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Effects of sub-acute and sub-chronic inhalation of 1-bromopropane on neurogenesis in adult rats☆

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ABSTRACT

Purpose: 1-Bromopropane (1-BP) intoxication is associated with depression and cognitive and memory deficits. The present study tested the hypothesis that 1-BP suppresses neurogenesis in the dentate gyrus, which is involved in higher cerebral function, in adult rats.
Methods: Four groups of 12 male Wistar rats were exposed to 0, 400, 800, 1000 ppm 1-BP, 8 h/day for 7 days. Another four groups of six rats each were exposed to 0, 400, 800 and 1000 ppm 1-BP for 2 weeks followed by 0, 200, 400 and 800 ppm for another 2 weeks, respectively. Another four groups of six rats each were exposed to 0, 200, 400 and 800 ppm 1-BP for 4 weeks. Rats were injected with 5-bromo-2′-deoxy-uridine (*BrdU*) after 4-week exposure at 1000/800 ppm to examine neurogenesis in the dentate gyrus by immunostaining. We measured factors known to affect neurogenesis, including monoamine levels, and mRNA expression levels of brain-derived neurotrophic factor (BDNF) and glucocorticoid receptor (GR), in different brain regions.
Results: BrdU-positive cells were significantly lower in the 800/1000 ppm-4-week group than the control . 1-Week exposure to 1-BP at 800 and 1000 ppm significantly reduced noradrenalin level in the striatum. Four-week exposure at 800 ppm significantly decreased noradrenalin levels in the hippocampus, prefrontal cortex and striatum. 1-BP also reduced hippocampal BDNF and GR mRNA levels.
Conclusion: Long-term exposure to 1-BP decreased neurogenesis in the dentate gyrus. Downregulation of BDNF and GR mRNA expression and low hippocampal norepinephrine levels might contribute, at least in part, to the reduced neurogenesis.

1. Introduction

1-Bromopropane (1-BP) was introduced as an alternative to ozone-depleting solvents, such as chlorofluorocarbons and 1,1,1-trichloroethane, and is mainly used as a cleaning agent for metal parts of precision instruments and as a solvent in spray adhesives (Ichihara, 2005; Ichihara et al., 2002). 1-BP is also currently used in the United States in the dry cleaning industry. However, previous studies have shown that 1-BP is neurotoxic in humans and rats. Human cases of 1-BP toxicity showed ataxia (Ichihara et al., 2002; Raymond and Ford, 2007), sensory deficits (Ichihara et al., 2002; Majersik et al., 2007; Raymond and Ford, 2007; Sclar, 1999), hyperreflexia in lower extremities (Majersik et al., 2007; Raymond and Ford, 2007; Sclar, 1999), prolongation of distal latency in motor nerve (Sclar, 1999), and decrease in sensory nerve conduction velocity (Sclar, 1999). Apart from the above neurologic deficits, disorders of the higher cerebral function including depression, anxiety and memory deficits in humans were also reported (Ichihara et al., 2004, 2002; Majersik et al., 2007). These were also supported by experimental studies, which showed reduced noradrenalin axons in rat prefrontal cortex and amygdala (Mohideen et al., 2011) and neurobehavioral abnormalities (Honma et al., 2003) in rats after exposure to 1-BP.

The mechanism of disorders of the higher cerebral function following exposure to 1-BP remains elusive. Rats treated with irradiation showed inhibition of hippocampal neurogenesis,

Abbreviations: 1-BP, 1-bromopropane; 5-HIAA, 5-hydroxyindoleacetic acid; 5-HT, serotonin; BDNF, brain-derived neurotrophic factor; BrdU, 5-bromo-2′-deoxy-uridine; DA, dopamine; DOPAC, 3,4-dihydroxyphenylacetic acid; GR, glucocorticoid receptor; HIP, hippocampus; HVA, homovanillic acid; NE, norepinephrine; PFC, prefrontal cortex; STA, striatum.

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impairment of conditional rule learning and memory for specific events (Winocur et al., 2006) and learning that depends on increased hippocampal neurogenesis (Gould et al., 1999). These studies show neurogenesis plays an important role in learning and memory. In addition, a number of investigators favor the notion of involvement of neurogenesis in mood disorders. A previous study indicated that the behavioral effects of chronic antidepressants are mediated through stimulation of hippocampal neurogenesis (Santarelli et al., 2003). Transgenic adult mice with impaired hippocampal neurogenesis exhibited anxiety-related behaviors (Revest et al., 2009).

