Development of a direct exposure system for studying the mechanisms of central neurotoxicity caused by volatile organic compounds

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Abstract: Many volatile organic compounds (VOCs) used in work places are neurotoxic. However, it has been difficult to study the cellular mechanisms induced by a direct exposure to neurons because of their high volatility. The objective of this study was to establish a stable system for exposing brain slices to VOCs. With a conventional recording system for brain slices, it is not possible to keep a constant bath concentration of relatively highly volatile solvents, e.g. 1-bromopropane (1-BP). Here we report a new exposure system for VOCs that we developed in which a high concentration of oxygen is dissolved to a perfused medium applying a gas-liquid equilibrium, and in which the tubing is made of Teflon, non adsorptive material. Using our system, the bath concentration of the perfused 1-BP remained stable for at least 2 h in the slice chamber. Both 6.4 and 2.2 mM of 1-BP did not change the paired-pulse response, but fully suppressed long-term potentiation in the dentate gyrus (DG) of hippocampal slices obtained from rats, suggesting that 1-BP decreases synaptic plasticity in the DG at the concentrations tested. Our new system can be applicable for investigating the underlying mechanisms of the neurotoxicity of VOCs at the cellular level.

Key words: Organic solvent, Central neurotoxicity, Gas-liquid equilibrium, Electrophysiology, Brain slices

Introduction

Volatile organic compounds (VOCs) are widely used as degreasers and constituents of paints, lacquers, inks, aerosol spray products, adhesives, intermediates in chemical synthesis, and fuels. It is well known that most VOCs have the potential to cause neurotoxicity, such as depressant effects on the central nervous system (CNS),

in acute high-level exposures, and degenerative changes in the CNS or the peripheral nervous system in chronic moderate-to-high-level exposure. Although it is still controversial whether there is causality between symptoms and exposure, some neurologic dysfunctions, such as sustained changes in mood and/or personality and impairment of intellectual function, have been reported in work places where VOCs are used[1].

The mechanisms of the neurotoxicity of VOCs have been studied by using animal models exposed to VOCs[2, 3], and on the cellular level by *in vitro* preparations of *Xenopus* oocytes and cultured cells under direct application[4–6]. Although some VOCs affect behavior, and their molecular

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targets such as receptors and ion channels have been demonstrated, the direct effects of VOCs on neural circuits and synaptic phenomena, especially the neuronal mechanisms of behavioral changes induced by VOCs, are poorly understood.

Brain slice preparations, which have isolated neural circuits keeping their physiological conditions, have frequently been used to evaluate the direct effects of chemicals, but there are few such studies for VOCs[7, 8]. One of the reasons is the difficulty in keeping a stable VOC concentration in a conventional VOC exposure system[7, 8]. Artificial cerebrospinal fluid (ACSF) is saturated by bubbling an O_2/CO_2 mixed gas to maintain the viability of the brain slices, but this bubbling causes the dispellation of the VOC dissolved in the ACSF.

The purpose of this study is to develop a stable system for direct exposure to VOCs. We developed the system by saturating ACSF with an O_2/CO_2 mixed gas without bubbling by supplying the gas into the headspace of the bottle and keeping the gas-liquid equilibrium state in the bottle in order to prevent evaporation of the VOCs. In addition, we used Teflon for tubing in all routes to avoid the adsorption of VOCs. We used 1-bromopropane (1-BP) as a representative VOC, because we can check the robustness and adaptability by using a VOC that has a comparatively higher volatility and neurotoxicity such as ataxic gait, prolongation of distal latency, alteration of the function of receptors, and memory dysfunction[9–13]. Since the direct effects of 1-BP on memory function have not been investigated in detail yet, we evaluated the effects of 1-BP on cellular models of learning and memory, such as long term potentiation (LTP) and paired pulse facilitation/paired pulse depression (PPF/PPD) induced by consecutive stimulation resulting in changes in the efficacy of synaptic transmissions[14–16].

Subjects and Methods

Exposure system for VOCs

In previous studies[7, 8], a VOC was applied to ACSF without preventing volatilization. In this study, we modified the exposure system for VOCs to keep the concentration stable. Figure 1 shows a schematic diagram of the experimental apparatus, which consists of a mixed-gas supply system, a perfusion path, a slice chamber, a stimulation system and a recording system.

