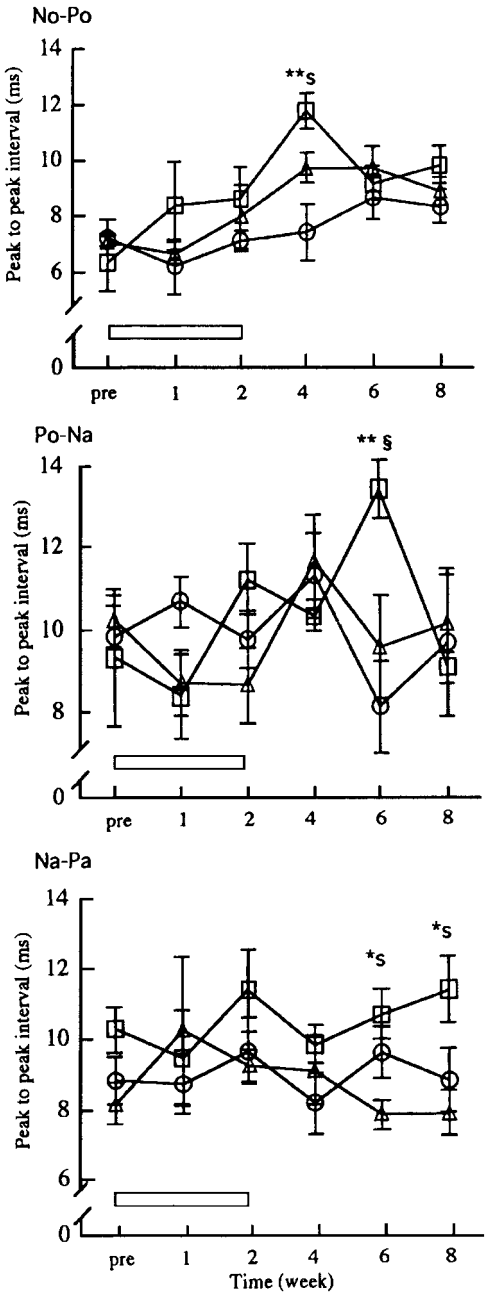

Fig. 3. Changes in the Intervals of No-Po, Po-Na, and Na-Pa of MAEP Components in Rats before (Pre), during (1, 2) and after (4, 6, 8) Treatment with Styrene at 400 mg/kg and 800 mg/kg Compared to Those in Controls

Statistical analysis was calculated by two-factor ANOVA. open circle: control, triangle: 400 mg/kg group, square: 800 mg/kg group. Values are presented as mean ± S.E. White rectangle shows the period of exposure to styrene for two weeks, *: $p < 0.05$, **: $p < 0.01$, between control and 800 mg/kg. s: $p < 0.05$, §: $p < 0.01$, between 400 mg/kg and 800 mg/kg.

DISCUSSION

In the present study, delays in latencies and peak-to-peak intervals of MAEP components were ob-