To clarify the relationship between 1-BP exposure and neurogenesis, we selected three variables, glucocorticoid receptor (GR), brain-derived neurotropic factor (BDNF) and a battery of neurotransmitters. Previous studies suggested that GR mediates the function of glucocorticoid, and glucocorticoid binding to GR can modulate the expression of various genes, including those related to neurogenesis (Webster et al., 2002). Another factor that affects neurogenesis is BDNF, a protein abundantly present in both the brain and plasma. BDNF is reported to increase neuronal survival *in vitro* (Memberg and Hall, 1995) and potentiate neuron differentiation in the adult hippocampus (Palmer et al., 1997). Also, disorders of neurotransmitter level are important pathoetiological factors in depression. In this regard, neurotransmitter release also regulates adult neurogenesis (Vaidya et al., 2007).

Based on the understanding of the relationships among disorders of higher cerebral function, neurogenesis, GR, BDNF and neurotransmitters, the present study was designed to investigate the effect of exposure to 1-BP on neurogenesis, the expression of BDNF and GR in the hippocampus, and monoamine levels in different brain regions, including hippocampus, in adult rats. Such information should enhance our understanding of the mechanism underlying disorders of higher cerebral function induced by exposure to 1-BP.

2. Materials and methods

The entire experiment was conducted according to the Guide of Animal Experimentation of Nagoya University, School of Medicine, concerning protection and control of animals.

2.1. Inhalation system

The inhalation exposure system was described in previous study (Ichihara et al., 2000). In brief, a regulated volume of 1-BP was evaporated at room temperature and mixed with a larger volume of clear air to achieve the target concentrations. The vapor concentration of 1-BP in the chamber was measured every 10 s by gas chromatography and electronically controlled to within ±5% of the target dose.

2.2. Animals and exposure to 1-BP

The study consisted of three parts. In the first experiment, 48 male Wistar rats purchased from Shizuoka Laboratory Center (SLC), Inc. Japan, were divided into four groups of 12 each and rats of each group were exposed to 1-BP at 0, 400, 800 and 1000 ppm using a custom-made inhalation system, at 8 h/day from 9:00 to 17:00 for 1 week. The age and body weight at the time of study entry were 10 weeks and 270–290 g, respectively. Six rats per group were used for biochemical studies and the remaining six rats were used for histopathological studies on neurogenesis.

In the second longer exposure experiment, 24 male Wistar rats (SLC, Inc. Japan) were divided into four groups of six rats each and exposed to 1-BP for 4 weeks to investigate the effect of longer exposure to 1-BP on neurogenesis. In these experiments, 1-BP concentration was set at 0, 400, 800 and 1000 ppm in the first 2 weeks, then reduced from 400 to 200, from 800 to 400, and from 1000 to 800 ppm in the second two weeks of the 4-week experiment because one rat died after 2-week exposure to 1000 ppm. Inhalation was conducted 8 h/day from 21:00 to 5:00 for 4 weeks in total. The age and body weight at study entry were 11 weeks and 320–350 g, respectively.

In the third longer exposure experiment, 24 rats were divided in four groups of six rats each and each rat was exposed to 1-BP at 0, 200, 400 and 800 ppm using the inhalation system 8 h/day from 21:00 to 5:00 for 4 weeks to study the effect of 4-week exposure to 1-BP on biochemical parameters. The age and body weight at study entry were 13 weeks and 330–360 g, respectively. Before exposure, the rats were acclimated in a light-controlled room [room temperature (23–25 °C), stable humidity (57–60%), 12 h light cycle starting at 9:00 am] and had free access to food and water.

The mean concentration of 1-BP measured every 10 s for 8 h was considered the value for that day. The daily time-weighted average of the concentration was 414 ± 12, 814 ± 21 and 1047 ± 23 ppm (n = 10, mean ± standard deviation) in the first experiment, and 379 ± 34, 756 ± 65 and 940 ± 90 ppm (n = 16) from the beginning to the 14–16th day of the experiment, and 199 ± 14, 386 ± 14 and 768 ± 25 ppm (n = 15) from the 15–17th day until the end of exposure. The time-weighted averages of exposure levels in the second part of the experiment (weeks 3 and 4) of the second experiment were 288 ± 7, 559 ± 13 and 851 ± 7 ppm, while those for the third experiment were 194 ± 12, 393 ± 13 and 775 ± 24 ppm (n = 31).

2.3. BrdU (5-bromo-2′-deoxy-uridine) injection

To determine the effect of 1-BP exposure on neurogenesis, after the last exposure, 12 rats of each group were each injected with BrdU (Sigma, St. Louis, MO) at 24 mg/100 g body weight in 0.9% saline, i.p. every 2 h for three times.