The mixed gas supply system consists of a gas cylinder filled with a mixed gas (O_2/CO_2: 95/5%) (1) with a regulator (P-583A, ASTEC Co., Ltd., Fukuoka, Japan), a
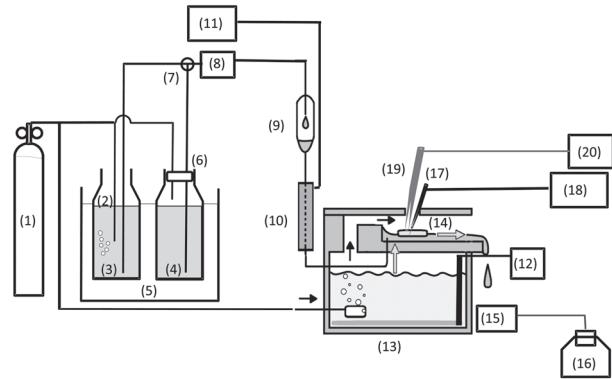
Fig. 1. Schematic diagram of the experimental apparatus.
(1) O_2/CO_2 gas cylinder, (2) polyethylene tube with injection needle, (3) artificial cerebrospinal fluid, (4) 1-BP solution, (5) Thermostatic water bath, (6) Teflon plug, (7) three-way cock, (8) peristaltic pump, (9) drip chamber made of glass, (10) inline heater, (11) automatic temperature controller, (12) temperature controller, (13) slice chamber, (14) hippocampal slice sample, (15) waste effluent vessel, (16) waste effluent reservoir, (17) electrode for recording, (18) head stage, (19) stimulation electrode, (20) isolator. Thick arrow and thin arrow denote flow of 1-BP solution and O_2/CO_2 gas, respectively.

polyethylene tube with an injection needle (0.90 × 38 mm, OH114. TOP Co., Japan) that is attached to the edge of the tube (2). The O_2/CO_2 mixed gas was supplied to the ACSF (3) and a bubbler in the slice chamber. 1-BP was dissolved in the ACSF in a bottle (657 ml, neck size I. D. 25 mm) (4) that was installed in a thermostatic water bath (5). The temperature of the thermostatic water bath was kept at 27.6°C. A Teflon plug (6) was attached to the top of the 1-BP bottle to reduce the loss of 1-BP. The ACSF was saturated with O_2/CO_2 mixed gas by bubbling. The 1-BP solution was also saturated with the same gas by supplying the gas into the headspace of the bottle and keeping the gas-liquid in a state of equilibrium in the bottle. Bubbling was not carried out in the 1-BP solution because a portion of the 1-BP in the solution would be purged and the concentration would be reduced.

1-BP exposure system

The exposure system consists of Teflon tubing, a three-way cock (7) for manually switching ACSF and 1-BP solution, a peristaltic pump (8) (PST-100N, IWAKI, Tokyo, Japan), a drip chamber using a 10 ml whole pipette made of glass (9), and an interface type slice chamber (13) in a shield made of copper.

ACSF with or without 1-BP was perfused at a flow rate of 1.0 ± 0.1 ml/min by controlling the number of rotations of the peristaltic pump. The perfusion rate of the fluid

was checked by the speed of droplets in the drip chamber. The perfusate was heated by an inline heater (10), which was controlled by an automatic temperature controller (11) (TC-3 24B, Warner instruments Co., Hamden, USA), just before it reached the slice chamber. The perfusate in the slice chamber was collected into a container (15) and stored in a reservoir (16).

A Teflon tube (ID 0.68 mm, OD 3 mm) was used to reduce the effect of loss of 1-BP by adsorption during perfusion because 1-BP is easily adsorbed inside the tubing. A typical peristaltic pump uses a silicone tube, but in this study, we used a Teflon tube (ID 2 mm and OD 3 mm) in order to avoid 1-BP adsorption. The tube was exchanged for a new one before the deformation or abrasion of the tube resulted in an unstable flow rate.

The slice chamber is a device for reserving a sliced hippocampal sample by perfusing ACSF. The sliced sample was set at a distance of 2 cm from the fluid entrance. To prevent drying of the slice sample and deficiency of oxygen, saturated water vapor and an O_2/CO_2 mixed gas were introduced to the slice samples from the water bath of the chamber. The water bath was warmed by a heater controlled with temperature controller (12) (TCCN3, Jikken Kaihatsu Co., Ltd., Kitakyushu, Japan). The temperature of the perfusate in the chamber was monitored by an automatic temperature controller (11).

