Industrial Health 2021, **59**, 239–248

Original Article

Cerebral excitability in pup rats prenatally exposed to 1-bromopropane is suppressed by bromide accumulated in the brain

Yukiko FUETA[1]*, Mitsuo HINOUE[1], Hazuki NOGUCHI[1], Mizuki MATSUNO[1], Toru ISHIDAO[1], C Edwin GARNER[2] and Hajime HORI[1]

[1]Department of Environmental Management and Control, School of Health Sciences, University of Occupational and Environmental Health, Japan
[2]Pharmaceutical Development, Radikal Therapeutics, Inc., USA

Received May 18, 2020 and accepted April 13, 2021
Published online in J-STAGE July 13, 2021
DOI https://doi.org/10.2486/indhealth.2020-0098

Abstract: Previously, we reported that prenatal exposure to 1-bromopropane (1-BP) causes the accumulation of bromide (Br^-) in the brain of rat pups. Here, we aimed to investigate the effects of Br^- accumulation in rat pups prenatally exposed to 1-BP vapor. Dam rats were exposed to 1-BP (400 or 700 ppm; 1-BP group) by inhalation, or to NaBr (20 mM; Br^- group) in drinking water during gestation days 1–20. We also analyzed pentylenetetrazole (PTZ, 60 mg/kg, ip)-induced behavioral changes in pups prenatally exposed to 1-BP or Br^- on postnatal day (PND) 14. PTZ-induced epileptic convulsions were inhibited in both 1-BP (700 ppm) and Br^- groups. The inhibition of neuronal excitability induced by Br^- was evaluated electrophysiologically using the hippocampal slices obtained from PND14–16 pups. PTZ (2 mM) failed to induce epileptiform discharge in the presence of 1.2 mM Br^- in the slices obtained from the control group. However, it induced epileptiform discharge following the removal of Br^-, by perfusing artificial cerebrospinal fluid into the slices obtained from the Br^- group. Our results indicate that Br^- accumulates in the brain of neonatal rat pups prenatally exposed to 1-BP vapor suppressed neuronal excitability.

Key words: Prenatal exposure, 1-bromopropane, Bromide, Neurotoxicity, Pentylenetetrazole, Hippocampal slice

Introduction

1-Bromopropane (1-BP) is a solvent substitute with various applications, including as a cleaning agent, and as an aerosol propellant and adhesive. In fact, in 2016 alone, 4,000 tons of 1-BP was produced and imported in Japan, with primary uses as an intermediate for drugs and agrochemicals, and as a vapor washing solvent[1)]. Although the American Conference of Governmental Industrial Hygienists recommended an 8 h time-weighted average (TWA) threshold limit value of 0.1 ppm 1-BP, in 2014[2)], the Japan Society for Occupational Health had previously recommended an occupational exposure limit (OEL) of 0.5 ppm in 2012[1)]. However, more recently, 1-BP exposure concentrations were investigated by the Japan Ministry of Health, Labor and Welfare in Japanese facilities[3)], showing that the actual exposure values of TWA were higher than the OEL in most cases; thus,

*To whom correspondence should be addressed.
E-mail address: yukiko@med.uoeh-u.ac.jp

the risk of occupational accidents and exposures remain high. Maintaining the TWA value is recommended as a precautionary measure against potential neurotoxicity, hepatotoxicity, reproductive toxicity, and developmental toxicity[2]. However, data on the developmental neurotoxicity (DNT) of 1-BP are limited despite concerns and increasing interest regarding DNT induced by industrial chemicals[4].

To address this issue, we previously reported that prenatal 1-BP exposure may induce delayed DNT as the excitability observed in the hippocampal slices of rat pups exposed to prenatal 1-BP vapor was enhanced during the lactation period, whereas disinhibition was observed in adult rats after reaching sexual maturation[5]. Moreover, another study reported that Br^- readily crosses the placenta in dams exposed to sodium bromide in their diet[6]. In addition, we found high concentrations of accumulated Br^- in both fetus and dam brains exposed to 1-BP vapor during pregnancy[7]. Thus, Br^- may represent a marker of both effect and exposure during the early postnatal period, including prenatal 1-BP exposure.

Furthermore, in humans, the blood and urine concentrations of Br^- in some employees exposed to 1-BP via inhalation were reported as being significantly higher than those in the control group and compared to the recommended exposure concentrations[8–11]. In most of these employees, 1-BP exerted neurotoxic effects. Similarly, in adult rats exposed to 1-BP vapor, the brain Br^- concentration was significantly higher than that in controls[12].

The net brain excitability is a balance between excitation and inhibition. γ-Aminobutyric acid (GABA) is one of the most prominent synaptic neurotransmitters; its receptor activation has an inhibitory effect on neuronal circuits in the adult brain. Br^- has been shown to interact with type A GABA receptors, allowing Cl^- to enter through the pores of GABA receptors[13, 14], thus demonstrating its antiepileptic therapeutic potential[14–16]. We previously reported that kainite (KA)-induced wet dog shake behavior was inhibited in suckling pups [postnatal day (PND) 14] prenatally exposed to 1-BP vapor[17].

Thus, the purpose of the present study was to investigate the effects of Br^- accumulation in rat pups prenatally exposed to 1-BP vapor. We hypothesized that the central neurotoxicity induced by prenatal exposure to 1-BP is partially due to accumulated Br^- in the brain, which serves to inhibit KA-induced brain excitability. To this end, we evaluated the effects of Br^- accumulation in the brain of rat pups prenatally exposed to 1-BP vapor. Pentylenetetrazole (PTZ), a central nervous system convulsant with less potency than KA[18], was used to analyze the changes in brain excitability through epileptic behaviors. Thus, behavioral changes induced by the interaction between Br^- and PTZ can be observed as PTZ is less potent than KA. In addition, we investigated whether PTZ-induced epileptiform activity in the brain slices is suppressed in the presence/absence of Br^-, at a concentration comparable with that in the brains of pups prenatally exposed to 1-BP.