2.4. Dissection and tissue preparation

Rats injected with BrdU were transcardially perfused with 4% paraformaldehyde (PFA) 12 h after the last injection. The whole brain was dissected out and postfixed in 4% PFA for 24 h. Subsequently, the brain samples were placed into 10% sucrose solution overnight then placed into 20% sucrose solution overnight and later transferred into 30% sucrose solution. After overnight incubation in 30% sucrose solution, the brain samples were embedded in optimal cutting temperature (OCT) compound and frozen on dry ice. Frozen blocks were stored in −80 °C.

On the other hand, 12 rats of each group that were not injected with BrdU were decapitated and the whole brain was dissected out within 5 min. The hippocampus, striatum and prefrontal cortex were separated and frozen on dry ice immediately. The adrenal glands were cut removed and frozen on dry ice simultaneously. Blood samples were collected using heparinized funnel soon after decapitation.

2.5. Real-time quantitative reverse transcription-polymerase chain reaction (RT-PCR)

The hippocampal mRNA expression levels of BDNF and GR were measured by quantitative RT-PCR. Briefly, the RNeasy Lipid Tissue Mini Kit (Qiagen, Hilden, Germany) was used to extract total RNA from the hippocampus. The RNA products were stored at −80 °C after extraction. SuperScript III Reverse Transcriptase Kit was used for mRNA reversal. After reverse-transcription, quantitative RT-PCR was performed using the M×3005P QPCR System (Agilent Technologies, Waldbronn, Germany). The primers and probes used in this reaction were designed by the Universal Probe Library (Roche Diagnostics): BDNF primers: forward, 5′AGCGCGAATGTGTTAGTGGT; reverse, 5′GCAATTGTTTGCCTCTTTTTCT, GR primers: forward, 5′GACATGGAAGCTGCAGAGTA; reverse, 5′TCGTTTCTTCCAGCATAAAGGT. Beta-actin was used as the control gene, using the primers: forward, 5′CCCGCGAGTACAACCTTCT; reverse, 5′CGTCATCCATGGCGAACT.

2.6. Measurement of serum levels of corticosterone and neurotransmitter levels in the hippocampus, striatum and pre-front cortex

Serum corticosterone levels were measured by a radioimmunoassay kit (Corticosterone EIA kit, Cayman Chemical, Ann Arbor, MI). The levels of monoamines, including noradrenalin, dopamine, serotonin, and their metabolites, were measured in the hippocampus, striatum and pre-front cortex (PFC), respectively, using high performance liquid chromatography (HPLC) with an electrochemical detector (ENO-10, Eicom Co., Kyoto, Japan).

In brief, the brains were quickly removed, and the hippocampus, striatum and PFC were dissected out on an ice-cold glass plate. Each brain region was rapidly frozen and stored at −80 °C until assayed. Each frozen brain sample was weighed and homogenized with an ultrasonic processor in 65 μl of 0.2 M perchloric acid containing isoproterenol as an internal standard. The homogenates were placed in ice for 30 min and centrifuged at 20,000 × g for 15 min at 4 °C. The supernatants were mixed with 1 M sodium acetate to adjust the pH to 3.0 and injected into an HPLC system equipped with a reversed-phase ODS column (Eicompak SC-5 ODS; 2.1 × 150 mm; Eicom) and an electrochemical detector. The column temperature was maintained at 25 °C, and the detector potential was set at +750 mV. The mobile phase was 0.1 M citric acid and 0.1 M sodium acetate, pH 3.5, containing 17% methanol, 190 mg/l sodium-L-octanesulfonate and 5 mg/l EDTA, and the flow rate was set at 0.23 ml/min. The turnover of monoamines was calculated from the content of each monoamine and their metabolites.

2.7. Immunohistochemistry and quantification of BrdU-positive cells

Coronal brain sections of 30 μm were cut on cryostat and mounted on slides. Every three sections were collected, thus covering the whole dentate gyrus (Bregma −2.56 to −6.04 mm). Sections were post-fixed in 4% PFA for 20 min, and treated with Nonidet P-40 (NP-40) overnight. They were later denatured with 5 M HCl for 20 min and then blocked with 10% goat serum for 1 h. BrdU-positive cells were labeled using

BrdU labeling & detection kit II (Roche Diagnostics, Mannheim, Germany) according to the protocol supplied by the manufacturer (Ibi et al., 2010). Sections were examined under a light microscope and the number of BrdU-positive cells was counted by an examiner blinded to the exposure group. Every three sections covering the entire dentate gyrus (total 15 sections per rat) were examined. BrdU-positive cells in the dentate gyrus were counted and recorded in an area that included the granule cell layer and subgranule zone (SGZ).