The stimulation system consists of a stimulation electrode (19), an isolator (SS-202, Nihon Kohden Co., Tokyo, Japan) (20), and an electric stimulation device (SEN3301, Nihon Kohden Co., Tokyo, Japan). The recording system consists of an electrode (17) for recording, a head stage (18) (MEZ8300, Nihon Kohden Co., Tokyo, Japan), a primary amplifier (MEZ8300, Nihon Kohden Co., Japan), a secondary amplifier (JK804A, Jikken Kaihatsu Ltd., Kitakyushu, Japan), an analog-to-digital converter (Theta Burst Corp., Irvine, USA), and a personal computer (d330uT, Hewlett-Packard Development Company, L.P., Houston, USA) installed with software (NAC 2.0, Theta Burst Corp., Irvine, USA).

Preparation of 1-BP solution

ACSF (NaCl 124 mM, KCl 2 mM, $MgSO_4$ 1 mM, $CaCl_2$ 3 mM, KH_2PO_4 1.25 mM, $NaHCO_3$ 26 mM, glucose 10 mM) was prepared, and all the reagents for the ACSF were the reagent grade (Nakalai Tesque Inc., Kyoto, Japan). The fluid was saturated with an O_2 95%/CO_2 5% mixed gas, and stored in a thermostatic bath at 27.6 °C. In order to perfuse 1-BP to the hippocampal slice sample, 3.33 and 10 mM 1-BP solutions were prepared by inject-

ing 1-BP to the ACSF in a bottle made of glass with a gas-tight syringe (Gastight#1750, Hamilton Company, Reno, USA). The 1-BP was dissolved in the ACSF by shaking and ultrasonic vibration of the 1-BP bottle.

We examined if the concentration of 1-BP in the bottle without any stopper could be controlled by the conventional exposure method, where the concentration of toluene is given at the experiment and the concentration of exposed toluene to neurons gradually decreases because of volatility[7, 8]. The 1-BP solution in the bottle with an initial concentration of 10 mM was sampled at 0, 15, 30, 45, 60, 90 and 120 min. The analysis method for 1-BP concentration in the bottle was performed in accordance with the following.

Chemical analysis

When the 1-BP solution is perfused in this system, a part of the 1-BP in the liquid may be lost by vaporization from the surface of the slice chamber. To investigate the total loss of 1-BP in this system, we measured of concentrations of perfused 1-BP solution at the point of the hippocampal slice sample in the chamber at 0, 3 and 5 min and 2 h after arrival of the solution. In order to examine the controllable concentration in our system, we decreased the 1-BP concentrations to 1.0, 0.1, 0.05 and 0.01 mM of an original bottle and measured the 1-BP concentration in the chamber at 5 min after arrival of the solution.

One milliliter of each solution was sampled and put into a 5 ml-vial, and the vial was immersed in a thermostatic water bath. After being left for 2 h or more at 50°C, 500 μl of the headspace air was sampled by a gas-tight syringe and injected into a gas chromatograph (GC353B FSL, GL Sciences Inc., Tokyo, Japan) equipped with a capillary column (TC-1, 0.53 mm × 30 m) and a flame ionization detector. An integrator (Chromatocorder 21, System Instruments Co., Hachioji, Japan) was used for calculating the peak area. The temperatures of column, injection port and detector were 100, 110 and 110°C respectively.

Experimental procedures for electrophysiology

In order to examine whether our system would work well, we measured a single evoked response and a long-term potential (LTP) in the dentate gyrus (DG) of rat hippocampal slices.

Animals

Male Wistar rats (5 to 7 wk of age, 14 rats) were purchased from Kyudo Co., Ltd. (Japan). For 1 wk before the experiments, the rats were kept in a breeding facility

under the conditions of a 12-h light-dark cycle, controlled temperature (23 ± 1°C) and humidity (55 ± 5%), and water and food could be taken ad lib. The experiments were conducted under the control of the Ethics Committee of Animal Care and Experimentation (No. AE09-001) in accordance with The Guiding Principle for Animal Care Experimentation, University of Occupational and Environmental Health, Japan, and the Japanese Law for Animal Welfare and Care.