Subjects and Methods

Animals

Fifty-nine (42 females and 17 males) Wistar rats purchased from Kyudo Co. (Tosu, Japan) at 9–11 weeks of age were housed in plastic cages with paper-made chips (ALPHA-dri; Shepherd Specialty Papers, Milford, USA) under a 12/12 h light/dark cycle (light period: 07:00–19:00 h). The room temperature was 23 °C ± 1 °C and relative humidity was 40%–70%. The rats had free access to food and water. Both breeding conditions and pregnancies in rats were achieved as previously described[5, 7]. In the morning after mating day, the presence of sperm in the vaginal smear or vaginal plug was verified to confirm gestation day (GD) 0. The rats and pups were anesthetized with isoflurane vapor before being sacrificed. All experiments were approved by the Ethics Committee for Animal Care and Experimentation in accordance with the University of Occupational and Environmental Health, Japan (AE03-065).

Prenatal exposure to 1-BP

1-BP (CAS No. 106-94-5; Guaranteed Reagent, higher than 98% purity) was purchased from Kanto Chemical Co., Ltd. (Tokyo, Japan). The exposure dosages were 0 (control), 400, and 700 ppm. The 400 ppm dosage was the lowest-observed-adverse-effect level (LOAEL) for neurotoxicity of disinhibition achieved by 12 weeks of exposure in adult male rats[12]. In the 1-BP group, dams were exposed to 1-BP vapor for 6 h/day over 20 days from GD 1 to 20 in an exposure chamber, following a previously described method[5, 7, 17]. The day of delivery was defined as PND 0 (GD21). 1-BP vapor exposure dosage was monitored using a gas chromatograph (GC353B FSL; GL Sciences Inc., Japan) equipped with a flame ionization detector. Exposure schedule and evaluation of Br^- effects are summarized in Fig. 1. Nineteen dams were used in the experiment, and were randomly divided into the following three groups: Control (n=8); 400 ppm exposure (400 ppm group; n=7); and 700 ppm exposure (700 ppm group; n=4). At PND14, 2–5 pups per litter were injected (ip) with PTZ (Sigma-Aldrich Corp., Tokyo, Japan) to examine brain excitability.

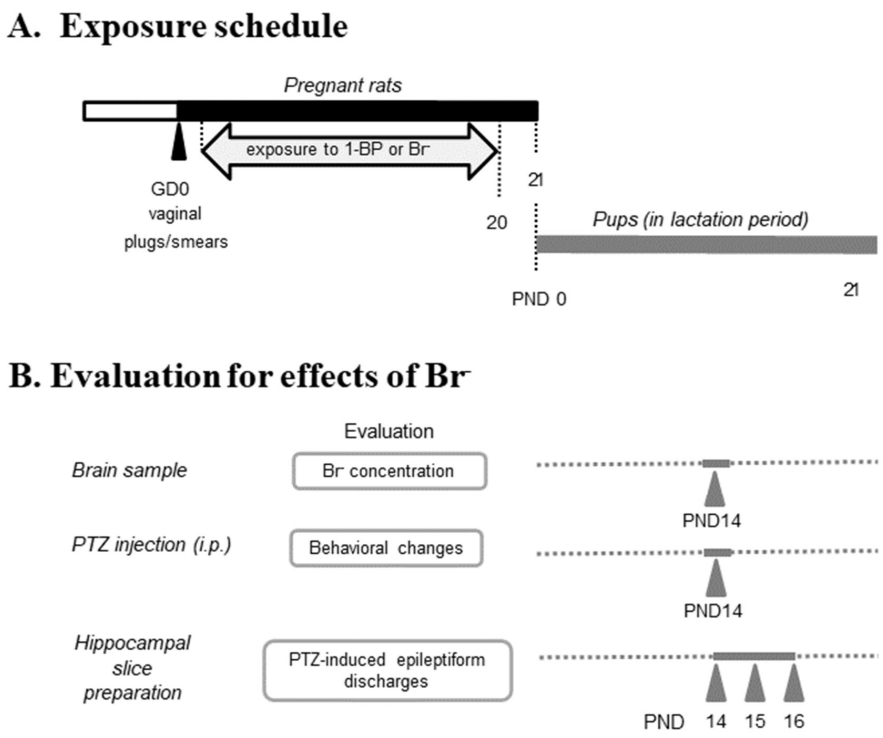


Fig. 1. Exposure schedule and evaluation of Br^- effects

Prenatal exposure to Br^- and analysis of brain Br^- concentration

Sodium bromide (NaBr; CAS No. 7647-15-6; Guaranteed Reagent, >99.5% purity) was purchased from FUJIFILM Wako Pure Chemical Co. (Osaka, Japan). Br^- was administered to rats via drinking water for 20 days from 11:00 h on GD1 to 11:00 h on GD21. Pregnant rats were randomly divided into the following two groups: Control (purified water; n=6) and Br^- exposed (Br group; n=9). We estimated Br^- uptake rate via drinking water so that a similar concentration of Br^-, to that of 1-BP, was achieved[7]; the final concentration of NaBr was 20 mM. At GD21, the total Br^- exposure was calculated from the water volume consumed. We then evaluated whether the brain concentration of Br^- via drinking water was similar to that via prenatal 1-BP inhalation[7]. To accomplish this, brain samples from two dams in the control and Br^- groups (total four dams), along with their fetuses, were cryopreserved at –80 °C until further analysis.