2.8. Statistical analysis

Data are expressed as mean ± SD. One-way analysis of variance (ANOVA) was used for comparison of group data, followed by Dunnett's multiple comparison. The significance level was set at $P < 0.05$. Data were analyzed using the JMP 8 software (SAS Institute Inc., Cary, NC).

3. Results

3.1. Quantitative RT-PCR

Quantitative RT-PCR showed no changes in BDNF, GR after 1-week exposure to 0, 400, 800 and 1000 ppm of 1-BP. The results of RT-PCR in rats exposed to 1-BP for 4 weeks are shown in Supplementary Table 1. The expression of BDNF mRNA relative to β-actin was lower in both the 400 ppm and 800 ppm groups but not in 200 ppm group, compared to the control group (Fig. 1a). The GR mRNA expression levels after exposure to 200 ppm, 400 ppm, and 800 ppm were also significantly lower than the control (Fig. 1b).

3.2. Corticosterone and weight of adrenal glands

Neither 1-week nor 4-week exposure to 1-BP at different concentrations altered plasma corticosterone level. Although body weight was significantly lower both after1-week and 4-week exposure, the weight of adrenal gland remained stable in this experiment and the weight of adrenal gland to body weight ratio increased after 1-week exposure (Table 1).

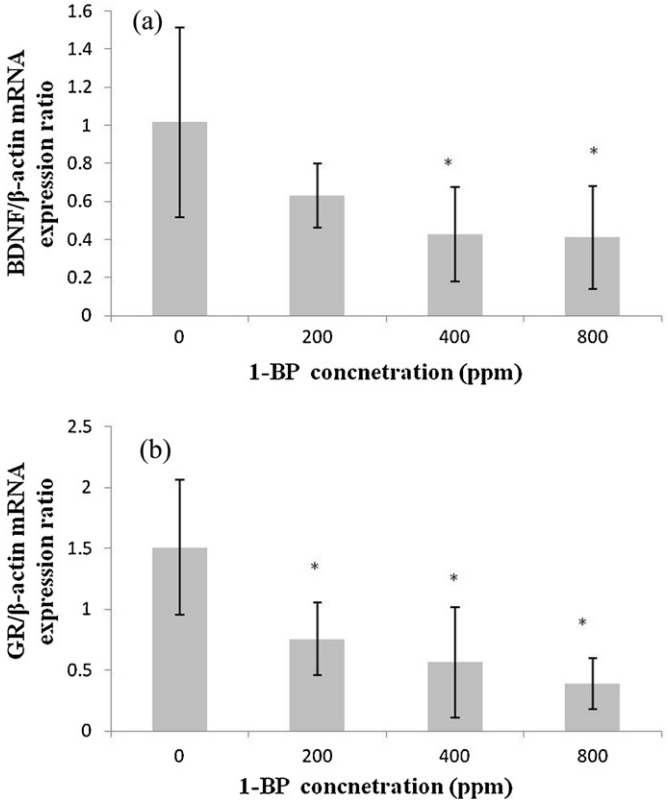

Fig. 1. Effects of 4-week exposure to different concentrations of 1-bromopropane (1-BP) on the mRNA expression of (a) brain-derived neurotrophic factor (BDNF), and (b) glucocorticoid receptor (GR), relative to that of β-actin. Data are mean ± SD of 6 rats. *$P < 0.05$ by ANOVA and Dunnett test.