Hippocampal slice preparation and chemicals

The rats were deeply anesthetized with diethyl ether, and brain slices were prepared as described previously[17]. Briefly, the brain was gently removed and dipped in an ice-cooled ACSF without Ca^{2+} (Ca-free ACSF, below 4°C) saturated with an O_2/CO_2 mixed gas (95%: 5%) for 1–2 min. The composition of the Ca-free ACSF in mM was: NaCl 124, KCl 2, KH_2PO_4 1.25, $MgSO_4$ 2, $NaHCO_3$ 26, and glucose 10. The brain was placed on a cooled petri dish and the hippocampi were quickly separated from the other brain regions while being moistened with an ice-cooled Ca-free ACSF. Transverse slices of 450 μm thickness were then obtained from the middle third region of the bilateral hippocampi with a McIlwain tissue chopper (The Mickle Laboratory Engineering, Co., Ltd, Guildford, UK). The slices were transferred to an interface-type recording chamber, which was controlled at 29.5 to 30.5°C, and perfused with ACSF saturated with a O_2/CO_2 mixed gas (95%: 5%). The number of control slices, 3.33 mM and 10 mM 1-BP-exposed slices were 7, 6, and 5 respectively.

Stimulation and recordings

Following a 1–2 h stabilizing period after slicing, a recording glass microelectrode (1–2 MΩ) was placed in the molecular layer in the DG. Bipolar stimulation electrodes made of stainless wires (50 μm in diameter) were placed on the medial perforant path, which provides a connectional route from the entorhinal cortex to the DG, for DG recording. The distance between the two electrodes for stimulation and recording was about 400 μm. The stimulations consisted of square-wave pulses from a stimulator via an isolator. Field excitatory postsynaptic potentials (fEPSPs) were evoked by a single test stimulus (0.1 ms duration at 0.0333 Hz). The stimulation intensity was adjusted to evoke a fEPSP of approximately half of the maximal amplitude. For the control slices, stable baseline responses were recorded for 10 min. For the 1-BP-exposures, 3.33 and 10 mM of 1-BP solution was perfused for

5 min to the slices after baseline recording. LTP was induced by applying two sets of high-frequency stimulation (HFS) of 100 Hz for 1 s with a set interval of 5 s. The test stimulation of single pulse was continued for 40 min after HFS. In order to check the effects of 1-BP on the transmitter release mechanisms from presynaptic function of neurotransmitter releasing, paired-pulse facilitation (PPF) was induced by paired-pulse stimulation with a 100 ms interval applied 30 s after baseline recording and 40 min recording for LTP. Electrophysiological signals were amplified by a high-impedance amplifier with a bandpass of 10 kHz. The signals were then digitized with a NAC 2.0 neurodata acquisition system (Theta Burst Corp., Irvine, USA) and stored on a personal computer using Dag Board/2000.

Analysis

The amplitude of synaptic volley (volley) and slope of fEPSP (slope) were analyzed for each response. The ratio of slope/volley represents the efficacy of synaptic transmission. In the data of LTP, the ratios for each experiment were normalized against the averaged slope/volley over a 10 min period of the baseline recording in the control and 1-BP-exposed groups. The effects of 1-BP were represented as percentage changes from the average of the baseline responses. In the data of PPF, the slope/volley ratio of the first response and the second response evoked by the paired-pulse stimulation was calculated.

The statistical significance of time- or dose-dependent changes was evaluated by Student *t*-test, Kruskal-Wallis test followed by Steel-Dwass test, or one way analysis of variance (ANOVA) followed by Scheffe *post hoc* test. The data were represented as mean ± SD and as mean ± SE for chemical and electrophysiological analysis, respectively. The significance level was set at $p < 0.05$.

Results

1-BP concentration

Figure 2 shows a time-dependent reduction of 1-BP concentration in the bottle, when 10 mM of 1-BP original solution in the bottle was bubbled with the O_2/CO_2 mixed gas (95%: 5%) by the conventional method. The half-life of the 1-BP concentration in the bottle was 14.4 min. Figure 3 shows the 1-BP concentration in the slice chamber perfused by our new system. The 1-BP concentration in the slice chamber was stable and did not change from 5 min to 2 h after bubbling. The 1-BP concentrations in the slice chamber were 2.2 and 6.4 mM, which were estimated from the retention concentration at 5 min after the time
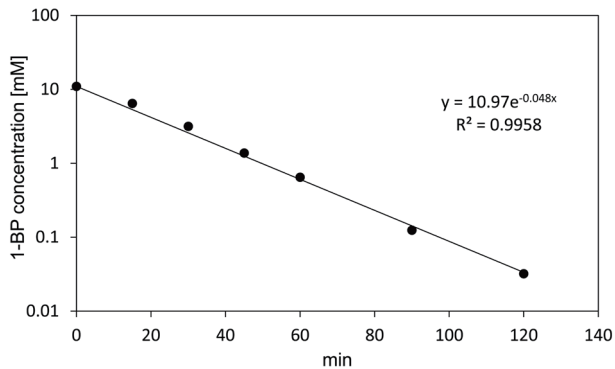
Fig. 2.   Reduction of 1-BP concentration in the original bottle in the conventional method.
1-BP of 10 mM concentration exponentially decreased by bubbling for 120 min with O_2/CO_2 mixed gas in the conventional method.