Fetus number, litter size, and body weight (PND 1, 7, and 14) of the pups were also recorded. At PND14, brain weight was measured in two and four litters from the control and Br^- groups, respectively; the brain samples were then cryopreserved at –80 °C to analyze Br^- concentration. The brain concentration of Br^- was determined as previously described[7]. The lowest limit in the quantitative determination using a gas chromatograph (GC353B FSL; GL Sciences Inc., Japan) equipped with a flame ionization detector was 3.87 µg/g tissue. The remaining litters at PND14 were injected with PTZ to examine brain excitability.

PTZ administration and behavioral observation

In our previous study, we reported that brain excitability induced by KA was significantly inhibited in the 1-BP group[17]. Hence, in the current study, we used PTZ to examine behavioral changes in the 1-BP, Br^-, and control groups. PTZ was dissolved in phosphate-buffered saline (pH 7.4). PTZ solution was intraperitoneally injected at a dose of 60 mg/kg to pups on PND 14. The number of PND14 pups injected with PTZ is described in Table 1. For the Br^- group, ten males and ten females from the control group and six males and eight females from the Br^- group were used. Data was collected from both male and female pups as it no sex differences have been reported in the severity of PTZ-induced seizures[19]. PTZ-injected pups are reported to exhibit a typical series of epileptic behaviors[18, 20, 21]. Therefore, the dose of PTZ, at which a series of epileptic behaviors was observed in more than 50% of the control pups, was deter-

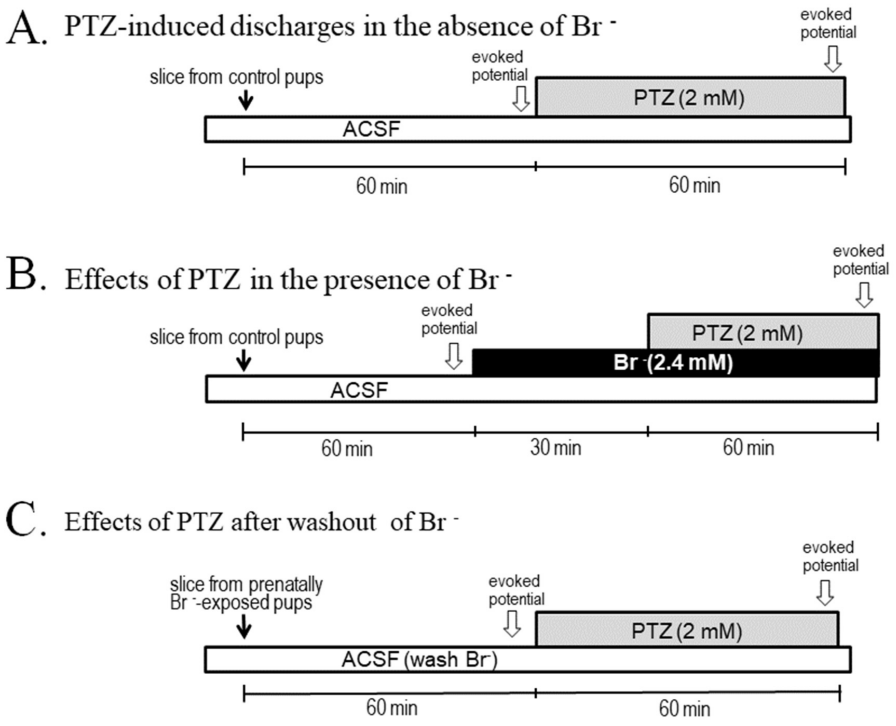


Fig. 2. Experimental design for the *in vitro* slice study
The experiment was designed to confirm that bromide (Br^-) in the brain has the potential to inhibit hyperexcitability induced by pentylenetetrazole (PTZ, 2 mM) using the hippocampal slices from rat pups. A) Effects of PTZ on control pups in the absence of Br^-. B) Effects of PTZ on control pups in the presence of 1.2 mM Br^-. C) Effects of PTZ after washing Br^- from the slices obtained from the Br group. The closed arrows indicate the timing of hippocampal slice preparation, whereas the open arrows indicate the timing of evoked potential recording with a stimulation intensity of 600 μA.

mined. The observations were conducted for 15 min following PTZ injection to pups in a clear plastic cage between 11:00 and 15:00 h, in a silent room maintained at 22 °C–24 °C. The first epileptic behaviors were body shaking and/or rapid backward walking. The pups then exhibited myoclonic jerks involving predominantly hind limb extension, followed by wild running for 1–2 s in the observation box; subsequently, the pups showed generalized tonic contractions. After this stage, generalized status epilepticus (SE) was observed within 15 min. To identify which sign is most effective as an index, we first analyzed the occurrence rate of each behavioral change and seizure episode in the 1-BP exposure groups and compared them with those in the control group. The occurrence rate of generalized tonic contraction in the Br^- group was compared with that in the control group.