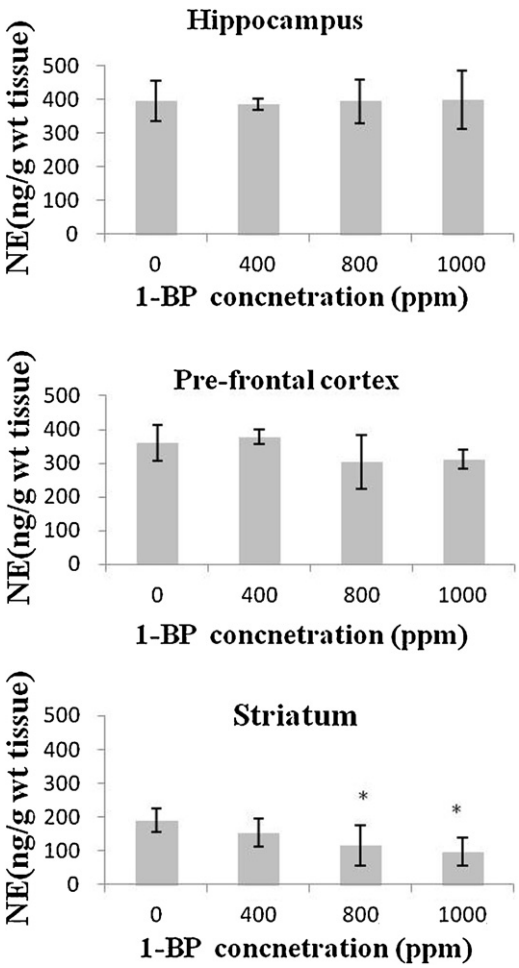

Fig. 2. Effects of 1-week exposure to different concentrations of 1-bromopropane (1-BP) on norepinephrine (NE) levels in the hippocampus, prefrontal cortex and striatum. Data are mean ± SD of 6 rats. *$P < 0.05$ by ANOVA and Dunnett test.

3.3. Changes in neurotransmitter levels

Exposure to 800 and 1000 ppm of 1-BP for 1 week reduced NE levels (Fig. 2), but not those of serotonin or 5-HIAA, in the striatum (Supplementary Table 2). Furthermore, the DOPAC/DA ratio decreased in the striatum, and the decrease varied proportionately with the 1-BP concentration. The HVA/DA ratio after 1-week exposure to 400 ppm of 1-BP was low in pre-frontal cortex (Supplementary Table 3). Exposure to 1-BP for 4 weeks resulted in more pronounced changes in hippocampus neurotransmitter levels compared with those observed after 1-week exposure: hippocampal NE was lower at 800 ppm (Fig. 3), and HVA, DOPAC/DA, HVA/DA, and the (DOPAC + HVA)/DA ratio were higher in the 800 ppm group. However, 1-BP did not affect hippocampal serotonin levels, irrespective of the dose and duration of exposure, whereas it reduced hippocampal DA in the 200 and 800 ppm groups. Exposure to 800 ppm decreased the concentrations of NE and 5-HIAA levels, and decreased HVA and HVA/DA ratio in the pre-frontal cortex, as well as reduced NE but had no effect on other neurotransmitters in the striatum (Supplementary Tables 4 and 5).

Table 1
Effects of 1-week exposure to 1-BP on bodyweight, weight of adrenal glands and plasma corticosterone level.

		n	Body weight (g)	Adrenal gland weight[a] (mg)	Adrenal gland weight/ body weight ratio	Corticosterone (ng/ml) ($\times 10^2$)
1-week	0	6	348 ± 7	32 ± 2	$(8.8 \pm 0.3) \times 10^{-5}$	15.2 ± 11.5
	400	6	339 ± 11	32 ± 5	$(9.3 \pm 0.1) \times 10^{-5}$	7.7 ± 7.1
	800	6	325 ± 11*	34 ± 4	$(10.5 \pm 0.9) \times 10^{-5}$*	8.5 ± 10.1
	1000	6	312 ± 19*	33 ± 4	$(10.5 \pm 0.9) \times 10^{-5}$*	15.4 ± 16.0
4-week	0	6	402 ± 15	36 ± 4	$(8.9 \pm 0.9) \times 10^{-5}$	2.0 ± 1.4
	400	6	402 ± 13	33 ± 1	$(8.3 \pm 0.4) \times 10^{-5}$	3.7 ± 1.4
	800	6	394 ± 22	34 ± 3	$(8.6 \pm 0.1) \times 10^{-5}$	2.3 ± 1.6
	1000	6	363 ± 28*	33 ± 3	$(9.0 \pm 0.1) \times 10^{-5}$	5.3 ± 2.8

Data are mean ± SD.
[a] Mean weight of left and right adrenal glands.
* $P < 0.05$, relative to the control (by one-way analysis of variance, followed by Dunnett's multiple comparison).

3.4. BrdU-positive cell count

Exposure to 1-BP for 1 week did not result in any change in neurogenesis, whereas exposure to 800/1000 ppm for 4 weeks reduced the number of BrdU-positive cells (Figs. 4 and 5).

