

Brief Report

Prenatal exposure to 1-bromopropane causes delayed adverse effects on hippocampal neuronal excitability in the CA1 subfield of rat offspring

Yukiko Fueta¹, Toru Ishidao¹, Susumu Ueno², Yasuhiro Yoshida³,
Yasunari Kanda⁴ and Hajime Hori¹

¹Department of Environmental Management and Control, School of Health Sciences, University of Occupational and Environmental Health, Kitakyushu, Japan, ²Department of Occupational Toxicology, University of Occupational and Environmental Health, Kitakyushu, Japan, ³Department of Immunology and Parasitology, School of Medicine, University of Occupational and Environmental Health, Kitakyushu, Japan and ⁴Division of Pharmacology, National Institute of Health Sciences, Tokyo, Japan

Abstract: Objectives: Neurotoxicity of 1-bromopropane (1-BP) has been reported in occupational exposure, but whether the chemical exerts developmental neurotoxicity is unknown. We studied the effects of prenatal 1-BP exposure on neuronal excitability in rat offspring. **Methods:** We exposed dams to 1-BP (700 ppm, 6 h a day for 20 days) and examined hippocampal slices obtained from the male offspring at 2, 5, 8, and 13 weeks of age. We measured the stimulation/response (S/R) relationship and paired-pulse ratios (PPRs) of the population spike (PS) at the interpulse intervals (IPIs) of 5 and 10 ms in the CA1 subfield. **Results:** Prenatal 1-BP exposure enhanced S/R relationships of PS at 2 weeks of age; however, the enhancement diminished at 5 weeks of age until it reached control levels. Prenatal 1-BP exposure decreased PPRs of PS at 2 weeks of age. After sexual maturation, however, the PPRs of PS increased at 5-ms IPI in rats aged 8 and 13 weeks. **Conclusions:** Our findings indicate that prenatal 1-BP exposure in dams can cause delayed adverse effects on excitability of pyramidal cells in the hippocampal CA1 subfield of offspring. (J Occup Health 2018; 60: 74-79)
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Correspondence to: Y. Fueta, Department of Environmental Management, School of Health Sciences, University of Occupational and Environmental Health, Yahatanishi-ku, Kitakyushu, 807-8555, Japan (e-mail: yukiko@med.uoeh-u.ac.jp)

hippocampal slices

Introduction

Social concerns have been raised regarding the developmental neurotoxicity of prenatally absorbed environmental chemicals, which may exert delayed adverse effects on brain function after birth. It is now recognized that some industrial chemicals (e.g., lead, methylmercury, polychlorinated biphenyls, arsenic, and toluene) can exert developmental neurotoxicity, which results in clinical or subclinical brain dysfunction in humans and in laboratory animals¹⁾. Many neurotoxic chemicals are present in industrial work settings, and it is not known whether prenatal exposure to industrial chemicals leads to developmental neurotoxicity.

1-Bromopropane (CH₃-CH₂-CH₂Br; 1-BP), one substitute for specific chlorofluorocarbons, is currently used as a solvent in a variety of industrial and commercial applications. Products containing 1-BP include degreasers and cleaners, spray adhesives, spot removers, coin cleaners, paintable mold release agents, automotive refrigerant flushes, and lubricants. Adverse effects on the central and peripheral nervous system have been found in industrial workers who used 1-BP²⁻⁴⁾. Adult rats exposed to 1-BP have also exhibited central neurotoxicity, alteration of mRNA levels of brain neurotransmitter receptors⁵⁾, and hippocampal disinhibition caused by a decrease in γ -aminobutyric acid (GABA)-mediated function⁶⁾. In *in vitro* studies using rat hippocampal slices, 1-BP directly suppressed the synaptic plasticity, referred to as a long-term potentiation, in the granule cells of the dentate

gyrus⁷).