PTZ-induced discharges in the hippocampal slices

The hippocampal slices at PNDs 14–16 were prepared as previously described[5, 22]. The concentration of PTZ used *in vitro* was 2 mM, according to previous studies[23–27]. Spontaneous and periodic discharge induced by PTZ, as well as field potentials evoked via electrical stimulation of Schaffer collateral/commissural fibers, were recorded from the CA1 region. To select the intact slices, an evoked PS amplitude > 3 mV, the average amplitude in the control group at PND14[5], was used. Our experimental design is shown in Fig. 2, where the ability of Br^- to inhibit brain hyperexcitability induced by PTZ is illustrated. First, we confirmed that PTZ (2 mM) induced epileptic discharge (Fig. 2A). Second, we examined the effects of PTZ in the presence of Br^-. To achieve this, we perfused the slices with 2.4 mM Br^- to a final concentration of 1.2 mM in the slices (Fig. 3A). Br^- concentration in the artificial cerebrospinal fluid (ACSF) was determined based on our results. Finally, after washing Br^- from the slices obtained from the Br group (Fig. 2C), we examined whether PTZ could induce epileptic discharge in these slices. We analyzed their latency, occurrence rate, amplitude, and discharge duration for comparison. This experimental design is capable of determining whether Br^- is associated with the observed changes in excitability in pup brains prenatally exposed to 1-BP. To ensure that the slice was intact throughout

the experiment, we recorded the evoke potentials (open arrow in Fig. 2).

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

Statistical analysis

Mann–Whitney U test and Chi-squared test of independence using 2 × 2 contingency table were used to evaluate the differences in litter size and the occurrence ratio of PTZ-induced generalized tonic contraction, respectively, between the control and Br^- groups. Mann–Whitney U test followed by Steel–Dwass test was used to compare the occurrence ratio of a series of PTZ-induced epileptic behaviors among the control, 400 ppm, and 700 ppm groups.

Results

We previously reported no difference in litter size between the prenatal 1-BP group and control group[5]. Similarly, no differences were observed in the litter size between the Br^- and control groups (14 ± 3 fetuses, 15 ± 3 pups in the Br^- group; 14 (12 and 15) fetuses, 16 ± 2 pups in the control group, $p>0.5$; Mann–Whitney U test).

Body weight is an effective index to evaluate developmental toxicity. As shown in the supplemental Fig. S1, prenatal 1-BP or Br^- had no effect on body weight at PND1, the day after birth; however, a significant inhibition of increased body weight was observed at PND 7 and 14, in both male and female pups.

PTZ-induced behavioral changes

The occurrence ratios for each behavioral episode measured in the 1-BP groups are summarized in Table 1. In the 1-BP control group, after body shaking and rapid backward walking, the first apparent epileptic sign was myoclonic jerks predominantly involving hind limb extension. This type of seizure occurred once to several times, followed by wild running for 1–2 s in the observation box. Subsequent-

Table 1. Pentylenetetrazole (PTZ)-induced behavioral changes and epileptic seizures in PND14 pups prenatally exposed to 1-bromopropane (1-BP) vapor

Behavior	1-BP concentration					
	0 ppm		400 ppm		700 ppm	
	Occurrence rate		Occurrence rate		Occurrence rate	
	Pup number	%	Pup number	%	Pup number	%
Body shaking	28/31	90	17/19	89	16/19	84
Rapid backward walking	22/31	71	8/19	42	8/19	42
Myoclonic jerks	26/31	84	18/19	95	14/19	74
Wild running	17/31	55	6/19	32	0/19	0
Generalized tonic contraction	20/31	65	7/19	37	0/19	0
Status epilepticus	20/31	65	6/19	32	0/19	0

ly, generalized tonic contraction in pups was characterized by the loss of postural control and strong contractions in the extensor muscles of the fore and hind limbs with cyanosis, followed by generalized SE.

The occurrence ratios for body shaking, rapid backward walking, and myoclonic jerks remained the same among the three groups. The 700 ppm group showed a significant decrease in wild running, generalized tonic contraction, and SE compared with the control group ($p<0.01$, Mann–Whitney U test followed by Steel–Dwass test).

We also analyzed the occurrence ratio of PTZ-induced generalized tonic contraction in the Br^- group and found it to decrease from 55% to 3% ($p<0.01$, Chi-squared test of independence using 2 × 2 contingency table) following PTZ injection in the Br^- group (in the control group).

PTZ-induced generalized tonic contraction was similarly suppressed in both 1-BP and Br groups.

PTZ-induced epileptiform discharges in the hippocampal slices

The brain Br^- concentration in the Br^- group at PND14 was approximately 1.9 mM (152.4 ± 28.1 μg/g tissue (n=23 pups from four litters)). The brain Br^- concentrations in pups prenatally exposed to Br^- were similar to those previously reported[7]. Based on these findings, we selected the Br^- concentration in ACSF to perfuse the hippocampal slices.

First, we ascertained that PTZ (2 mM) induced characteristic epileptiform discharges in 60% of the tested slices (n=10) obtained from nine control pups. PTZ-induced discharges showed an isolated periodic pattern, followed by long-lasting tonic-like, epileptiform discharges. The isolated periodic epileptiform discharges (Fig. 4A) occurred for a short duration (0.23 ± 0.03 s), with an amplitude of 1.0 ± 0.3 mV and an occurrence rate of 13 ± 3/min with a duration of 60–240 s. Thereafter, long-lasting tonic-like epileptiform discharge (a single curly bracket) proceeded for 25–45 s with an amplitude of 1.7 ± 0.8 mV. The evoked potential was also epileptic with multiple spikes (thick arrows) in all ten slices. Second, the perfusion of 2.4 mM (1.2 mM in the slices, Fig. 3A) Br^- for 30 min before PTZ application did not result in PTZ-induced epileptiform discharges in any of the ten new slices obtained from the seven control pups (Fig. 4B). The evoked epileptic potential was observed in eight of the ten slices in the presence of PTZ and Br^-. Finally, in the absence of Br^-, in eight hippocampal slices obtained from the four pups prenatally exposed to Br^- (Fig. 3B), PTZ-induced epileptiform discharges were similar to those observed in the control pups (Fig. 4A). The isolated periodic epileptiform discharges (Fig. 4C) were observed in four of the eight slices with an occurrence rate of 28 ± 2/min, an amplitude of 1.3 ± 0.4 mV, and a duration of 20–95 s. The duration of the succeeding long-lasting tonic-like epileptiform discharge was 9–22 s with an amplitude of 1.4 ± 0.6 mV. The evoked potential was epileptic in all ten slices (Fig. 4C).