4. Discussion

The present study showed that 4-week exposure to 1-BP decreased neurogenesis in the dentate gyrus. To the best of our knowledge, this is the first study to demonstrate the effect of 1-BP on neurogenesis in animals. Decrease in hippocampal neurogenesis might play an important role in 1-BP-induced disorders of the higher cerebral function, including depressive mood, impairment of cognitive function and memory loss. With regard to the monoamine levels, 1-week exposure to 1-BP resulted in early-onset fall in NE level in the striatum and 4-week exposure decreased to a greater extent NE level in the hippocampus, prefrontal cortex and striatum, but had no effect on serotonin level. Taken together

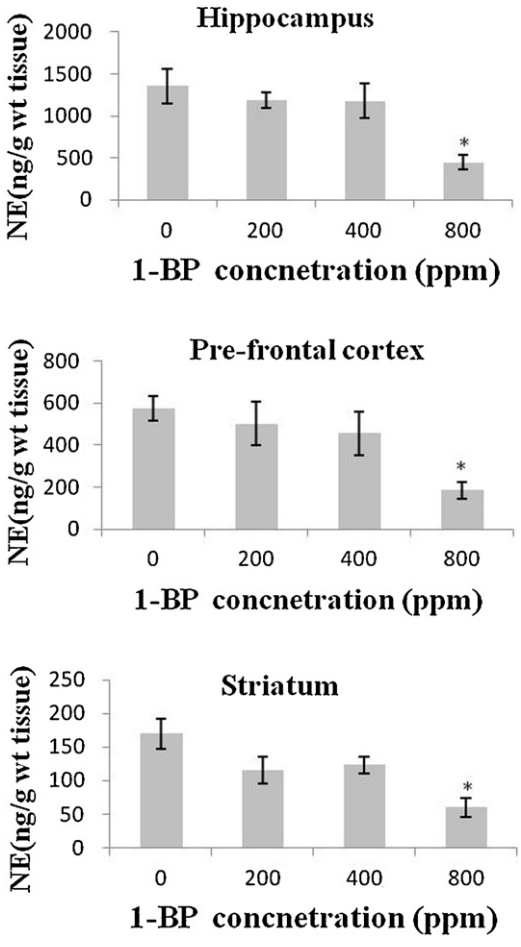

Fig. 3. Effects of 4-week exposure to different concentrations of 1-bromopropane (1-BP) on norepinephrine (NE) levels in the hippocampus, prefrontal cortex and striatum. Data are mean ± SD of 5 rats for the 0, 400 and 800-pmm groups and 6 rats for the 1000 ppm group. *$P < 0.05$ by ANOVA and Dunnett test.

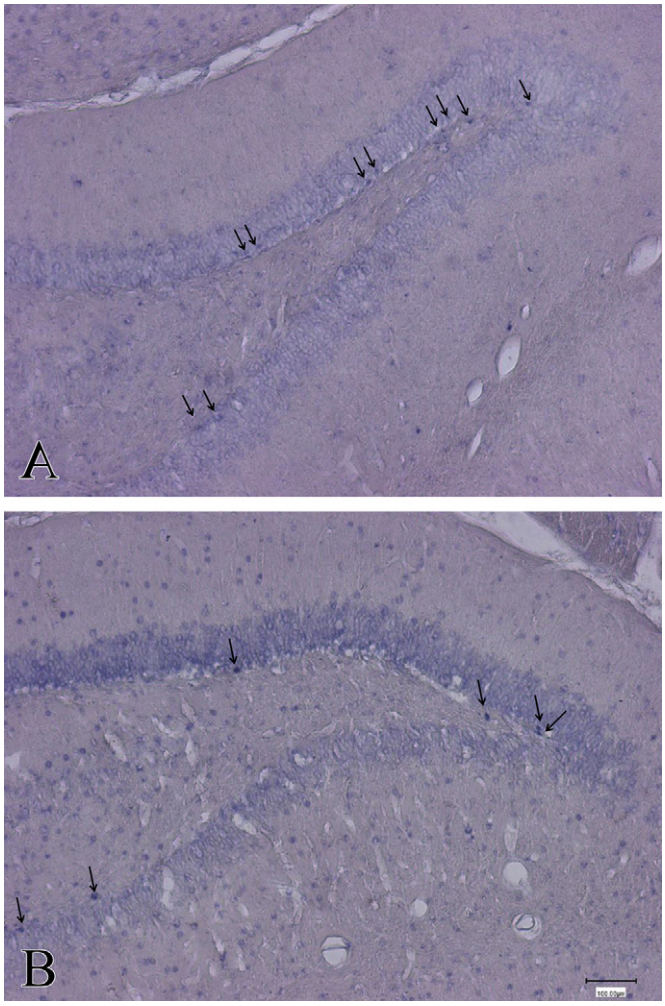

Fig. 4. Immunostaining of newborn cells in dentate gyrus with BrdU after 4-week exposure to 1-bromopropane. (A) control group (0 ppm), (B) 1000/800 ppm group BrdU-positive cells are stained dark brown (arrows).