Developmental toxicity is one reason for the threshold limit value set by the American Conference of Governmental Industrial Hygienists for 1-BP⁸. We recently reported that prenatal exposure to 1-BP suppressed the occurrence of kainate (KA)-induced “wet dog shake” behavior in 2-week-old rat pups⁹. However, whether or not prenatal 1-BP exposure changes neuronal function at the cellular level in the brain of the offspring remains unknown. Therefore, we studied the effects of prenatal 1-BP exposure on neuronal excitability after birth. In studying neuronal excitability, population spikes (PSs) were recorded in the CA1 subfield of hippocampal slices. We analyzed stimulation-dependent responses, stimulation/response (S/R) relationships, and the ratio of responses to double-pulse stimulations (paired-pulse ratios or PPRs). PPRs have been used as a simple method for assessing excitability in neuronal networks^{6,10}. In the present study, we evaluated rats at 2, 5, 8, and 13 weeks of age, to determine whether prenatal 1-BP exposure exerts delayed effects after birth.

Materials and Methods

Animals and exposure protocol

Preparation of rats and 1-BP inhalation were made according to our previous study⁹. Briefly, adult male and female Wistar rats were purchased from Kyudo Co., Ltd. (Tosu, Japan). The rats were housed in plastic cages with paper-made chips (ALPHA-dri, Shepherd Specialty Papers, Richland, MI, USA) on a 12-h light/dark cycle (light period: 7 AM-7 PM). The temperature was controlled at 22°C-23°C. The relative humidity was approximately 50%-70%. The animals were allowed to consume food and water ad libitum. Female rats at the proestrus stage were mated with male rats. On the morning of the following day, the existence of sperm in the vaginal plug or vaginal smear was verified as gestation day (GD) 0. 1-BP was purchased from Kanto Chemical Co., Ltd. (Tokyo, Japan). Dams were exposed to 1-BP vapor at a concentration of 700 ppm (6 h/day) for 20 days from GD 1 to GD 20 in an exposure chamber, whereas the other dams were provided fresh air in the same type of chamber. The exposure concentration was designed to be 700 ppm, which was higher than the LOAEL (400 ppm) for decrease in inhibition, so-called disinhibition¹¹. Rats were not allowed access to food and water during the inhalation period. At postnatal day (PND) 2, the litter size was counted. Until the experimental days, male and female rat pups were housed separately after weaning. Some pups in the control and prenatally 1-BP-exposed groups were sourced from pups that were not injected with KA in our previous study⁹. The prenatally 1-BP-exposed groups are abbreviated as the 1-BP group. The number of dams in the control group and 1-BP group was 15 and 12, respectively.

The total number of pups in the control group and 1-BP group was 29 and 20, respectively.

The experiments were conducted under the guidance of the Ethics Committee of Animal Care and Experimentation in accordance with the Guiding Principle for Animal Care Experimentation, University of Occupational and Environmental Health, Japan, which conforms to the National Institutes of Health Guide for the Care and Use of Laboratory Animals and the Japanese Law for Animal Welfare and Care.

Hippocampal slice preparation

Electrophysiological tests were conducted in male rats at 2 (PND 14), 5, 8, and 13 weeks of age. The total number of tested slices in the control group and 1-BP group was 119 and 86, respectively. The slices were prepared following previously reported methods⁶. Briefly, the rats were deeply anesthetized using a diethyl ether vapor. After decapitation, the brain was removed and dipped in an ice-cooled artificial cerebrospinal fluid (ACSF) (3°C-4°C) saturated with an O₂/CO₂ mixture (95%:5%). The ACSF was composed of 124 mM NaCl, 2 mM KCl, 1.25 mM KH₂PO₄, 2 mM CaCl₂, 2 mM MgSO₄, 26 mM NaHCO₃, and 10 mM glucose. The bilateral hippocampi were separated from the other brain regions. Further, transverse slices were obtained from the middle third region of the hippocampus using a McIlwain tissue chopper (Mickle Laboratory Engineering, Co., Ltd., Guildford, UK). The thickness of the slice was 600 µm for 2-week-old rats and 450 µm for 5-, 8-, and 13-week-old rats. The slices were transferred to an interface-type recording chamber, which was controlled at 32 ± 0.2°C, and perfused with ACSF saturated with a mixture of O₂/CO₂ (95%:5%) at a flow rate of 1 ml/min.

All the chemicals used in this study were of reagent grade and purchased from commercial sources.