Discussion

Our findings have significant implications for work and safety regulations implemented by governments, as well as regulations that govern food and drink industries. Furthermore, our study provides insights into the potential mechanisms of neuronal excitability, inhibition, and GABA function. In the field of medicine, our findings are important for the treatment of epilepsy and related neurological disorders that involve overexcitation of the CNS. Our previous studies[5, 7, 17, 22] demonstrated the possibility that pathological conditions after birth could be caused by chemicals to which the mother might have been exposed to during pregnancy.

In the current study, we evaluated changes in neuronal excitability of the brain caused by Br^- in DNT induced by prenatal 1-BP exposure. A high concentration of Br^- was observed in the brain of pups prenatally exposed to 1-BP[7]; thus, in the Br^- group, we first achieved the same brain Br^- concentration as that observed in the 1-BP group. Subsequently, we compared the developmental effects of prenatal Br^- exposure on neuronal excitability with those of 1-BP exposure in pups, and found that both exhibited similar effects in suppressing PTZ-induced tonic contraction *in vivo* and hyperexcitability induced by PTZ, using the brain slices prepared *in vitro*.

Exposure of pregnant rats to 1-BP resulted in fetus brain Br^- concentrations up to ~10 mM at GD20 and ~7.5 mM in pups at PND3[7], which then gradually declined to approximately 1.3 mM at PNDs 13–15, according to the estimation using the one-compartment model. Moreover, the average concentration of Br^- in the brain of pups at PND14 was 1.9 mM, which is similar to the 1.3 mM Br reported to potentially enhance GABAergic inhibitory activity[13, 14], which, may explain the inhibition of increased body weight during lactation owing to weak suckling caused by the sedative effect of Br^-.

Considering that PTZ-induced epileptic episodes are typical (e.g., generalized tonic contraction following several myoclonic convulsions) in immature animals, the PTZ model is commonly used to induce brain hyperexcitability during lactation[18–21]. In this study, PTZ-induced generalized tonic contractions were suppressed in PND14 rat pups. However, the brain region(s) involved in PTZ-induced ton-

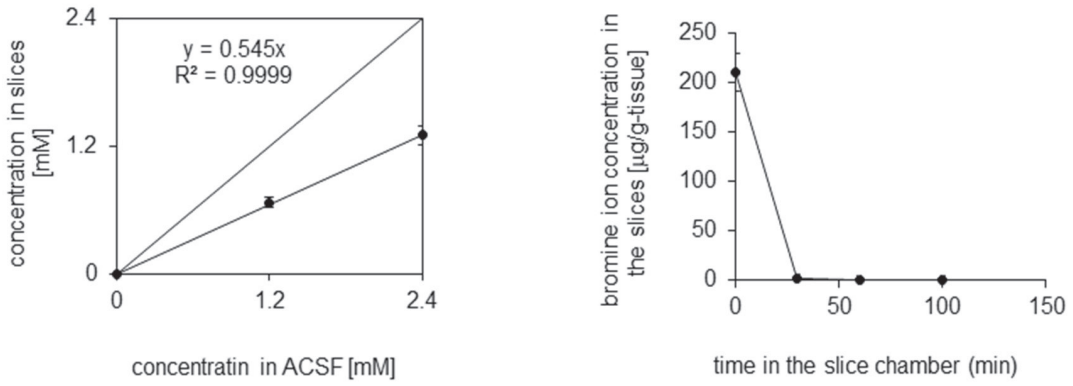


Fig. 3. Changes in Br^- concentration in the hippocampal slice
Left: Br^- concentration in ACSF vs. that in the hippocampal slices.
Right: Decrease in Br^- concentration in the hippocampal slices obtained from the Br^- group during ACSF perfusion.