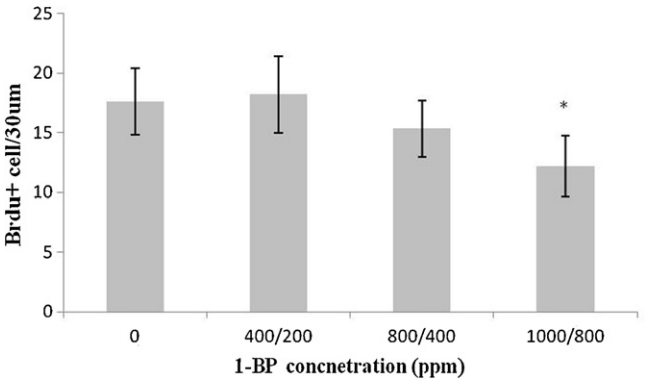

Fig. 5. Effects of 4-week exposure to different concentrations of 1-bromopropane (1-BP) on neurogenesis in the dentate gyrus. Data are mean ± SD of 6 rats in 0, 200/400 ppm, 400/800 ppm group and 5 rats in 800/1000 ppm group. *$P < 0.05$ by ANOVA and Dunnett test.

with our previous study showing that 4-week exposure to 1-BP decreased the number of noradrenergic axons, but not serotonin axons in F344 rats (Mohideen et al., 2011), it seems that exposure to 1-BP targets the noradrenalin system in the central nervous system (CNS).

Several studies demonstrated changes in neurotransmitter levels in depression and their modulation by antidepressants. The change in neurotransmitter level could affect neurogenesis in the hippocampus as mentioned in the Section 1. Although it is difficult to identify the exact neurotransmitter responsible for neurogenesis, recent studies have shown that NE, but not serotonin, directly activates adult hippocampal neurogenesis. Jhaveri and colleagues (Jhaveri et al., 2010) found both *in vitro* and in adult mice that NE can activate stem cells in the adult hippocampus by acting with β_3–adrenergic receptors. Activation of α_2-adrenoceptor was also reported to reduce hippocampal neurogenesis in adult Wistar rats (Yanpallewar et al., 2010). Considered together with results of the studies on the role of NE in neurogenesis, the present results suggest that exposure to 1-BP affects neurogenesis through disorders of the noradrenalin system.

In our experiments, 4-week exposure to 1-BP also down-regulated BDNF and GR mRNAs. The result of BDNF in the present study is consistent with the previous *in vitro* study, which showed down-regulation of BDNF in a human astrocyte cell line U251 and mouse primary astrocytes (Yoshida et al., 2009). Since BDNF and GR are known to positively control neurogenesis (Oomen et al., 2007; Rossi et al., 2006), it is possible that their down-regulation contributes to the decrease in neurogenesis in the present study (Fig. 6). Furthermore, a previous cell culture study showed that NE enhanced BDNF expression in hippocampal neurons: isolated embryonic hippocampal neurons cultured in the presence of NE increased BDNF mRNA and protein in dose-and–time dependent manners (Chen et al., 2007). In another pilot study, treatment of patients with amyotrophic lateral sclerosis (ALS) by BDNF for nine months increased NE levels (Beck et al., 2005). These results indicate that BDNF and NE can affect each other and their levels correlate with each other. The decrease in both NE and BDNF found in the present study is consistent with the above studies on the relationship between NE and BDNF, although the study did not clarify the exact pathway involved in the regulation of NE and BDNF, leading to the suppression of neurogenesis after exposure to 1-BP (Fig. 6).

Previous studies demonstrated that corticosterone suppresses neurogenesis in adult rats (Brummelte and Galea, 2010). However, the present study did not find significant changes in corticosterone level and weight of the adrenal gland, suggesting that it is not involved in the suppression of neurogenesis. The increase in the weight of adrenal gland to body weight ratio should result from the significant decrease in body weight, as the absolute weight of adrenal gland did not change significantly.