Stimulation and recordings

After a stabilizing period of 1-2 h, bipolar stimulation electrodes made with stainless steel wires (50 µm in diameter) were placed on the stratum radiatum, where the Schaffer collateral and commissural fibers run up in the CA1 subfield (Fig. 1A). PS was recorded from the pyramidal cell layer in the CA1 subfield using glass microelectrodes (1-2 MΩ). Stimulations consisted of square-wave pulses (200 µs) from a stimulator (SEN7203, Nihon Kodens Co., Tokyo, Japan) via an isolator (SS202J, Nihon Kodens Co.). Stimulation intensities were 10 µA and 50 µA and increased by 100 µA every 2 min from 100 µA to a current of 600 µA in the slices from the 2-week-old rats. In the slices from the 5-, 8-, and 13-week-old rats, the stimulation was delivered every 30 sec with intensities of 20, 40, 60, 80, 100, 140, 200, and 300 µA. The S/R relationship in the extracellular recording configuration represents basic excitability of the local area responding to

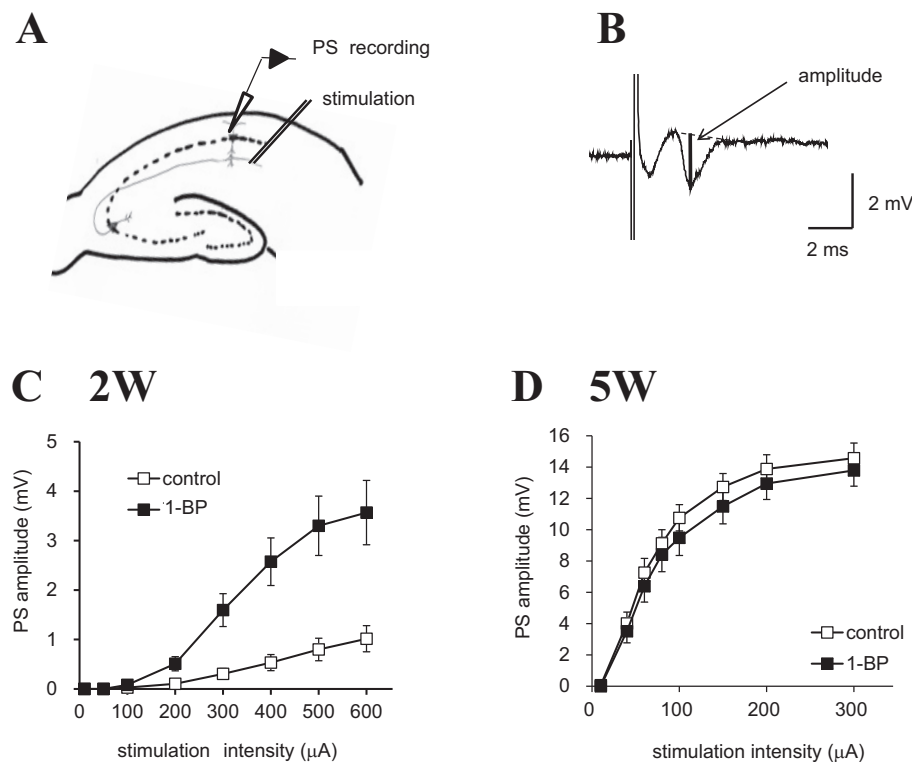


Fig. 1. Recording of population spikes (PSs) from the hippocampal CA1 field and PS stimulus/response (S/R) relationships (A) Stimulation electrode and recording electrode set on the CA1 subfield of the hippocampal slice. The stimulation electrode was set in the stratum radiatum, supplying stimulation to Schaffer collateral and commissural fibers. The PS recording electrode was set in the pyramidal cell layer. (B) Typical PS recorded in the CA1 field of the hippocampal slice obtained from a 2-week-old control rat. The thick line represents the PS amplitude measurement; stimulation intensity was 600 μ A. (C) At 2 weeks of age, S/R relationships of the PS amplitude obtained from the 1-BP-exposed rats were significantly enhanced compared to S/R relationships in control rats ($p < 0.001$ by repeated-measure ANOVA). (D) At 5 weeks, the enhancement observed in the 1-BP-exposed rats disappeared, and the S/R relationship decreased to control levels (PS amplitude: $p = 0.5$ by repeated-measure ANOVA). The horizontal axis represents stimulation intensity; the vertical axis represents PS amplitude. Data of 16-19 slices were averaged.