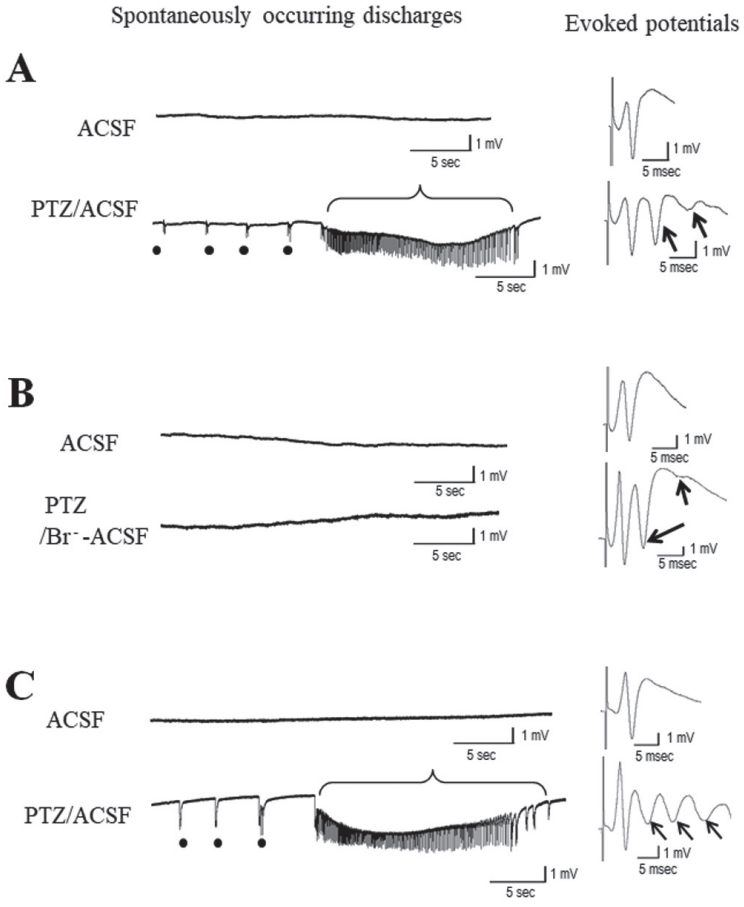
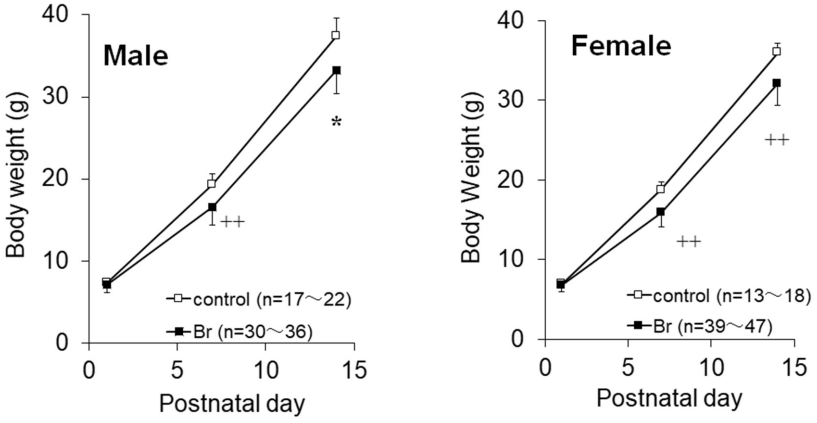


Fig. 4. Pentylenetetrazole (PTZ)-induced epileptiform discharge and evoked potentials in the hippocampal CA1 area slices
Left panels: spontaneously occurring discharge; right panels: evoked potential. A) No discharge in ACSF, and the evoked potential shows an intact slice. The lower right panel shows typical epileptiform discharge induced by PTZ in the same hippocampal slice as that in the upper panel. The evoked potential exhibits multiple population spikes, indicating a tendency to become epileptic. B) In the presence of Br^- (1.2 mM), no epileptiform activity is observed; however, the evoked potential exhibits epileptic characteristics. C) After washing Br^- from the slices obtained from the Br^- group, PTZ induces epileptiform discharge. The evoked potential shows epileptic characteristics. The closed circles represent isolated periodic discharge, and a single curly bracket represents long-lasting tonic-like epileptiform discharge following isolated periodic discharge. Arrows indicate individual population spikes within multiple population spikes.

Table 2. Br^- concentration in each group and hippocampal slices

Sample	Br^- concentration (mM)
PND14 brain of the 1-BP (700 ppm) exposure group	1.3
PND14 brain of the NaBr exposure group	1.9
Hippocampal slices	1.2



Supplementary Fig. S1. Changes in body weight of rat pups on postnatal day (PND) 1, 7, and 14. Left: Changes in male pups. Right: Changes in female pups. Open and closed squares represent the average values calculated for the body weight of the control and BR groups, respectively. The number indicates pup heads measured. * $p<0.05$ by Student's *t*-test. ++ $p<0.01$ by Welch *t*-test. Data are presented as the mean ± standard deviation.

ic contraction have yet to be identified. To the best of our knowledge, only one mapping study has been reported on rat pups at PND10 and PND21[20], with no evidence from c-Fos mapping or magnetic resonance imaging studies using PND14 rat pups.

Finally, *in vitro*, we confirmed that Br^-, comparable with the accumulated concentration in the brains of pups prenatally exposed to Br^-, suppressed PTZ-induced epileptiform activities in the CA1 area in the hippocampal slices (Fig. 4B and Table 2). Considering that we only observed epileptiform activity in 60% of the tested slices (Fig. 4A), hippocampal CA1 may be less responsive to PTZ-induced hyperexcitability, as was also reported in a previous study[20]. Additionally, the occurrence rate of generalized tonic contraction was 65% and 55% in the control group of the 1-BP group and Br group, respectively (Table 1), suggesting that the induction rate of approximately 60% may be due to the predisposition of PND14 pups. In the presence of 1.2 mM Br^-, no slices exhibited epileptiform activity, even in the presence of 2 mM PTZ. The suppression potency was similar to that in the 1-BP (700 ppm) and Br^- groups observed *in vivo*. In addition, after using ACSP to wash Br^- from the slices obtained from the pups of the Br group, PTZ was observed to induce the same pattern of epileptiform activity (Fig. 4C), suggesting that PTZ-induced epileptiform was independent of accumulated Br^- removal from pup brains.

Br^- was washed out with ACSF within 30 min (Fig. 3B); therefore, we believe that the enhanced excitability that we previously reported in the hippocampal slices[5] can be attributed to the effects of certain changes in the neuronal network observed after washing Br^-. During brain development, the activation of GABAergic receptors generates depolarizing potentials in the central nervous system, including hippocampal neurons[28, 29], hypothalamic neurons[30], cortical neurons[31], and spinal cord[32]. The biological significance of depolarizing GABA action in the developing hippocampus was recently investigated[33]. A high concentration of Br^- may prevent GABA receptor depolarization,

and, thus, affects neuronal network development. However, the underlying mechanism(s) induced by Br^- during brain development warrant further investigation.