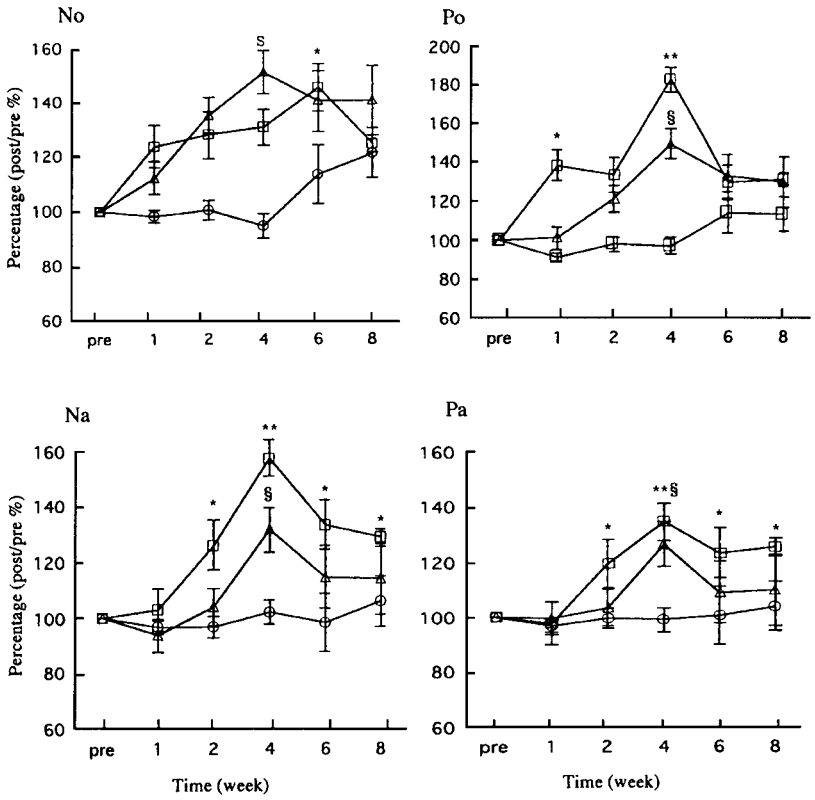

Fig. 4. Percentages of Latencies of MAEP Components in the Control and 400 mg/kg and 800 mg/kg Styrene-Treated Group, Respectively
Latency of each component in each group before administration of styrene was considered to be one hundred and the percentage of each of them (post/pre) was calculated. open circle: control group, triangle: 400 mg/kg group (s: $p < 0.05$, §: $p < 0.01$ compared to their values before exposure to styrene), square: 800 mg/kg group (*: $p < 0.05$, **: $p < 0.01$ compared to their values before exposure to styrene).

served in rats treated with styrene for both 400 mg/kg and 800 mg/kg compared to the control. Furthermore, the greatest delays in the latencies of MAEP components, especially Po, Na and Pa components, were observed at the fourth week after the beginning of exposure to styrene. This indicated that these changes were characterized by occurrence in the early period of styrene exposure, and a relation to the exposure dose and time. These results objectively confirmed the harmful effects of styrene on the central nervous system and indicated that analysis of MAEPs could be used to determine the dysfunction of the brain caused by styrene.

Middle latency auditory evoked potentials have become a subject of a renewed interest in the last ten years. They are composed of several components that can be detected within 50 ms after the onset of a stimulus. These components have been labeled by Picton *et al.*[22] as follows: No, Po, Na, Pa, reflecting their polarity and sequence of occurrence. Possible source generators for the middle latency components No, Po, Na, might be generated from the medial geniculate body and polysensory nuclei of the thalamus. Recently a series of animal investigations has shown the electrophysiological origins of the Pa component.[23] It is believed that the vertex-recorded Pa component originates bilaterally from vertically oriented dipoles within the primary auditory cortices. Although theperformance of MAEPs has been studied extensively in normal subjects, little is known about how they are disrupted by pathology of the nervous system, particularly by neurotoxins. Picton *et al.*[22] and Jacobson *et al.*[23] investigated chronic and acute effects of imipramine, a centrally active compound, on the central nervous system using brainstem auditory evoked potentials (BAEPs) and MAEPs. Although there were no observable changes in BAEPs after either acute or chronic administration, chronic application of imipramine caused an apparent increase in latency and reduction of amplitude in MAEP components. This proved that MAEP performance has more sensitivity than BAEPs to detect the effects on the central nervous system of neurotoxins. We know that MAEP components are largely distributed over both upper-brainstem hemispheres[24,25] Analysis of MAEPs could provide a measure of central auditory function.[21] Abnormalities in either the latency or interval of MAEPs in

this study, therefore, objectively indicated that the upper brainstem of the central nervous system in rats is also impaired by treatment with styrene. Furthermore, a previous study has reported that the Na component originates from the thalamus or thalamocortical radiations. The Pa component, however, is thought to be mainly related to the simultaneous activation of both supratemporal auditory cortices.[20] In our present study, clear delays in the latencies of MAEPs components, especially, were observed in Na and Pa components which are considered to reflect the activities of cortices and/or subcortices. This indicated that the cortex or subcortex may be more vulnerable to styrene exposure. Thus, recording of MAEPs can reflect brain activity and can be used in the objective evaluation of various neurological disorders caused by neurotoxins including styrene.

On a neurophysiological basis of neurotoxicity of styrene, changes in MAEP components, in particular, delays of the latencies in later components, proved objectively that lesions in the central nervous system can be caused by styrene administration. These changes may help to explain some clinical syndromes in humans exposed to styrene at work, for example, memory complaints, depression, and irritability.

To clarify the mechanism of these effects of styrene on the central nervous system, dopamine depletion has been reported as a neurochemical basis of the neurotoxicity of styrene. Mutti *et al*.[11,12] reported that dopamine metabolism is impaired by exposure to styrene. A marked and dose–dependent depletion in striatal and tubra-infundibular dopamine was found in rabbits exposed to styrene for 3–7 days, and such effects were also detectable after intraperitoneal injection of the main end-products of biotransformation of styrene, *i.e*., mandelic acid (MA) and PGA, respectively. Further studies reported that DA content recovered after approximately 3 weeks from the last exposure and that neither its synthesis nor its catabolism was affected. Moreover, styrene exposure can interfere with the balance in intracellular as well as in extracellular ion concentrations. These studies suggested that both intracellular ion transmission and intercellular neurochemical transmission were interfered by exposure to neurotoxins including styrene. In contrast, Husain *et al*.[26] examined the effects of styrene on neurotransmitters, including serotonin, noradrenaline and dopamine levels, as well as the activities of monoamine oxidase (MAO) and acetylcholinesterase (AchE), in whole brains of rats administered styrene at 1 ml/kg daily for 15 days. They found a significant increase in serotonin and noradrenaline, whereas, there were no changes in dopamine content. Furthermore, they also pointed out a significant alteration in MAO, but not in AchE. Although the mechanism of the neurophysiological effects of styrene exposure has not been precisely elucidated, these previous studies about neurochemical alterations may be considered on the basis of changes in MAEP components because MAEPs are electrical manifestations of activity in multisynaptic pathways.[19]