electrical stimulation, and the responses are prefigured to increase as the stimulation strengthens. For the paired-pulse configuration, after the S/R relationship experiment, the current amplitude was adjusted to result in an almost-maximum PS, 600 μ A for slices from the 2-week-old rats, and 300 μ A for slices from the 5-, 8-, and 13-week-old rats. Interpulse intervals (IPIs) of the paired-pulse stimulation were 5 and 10 ms and delivered every 2 min for slices from the 2-week-old rats and every 1 min for slices from older rats. Electrophysiological signals were amplified using a high-impedance amplifier (Axoclamp 2B, Molecular Devices, Sunnyvale, CA, USA). The signals were then digitized with an AD converter (Digidata 1200, Molecular Devices) and stored on a computer using pCLAMP software (Molecular Devices).

Electrophysiological analysis

PS amplitude was measured as described in our previous study⁶⁾ (Fig. 1B). PPRs were calculated as follows:

$$\text{PPR of PS} = \frac{\text{second PS amplitude}}{\text{first PS amplitude}}$$

In our previous inhalation studies using adult rats^{6,11)}, PPRs of PS evoked with paired-pulse stimulation at IPIs of 5 and 10 ms in the CA1 subfield were < 1 in the hippocampal CA1 of control adult rats, representing the presence of feedback inhibition. Compared to those of adult rats, PPRs of PS in immature rats can be 1 or higher¹²⁾. Thus, in either case of inhibition or facilitation, paired-pulse configuration in extracellular recordings in the slices is useful to examine the excitability of the local area responding to double-pulse stimulations.

Statistical analysis

Statistical significance was evaluated by repeated-measure analysis of variance (ANOVA) for the S/R relationship. For PPRs, unpaired Welch's *t*-test was used to determine a difference between the 1-BP and control groups, when the data were normally distributed. Otherwise, the Mann-Whitney U test was applied, and *p* values <0.05 (two-tailed) were considered statistically significant. Electrophysiological data are expressed as means \pm standard errors of the mean (SEMs). Litter size represents mean \pm standard deviation. Statistical tests were performed in Ekuseru-Toukei 2010 for Windows (Social Survey Research Information Co., Ltd., Tokyo, Japan).

Results

There was no difference between the litter sizes of the control group and 1-BP group (control: 14 ± 2 pups, 15 dams; 1-BP: 14 ± 3 pups, 12 dams).

As shown in Fig. 1C, the PS amplitude was 4 times greater in the 1-BP group than in the control group at 600 μ A of stimulation intensity in 2-week-old rats. In 5-week-old rats, the enhancement disappeared, and the levels decreased to the control level of the S/R relationship of the PS amplitude (Fig. 1D). No difference was observed between the 1-BP and control groups at 8 and 13 weeks of age (data not shown). Increased excitability of pyramidal neurons was a transient change.

The left column of Fig. 2 shows examples of paired-pulse responses recorded from the hippocampal CA1 subfield of the control and 1-BP groups. As shown in Fig. 2A, at 2 weeks of age, the averaged PPR was approximately 2 in the control group, suggesting a facilitatory effect. In contrast, inhibition rather than facilitation was observed at the 5-ms IPI in the 1-BP group. At the 10-ms IPI, PPRs showed a slight facilitation but were significantly decreased compared to PPRs in the control group. At 5 weeks of age, PPRs were lower than 0.2, displaying an apparent feedback inhibition in both groups (data not shown), and the effects of prenatal 1-BP exposure on PS PPRs disappeared. At 8 and 13 weeks of age, PPRs were still lower than 1 but increased significantly at 5 ms of IPI in the 1-BP group compared with that of the control group (Fig. 2B and 2C). There was no significant difference in the disinhibitory effects between 8- and 13-week-old rats.

Discussion

In general, the effects of prenatal chemicals on the brains of offspring with more littermates may be different from those with fewer littermates. In addition, litter size may be affected by inhalation of 1-BP by the dams. However, because there was no difference between the litter sizes of the control and 1-BP groups in this study, the de-

layed developmental toxicity observed was not likely to be associated with changes in litter size.