Developmental toxicity has been reported in rat dams exposed to NaBr through drinking water[34]. Although their precise exposure conditions differed from ours, the inhibition of increased body weight in pups was the same as our current study findings. In addition, the decrease in brain weight, and changes in brain structure, correspond to the effects observed on postnatal development of pup brains.

We have also been investigating the usefulness of electro-physiological DNT and reported cases of prenatal valproic acid[22] and 1-BP[5] exposure. In the present study, we aimed to evaluate the developmental effects of Br^- accumulated in the brains of pups prenatally exposed to 1-BP and used the hippocampal slices to directly examine the effects of Br^- as significant field potentials could be evoked. Although further research on other chemicals is necessary, the results of electro-physiological DNT studies can provide a basis for future studies on DNT.

Certain limitations were noted in the current study. First, it is unclear whether the hippocampus has the highest level of responsiveness to injected PTZ. Hence, once it is determined which sections contain the highest responsiveness, slices should be obtained for further analysis. Second, the underlying mechanisms at cellular concentrations were not elucidated in the current study and, thus, requires further investigation.

As noted previously, the Japan Society for Occupational Health recommends an OEL of 0.5 ppm, whereas in the present study, considering three factors (i.e., difference in species, LOAEL of 700 ppm, and developmental neurotoxicity[5]), the exposure limit was calculated as 0.7 ppm using a default uncertainty factor. Therefore, based on our experimental data, the OEL appears to be appropriate. However, further studies on developmental neurotoxicity, based on different approaches, are required for the establishment of a standardized human risk assessment system.

To allow for accurate biological monitoring, the relationship between chemical concentrations in the blood/urine and the brain should be examined in animal models, and subsequently adapted for humans, as targeted brain samples are not unavailable in humans. Accordingly, a mathematical simulation study examining the relationship between the concentration of 1-BP in the blood and brain of rats, is underway. Once such techniques are adequately developed, we will be able to expand the findings to different kinds of chemicals that affect brain function.

In conclusion, Br^-, accumulated in the brain of pups prenatally exposed to 1-BP, affected neuronal excitability during lactation. Our findings may serve as a warning to women working in industries using Br^-. Meanwhile, xenobiotic Br^- is also found in medications, certain soft drinks, as well as dietary and herbal supplements containing bromide salt, which can cause bromism when consumed regularly[35]. Although some of these products contain warning labels, the use of these labels has recently decreased. Nonetheless, several forms of bromide are readily available. Therefore, quantitative rating of adverse effects induced by complex exposure to chemicals is necessary, particularly in children and pregnant women.

Acknowledgments

We would like to thank Mrs. K Egashira and Mrs. M Nakamura for animal care, and Ms. M Nonaka for analyzing the data of early animal experimentation with PTZ. We would like to thank Editage (www.editage.com) for English language editing.

Funding

This study was partially supported by a Grant-in-Aid for Scientific Research (C) [grant number 18510064 to Y.F.] from the Japan Society for the Promotion of Science (JSPS).

References

1) Recommendation of Occupational Exposure Limits (2018–2019) (2018) J Occup Health **60**, 419–542.
2) American Conference of Governmental Industrial Hygienists (ACGIH). 1-Bromopropane: TLV® Chemical Substances 7th Edition Documentation. Cincinnati: ACGIH; 2014.
3) The Japan Society for Occupational Health (2016). 1-bromopropane exposure survey results. https://www.mhlw.go.jp/content/11201000/000466716.pdf. Accessed July 10, 2019 in Japanese
4) Julvez J, Grandjean P (2009) Neurodevelopmental toxicity risks due to occupational exposure to industrial chemicals during pregnancy. Ind Health **47**, 459–68.
5) Fueta Y, Ishidao T, Ueno S, Yoshida Y, Kanda Y, Hori H (2018) Prenatal exposure to 1-bromopropane causes delayed adverse effects on hippocampal neuronal excitability in the CA1 subfield of rat offspring. J Occup Health **60**, 74–9.
6) van Leeuwen FXR, den Tonkelaar EM, van Logten MJ (1983) Toxicity of sodium bromide in rats: Effects on endocrine system and reproduction. Food Chem Toxicol **21**, 383–9.
7) Ishidao T, Fueta Y, Ueno S, Yoshida Y, Hori H (2016) A