Exposure to 1-BP increases oxidative stress in the CNS, as reported in previous studies from our group. Thus, 1- and 4-week exposure to 1-BP dose-dependently decreased total glutathione in the rat cerebellum and cerebrum including the hippocampus (Wang et al., 2002, 2003). In other studies, ROS and total protein carbonyl increased in both hippocampus and plasma after 4-week exposure to 1-BP, in a dose-dependent manner (Huang et al., 2012, 2011). Furthermore, exposure to 1-BP increased the levels of oxidative stress markers of thiobarbituric acid reactive substances (TBARS), protein carbonyl, reactive oxygen species (ROS) and nitric oxide (NO) in a dose-dependent manner in the rat cerebellum (Subramanian et al., 2012). On the other hand, another study suggested that increased oxidative stress is the underlying mechanism of decreased BDNF levels in acute manic episodes (Kapczinski et al., 2008) and treatment with vitamin E, an antioxidant, preserved the process of activation of synapsin I and CREB, through which BDNF facilitates synapse associated with learning and memory (Wu et al., 2004). The decrease in BDNF mRNA in the present study might be partly caused by oxidative stress in the hippocampus induced by 1-BP exposure. In this regard, treatment with norepinephrine was reported to reduce ROS levels in cultured cells (Troadec et al., 2001). Considered collectively, the low NE levels described in the present study could increase oxidative stress (Fig. 6).

In this experiment, we were able to measure the mRNA levels of GR and BDNF, but not their protein levels. Since we used the left hippocampus for measurement of neurotransmitter levels and the right side for RT-PCR, further samples for measurement of the protein levels were unfortunately not available.

In the second experiment, one rat died because of debilitation at 2 weeks after the start of exposure to 1-BP. The timing of debilitation in the present study was much earlier than that reported in a previous study (Yu et al., 1998). This might be due to differences in the study protocols: the rats were exposed to 1-BP at night in the present study while they were exposed to 1-BP from day to evening in the previous study. Thus, differences in 1-BP uptake into body tissues are likely to exist between the two studies due to differences in physical activity and breathing pattern at night in rats. However, since we changed the level of exposure to 1-BP at 2 weeks in this rat, it is difficult to directly compare the present study with the previous study. Nevertheless, the present study suggests that exposure of adult rats to 1-BP at high concentration induces suppression of neurogenesis. Further studies that include larger number of rats exposed to 1-BP for longer period are needed.

In the present study, exposure levels of 200–1000 ppm were selected to match those found in the work environment of cases identified with 1-BP neurotoxicity. A recent case report of human 1-BP intoxication showed an estimated exposure level of 553 ppm (mean of time weighted averages) (Samukawa et al., 2012). The ambient concentration of 1-BP in front of a washing tank containing 1-BP was around 1500 ppm. Thus, the concentrations of 1-BP used in the present study of 200–1000 ppm truly represent those actually present in poorly controlled workplaces associated with the reported human cases of neurotoxicity. Admittedly, however, it is difficult to directly compare the effects of different exposure levels on humans and animals at this stage, because of possible species difference in susceptibility (Liu et al., 2009).

In conclusion, the present results in rats suggest that the disorders of higher cerebral function noted in workers exposed to 1-BP may be explained by suppression of neurogenesis in the hippocampus, and that this effect might be, at least in part, due to the negative effect of 1-BP on GR and BDNF mRNAs and hippocampal NE level.

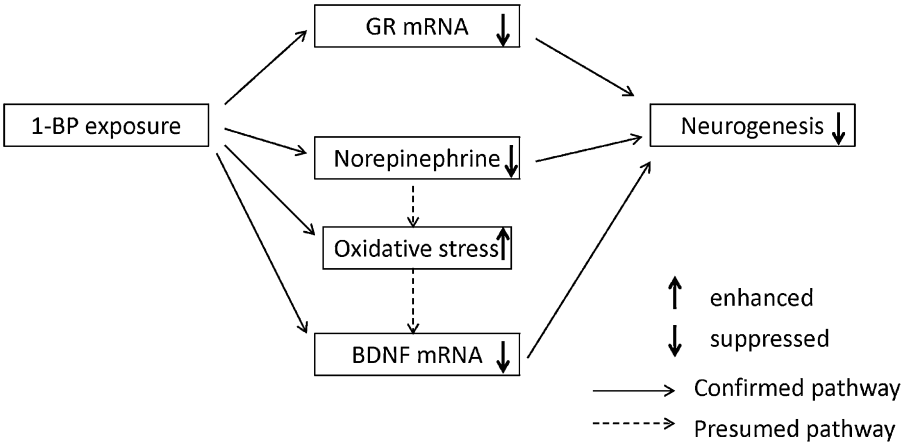

Fig. 6. Possible pathways of suppression of neurogenesis after exposure to 1-bromopropane (1-BP).

Conflicts of interest

None.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tox.2012.12.009.

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