The present study revealed that prenatal exposure to 1-BP enhanced the excitability of CA1 pyramidal neurons and caused a decrease in PPRs of PS amplitude in hippocampal slices from 2-week-old rats. The lactation period after birth is considered to be the period of synaptogenesis in rat brains¹³; thus, neuronal development during the lactation period may be sensitive to prenatal chemical exposures. In a previous study⁹, we reported that prenatal exposure to 1-BP suppressed KA-induced "wet dog shake" behaviors in 2-week-old rats. In this study, prenatal 1-BP exposure rendered the hippocampal CA1 subfield highly responsive to a single stimulation but suppressive to double stimulations. The decrease in PPRs in the 1-BP group shown in Fig. 2A may have been caused by the higher sensitivity of PS1. In the control group, PS1 may have been developmentally suppressed for some unknown reason (as shown in Fig. 1C), and the suppression may have been lifted following the second stimulation, as shown in PS2. Prenatal 1-BP exposure may weaken or lift the suppression in the PS1. The PS2 amplitude in the control group was similar to that seen in the 1-BP group. This indicates that prenatal 1-BP exposure may make CA1 neurons hyperexcitable at the developmental stage, which is quite different from normal brain development. Because 1-BP is metabolized in the womb of pregnant rats and biotransformed into metabolites, it is unclear whether those effects were caused by 1-BP itself.

Although a decrease in PPRs of PS amplitude was observed in the 1-BP group at 2 weeks of age, the difference diminished at 5 weeks of age, as did the S/R relationship. In contrast to that of the 2-week-old pups, the 8- and 13-week-old groups displayed an increase in PPRs of the PS, also known as disinhibition. In the 13-week-old rats, we observed that the disinhibitory effect induced by the prenatal 1-BP exposure was greater in female than in male rats (data not shown). It is unclear, however, whether sex-specific effects would be observed at other ages, and this should be investigated in the future. Thus, prenatal exposure to 1-BP can exert developmental effects linked to the excitatory function of neurons and network excitability. Disinhibition has been reported in relation to subclinical and clinical changes in brain excitability in epileptic patients and animals¹⁴, as well as in anxiety disorders¹⁵. We did not observe any spontaneous abnormal behaviors in the 1-BP group during breeding. To date, developmental neurotoxic effects caused by 1-BP exposure have not been reported in children whose mothers were exposed occupationally during pregnancy. However, because disinhibition can be associated with the hyperexcitable brain and epilepsy, it should not be concluded that disinhibition is merely a phenomenon restricted to rats. Because disinhibition is interpreted as a disturbance of the excitation/inhibition balance in the hippocampal CA1 area, disin-