In our present study, the greatest effects of MAEP components in rats were seen at the fourth week after the beginning of treatment with styrene, This might be due to the character of styrene metabolism. Langvardt and Nolan[27] and Csandy *et al*.[28] have determined styrene and its active metabolites in rat blood. Although the styrene concentration in blood becomes rapidly lower, its active intermediate product, 7, 8-oxide styrene remains for a relatively long period and is further metabolized to PGA and PG. Consequently, the adverse effects on the central nervous system of styrene may take time to evolve after exposure and appear to become more severe depending upon the accumulation of styrene and/or its metabolites in brain tissue. Our previous report showed the increased expression of glial fibrillary acidic protein (GFAP) from one month after treatment with styrene for both 400 mg/kg and 800 mg/kg in rats.[29] The increase in GFAP may be due either to the effect of styrene direct, or as a reactive response to the damage to neuronal cells caused by treatment with styrene. The present results and previous studies demonstrated that the electrophysiological alteration of the processing of auditory function occurred before neuronal cell damage induced by styrene exposure.

In summary, the large magnitudes of the changes in the latencies of MAEP components, especially in Po, Na, and Pa components that were produced following treatment with styrene, indicates that styrene has a marked effect on the processing of auditory information in the central nervous system in rats. In addition, recent reports indicated that permanent hearing loss induced by styrene exposure was due to the amount of outer hair cell losses.[30] However, the present results indicated that effects of styrene exposure on hearing function judged by MAEPs may indicated the possibility of reversal of these effects, at least in part, after ceasing exposure to styrene. These results suggest that MAEPs may be useful as a maker to detect the early stage of neurophysiologi-

cal impairment of auditory function induced by styrene exposure.

Acknowledgments This work was suported by grants from the Ministry of Education, Culture, Sports, Science and Technology, Japan. Part of this paper was published as the PhD Thesis of Yu-ping Wang in Hokkaido Journal of Medical Science (1998).