cross-fostering analysis of bromine ion concentration in rats that inhaled 1-bromopropane vapor. J Occup Health **58**, 241–6.
8) Hanley KW, Petersen M, Curwin BD, Sanderson WT (2006) Urinary bromide and breathing zone concentrations of 1-bromopropane from workers exposed to flexible foam spray adhesives. Ann Occup Hyg **50**, 599–607.
9) Majersik JJ, Caravati EM, Steffens JD (2007) Severe neurotoxicity associated with exposure to the solvent 1-bromopropane (n-propyl bromide). Clin Toxicol **45**, 270–6.
10) Raymond LW, Ford MD (2007) Severe illness in furniture makers using a new glue: 1-Bromopropane toxicity confounded by arsenic. J Occup Environ Med **49**, 1009–19.
11) Mathias PI, Cheever KL, Hanley KW, Marlow KL, Johnson BC, B'Hymer C (2012) Comparison and evaluation of urinary biomarkers for occupational exposure to spray adhesives containing 1-bromopropane. Toxicol Mech Methods **22**, 526–53.
12) Fueta Y, Ishidao T, Ueno S, Yoshida Y, Kunugita N, Hori H (2007) New approach to risk assessment of central neurotoxicity induced by 1-bromopropane using animal models. Neurotoxicology **28**, 270–3.
13) Suzuki S, Kawakami K, Nakamura F, Nishimura S, Yagi K, Seino M (1994) Bromide, in the therapeutic concentration, enhances GABA-activated currents in cultured neurons of rat cerebral cortex. Epilepsy Res **19**, 89–97.
14) Meierkord H, Grünig F, Gutschmidt U, Gutierrez R, Pfeiffer M, Draguhn A, Brückner C, Heinemann U (2000) Sodium bromide: Effects on different patterns of epileptiform activity, extracellular pH changes and GABAergic inhibition. Naunyn Schmiedebergs Arch Pharmacol **361**, 25–32.
15) Pearce JMS (2002) Bromide, the first effective antiepileptic agent. J Neurol Neurosurg Psychiatry **72**, 412.
16) Brodie MJ (2010) Antiepileptic drug therapy the story so far. Seizure **19**, 650–5.
17) Fueta Y, Kanemitsu M, Egawa S, Ishidao T, Ueno S, Hori H (2015) Prenatal exposure to 1-bromopropane suppresses kainate-induced wet dog shakes in immature rats. J UOEH **37**, 255–61.
18) Meilleur S, Aznavour N, Descarries L, Carmant L, Mamer OA, Psarropoulou C (2003) Pentylenetetrazol-induced seizures in immature rats provoke long-term changes in adult hippocampal cholinergic excitability. Epilepsia **44**, 507–17.
19) Potier S, Sénécal J, Chabot J -, Psarropoulou C, Descarries L (2005) A pentylenetetrazole-induced generalized seizure in early life enhances the efficacy of muscarinic receptor coupling to G-protein in hippocampus and neocortex of adult rat. Eur J Neurosci **21**, 1828–36.
20) Andre V, Pineau N, Motte JE, Marescaux C, Nehlig A (1998) Mapping of neuronal networks underlying generalized seizures induced by increasing doses of pentylenetetrazol in the immature and adult rat: A c-fos immunohistochemical study. Eur J Neurosci **10**, 2094–106.
21) Erdoğan F, Gölgeli A, Küçük A, Arman F, Karaman Y, Ersoy A (2005) Effects of pentylenetetrazole-induced status epilepticus on behavior, emotional memory and learning in immature rats. Epilepsy Behav **6**, 537–42.
22) Fueta Y, Sekino Y, Yoshida S, Kanda Y, Ueno S (2018) Prenatal exposure to valproic acid alters the development of excitability in the postnatal rat hippocampus. Neurotoxicology **65**, 1–8.
23) Herron C E, Williamson R, Collingridge G L (1985) A selective N-methyl-d-aspartate antagonist depresses epileptiform activity in rat hippocampal slices. Neurosci Lett **61**, 255–60.
24) Piredda S, Yonekawa W, Whittingham TS, Kupferberg HJ (1986) Effects of antiepileptic drugs on pentylenetetrazole-induced epileptiform activity in the in vitro hippocampus. Epilepsia **27**, 341–6.
25) Bingmann D, Speckmann E - (1986) Actions of pentylenetetrazol (PTZ) on CA3 neurons in hippocampal slices of guinea pigs. Exp Brain Res **64**, 94–104.
26) Armand V, Louvel J, Pumain R, Heinemann U (1998) Effects of new valproate derivatives on epileptiform discharges induced by pentylenetetrazole or low Mg^{2+} in rat entorhinal cortex-hippocampus slices. Epilepsy Res **32**, 345–55.
27) Chang P, Walker MC, Williams RSB (2014) Seizure-induced reduction in PIP3 levels contributes to seizure-activity and is rescued by valproic acid. Neurobiol Dis **62**, 296–306.
28) Hosokawa Y, Sciancalepore M, Stratta F, Martina M, Cherubini E (1994) Developmental changes in spontaneous GABAA-mediated synaptic events in rat hippocampal CA3 neurons. Eur J Neurosci **6**, 805–13.
29) Leinekugel X, Khalilov I, McLean H, Caillard O, Gaiarsa J L, Ben-Ari Y, Khazipov R (1999) GABA is the principal fast-acting excitatory transmitter in the neonatal brain. Adv Neurol **79**, 189–201.
30) Chen G, Trombley PQ, Van Den Pol AN (1996) Excitatory actions of GABA in developing rat hypothalamic neurones. J Physiol (Lond) **494**, 451–64.
31) Owens DF, Boyce LH, Davis MBE, Kriegstein AR (1996) Excitatory GABA responses in embryonic and neonatal cortical slices demonstrated by gramicidin perforated-patch recordings and calcium imaging. J Neurosci **16**, 6414–23.
32) Ziskind-Conhaim L (1998) Physiological functions of GABA-induced depolarizations in the developing rat spinal cord. Perspect Dev Neurobiol **5**, 279–87.
33) Cherubini E, Griguoli M, Safiulina V, Lagostena L (2011) The depolarizing action of GABA controls early network activity in the developing hippocampus. Mol Neurobiol **43**, 97–106.
34) Disse M, Joó F, Schulz H, Wolff JR (1996) Prenatal Exposure to Sodium Bromide Affects the Postnatal Growth and Brain Development. J Brain Res **37**, 127–34.
35) Lugassy DM, Nelson LS (2009) Case files of the medical toxicology fellowship at the New York City Poison Control: Bromism: Forgotten, but not gone. J Med Toxicol **5**, 151–7.