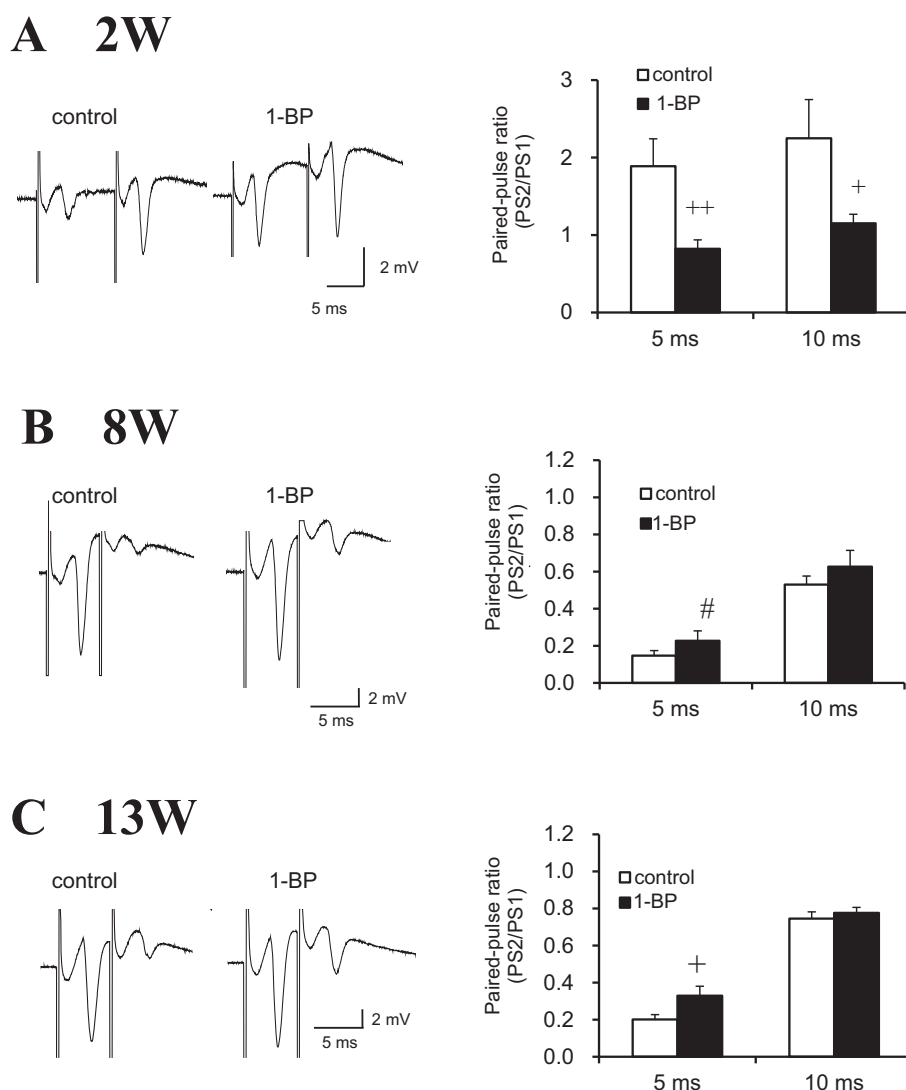


Fig. 2. Paired-pulse ratios (PPRs) of the population spikes (PSs) evoked with a double stimulation of 5 and 10 ms interpulse intervals (IPIs) in the CA1 subfield of hippocampal slices obtained from 2-, 8-, and 13-week-old male rats and 13-week-old female rats

Left column: Representative examples of paired-pulse responses recorded from the hippocampal CA1 subfield of the control and 1-BP groups. Right column: (A) At 2 weeks of age, PPRs decreased substantially in the 1-BP group ($++p < 0.01$ vs. the control group at the 5-ms IPI, $+p < 0.05$ vs. the control group at the 10-ms IPI by Welch's *t*-test). (B) At 8 weeks of age, PPRs were lower than 1 in both groups, indicating an apparent inhibition. At the 5-ms IPI, the PPR of the 1-BP group increased compared with that of the control group ($\#p < 0.05$ by Mann-Whitney U test). (C) Similar to the 5-ms IPI at 8 weeks of age, PPR of male rats in the 1-BP group increased compared with that of the control male rats at 13 weeks of age ($+p < 0.05$ by Welch's *t*-test). The horizontal axis represents the IPIs; the vertical axis represents the PPRs of PS amplitude. Data of 16-25 slices were averaged.

hibitory effects can be classified as adverse effects.

The enhancement of excitability induced by prenatal 1-BP exposure was observed only in the 2-week-old group, and may therefore have been only a transient effect. Alternately, one could argue that the excess basal excitability during synaptogenesis is not coincidental with disinhi-

bition after maturation. If so, the PS S/R relationship can be useful as a new index marker for developmental neurotoxicity of chemicals before the appearance of neurophysiological changes in the brain after maturation. To validate this method for assessing the developmental neurotoxicity of industrial chemicals, we should test chemi-

cals that are already known to exert developmental neurotoxicity. To this end, we are currently investigating valproic acid, an antiepileptic drug used in an established animal model of the developmental disorder, autism. Synaptic transmission generates action potentials; we are also studying field excitatory postsynaptic potentials.

In conclusion, we demonstrated that prenatal 1-BP exposure can cause delayed neurotoxicity, although the underlying mechanism is not known yet, and requires further investigation.

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Conflicts of interest: There are no conflicts of interest.

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