REFERENCES

1) Bond, J. A. (1989) Review of the toxicology of styrene. *Crit. Rev. Toxicol.*, **19**, 227–249.
2) Morrow, L. A., Steinhauer, S. R. and Hodgson, M. J. (1992) Delay in P300 latency in patients with organic solvent exposure. *Arch. Neurol.*, **49**, 315–320.
3) Baker, E. L. (1994) A review of recent research on health effects of human occupational exposure to organic sorvents. *J. Occup. Med.*, **36**, 1079–1092.
4) Cherry, N. and Gautrin, D. (1990) Neurotoxic effects of styrene: further evidence. *Br. J. Ind. Med.*, **47**, 29–37.
5) Triebig, G., Lehrl, S., Weltle, D., Schaller, K. H. and Valentin, H. (1989) Clinical and neurobehavioural study of the acute and chronic neurotoxicity of styrene. *Br. J. Ind. Med.*, **46**, 799–804.
6) Murata, K., Araki, S. and Yokoyama, K. (1991) Assessment of the peripheral central and autonomic nervons system function in styrene workers. *Am. J. Ind. Med.*, **20**, 775–784.
7) Jegaden, D., Amann, D., Simon, J. F., Habault, M., Legoux, E. and Galopin, P. (1993) Study of the Neurobehavioural toxicity of styrene at low levels of exposure. *Int. Arch. Occup. Environ. Health*, **64**, 527–531.
8) Mutti, A., Mazzucchi, A., Rustichelli, P., Frigeri, G., Arfini, G. and Franchini, I. (1984) Exposure-effect and exposure-response relationships between occupational exposure to styrene and neuropsychological functions. *Am. J. Ind. Med.*, **5**, 275–286.
9) Christer, E., Helena, A., Gunnar, J. and Kenneth, N. (1993): Increase in neuropsychiatric symptoms after occupational exposure to low levels of styrene. *Br. J. Ind. Med.*, **50**, 843–850.
10) Araki, S., Murata, K. and Yokoyama, K. (1994) Application of neurophysiological methods in occupational medicine in relation to psychological performance. *Ann. Acad. Med. Singapore*, **23**, 710–718.
11) Mutti, A., Falzoi, M., Romanelli, A. and Franchini, I. (1984) Regional alterations of brain catecholamines by styrene exposure. *Arch. Toxicol.*, **55**, 173–177.
12) Mutti, A., Falzoi, M., Romanelli, A., Bocchi, M. C., Ferroni, C. and Franchini, I. (1988) Brain dopamine as a target for solvent toxicity: effects of some monocyclic aromatic hydrocarbons. *Toxicology*, **49**, 77–82.
13) Romanelli, A., Falzoi, M., Mutti, A., Bergamaschi, E. and Innocente, F. (1986) Effects of some aromatic solvents and their metabolites on brain dopamine in rabbits. *J. Appl. Toxicol.*, **6**, 431–436.
14) Osterman-Golkar, S., Christakopoulos, A., Zorcec, V. and Svensson, K. (1995) Dosimetry of styrene 7,8 oxide in styrene and styrene oxide exposed mice and rats by quantification of heamoglobin addncts. *Chem. Biol. Interact.*, **95**, 79–87.
15) Zhang, X. X., Chakrabarti, S., Malick, A. M. and Richer, C. L. (1993): Effects of different styrene metabolites on cytotoxicity, sister-chromatid exchanges and cell-cycle kinetic in human whole blood lymphocytes in vitro. *Mutat. Res.*, **302**, 213–218.
16) Robert, C. S., Boyes, W. K., Pryor, G. T., Svensgaard, D. J., Kassay, K. M., Gordon, G. R. and Shinsky, N. (1993) Combined effects of solvents on the rat's auditory system: styrene and trichloroethylene. *Int. J. Psychophysiol.,* **14**, 49–59.
17) Yano, B. L., Dittenber, D. A., Albee, R. R. and Mattsson, J. L. (1992) Abnormal auditory brainstem responses and cochlear pathology in rats induced by an exaggerated styrene exposnre regimen. *Toxicol. Pathol.*, **20**, 1–6.
18) Litscher, G. (1995) Middle latency auditory evoked potentials in intensive care patients and normal controls. *Int. J. Neurosci.*, **83**, 253–267.
19) Arezzo, J. C., Simson, R. and Brennan, N. E. (1985) Evoked potentials in the assessment of neurotoxicity in humans. *Neurobehav. Toxicol. Teratol.*, **7**, 299–304.
20) Di, S. and Barth, D. S. (1993) Binaural vs. monaural auditory evoked potentials in rat neocortex. *Brain Res.*, **630**, 303–314.
21) Kraus, N. and McGee, T. (1995) The middle latency response generating system. *Electroencephalogr. Clin. Neurophysiol.*, (EEG suppl.) **44**, 93–101.
22) Picton, T. W., Hillyard, S. A., Krausz, H. I. and Galambos, R. (1974) Human auditory evoked potentials: Evaluation of components. *Electroencephalogr. Clin. Neurophysiol.*, **36**, 179–190.
23) Jacobson, G. P., Privitera, M., Neils, J. R., Grayson, A. S. and Yeh, H. S. (1990) The effects of anterior temporal lobectomy (ATL) on the middle latency auditory evoked potentials. *Electroencephalogr. Clin. Neurophysiol.*, **75**, 230–241.
24) Sequeira, D. J. and Strobel, H. W. (1996) In vitro metabolism of imipramine by brain microsomes: effects of inhibitors and exogenous cytochrome P450

reductase. *Brain Res*., **738**, 24–31.
25) Shaw, N. A. (1995) The temporal relationship between the brainstem and primary cortical auditory evoked potentials. *Prog. Neurobiol*., **47**, 95–103.
26) Husain, R., Srivastava, S. P., Mushtaq, M. and Seth, P. A. (1980) Effects of stytene on levels of serotonin, noradrenalines, dopamine and activity of AchE cholinesterase and monoamine oxidase in rat brain. *Toxicol. Lett*., **7**, 47–50.
27) Langvardt, P. W. and Nolan, R. J. (1991) Determination of styrene-7,8-oxide in whole rat blood by gas chromato-graphy-mass spectrometry. *J. Chromatogr.*, **567**, 93–103.
28) Csanady, G. A., Mendrala, A. L., Nolan, R. J. and Fisler, J. G. (1994) A physiologic pharmacokinetic model for styrene and styrene-7.8-oxide in mouse, rat and men. *Arch. Toxicol*., **68**, 143–157.
29) Wang, Y., Saito, T., Hosokawa, T. and Saito, K. (1998) Alteration in hippocampal and cerebral expression of glial fibrillary acidic protein following styrene exposure in rat. *Neuropathology*, **18**, 289–294.
30) Loquet, G., Campo, P. and Lataye, R. C. (1999) Comparison of toluene-induced and styrene-induced hearing losses. *Neurotoxicol. Teratol*., **21**, 689–697.