Fig. 3. 1-BP concentration in the slice chamber in the new system.
Concentration was measured at 0, 3, and 5 min, and 2 h after beginning of perfusion of 3.33 and 10 mM 1-BP original solution. Values represent mean ± SD.
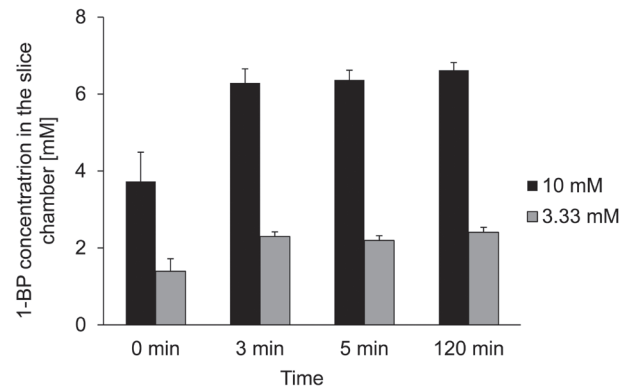
when the 3.33 and 10 mM 1-BP solutions reached the slice chamber. Figure 4 shows the linear relationship between the concentrations in the bottle and in the slice chamber. For example, 1-BP concentration of 0.01 mM in the bottle decreased to 0.0069 ± 0.0008 mM (n=10) in the slice chamber. We examined the effects of 1-BP on synaptic responses in the 2.2 and 6.4 mM 1-BP concentrations.

Effects of 1-BP on synaptic responses

We first analyzed the effects of 1-BP on the fEPSP evoked by single stimulation. The synaptic transmissions of baseline in the groups of the 0 (control), 2.2, and 6.4 mM 1-BP concentration were 1.07 ± 0.28, 2.27 ± 0.48, 1.87 ± 0.08 (mV/ms)/mV, respectively. As shown in Fig. 5A, the 5 min perfusion of 2.2 and 6.4 mM of 1-BP did not change in synaptic efficacy (p=0.94 by Kruskal-Wallis test). 1-BP reduced post tetanic potentiation 2 min after tetanus stimulation, although the reduction did not reach a significant level (p=0.10, by one way ANOVA) (Fig. 5B and C). On the other hand, both concentrations of 1-BP significantly suppressed LTP (Fig. 5B and D, p<0.05 compared to the control value tested by one way ANOVA followed by Scheffe's test). The reduction of LTP was not associated with the changes of PPF of fEPSP (Fig. 5E) at the inter-pulse interval of 100 ms, which means the reduction of synaptic efficacy induced by 1-BP was probably related to not presynaptic, but mainly post synaptic mechanisms.
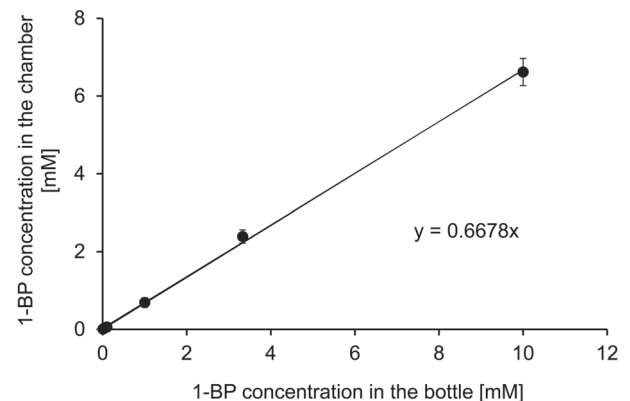
Discussion

There are few studies[7] using the direct application of VOCs in brain slice preparations because of the difficulty in keeping the concentration of VOCs stable. VOCs are

Fig. 4.   Linear relationship of the concentrations between in the bottle and the slice chamber.
Concentration was measured at 5 min after beginning of perfusion of 0.01, 0.05, 0.1, 1.0, 3.33 and 10 mM. Values represent mean ± SD.

easily dispelled and vaporized during the bubbling of O_2/CO_2 mixed gas with VOCs in an ACSF solution in a glass bottle in the conventional method. When gases and liquids are put into a closed vessel and left for a while, the gas phase and liquid phase reach a state of equilibrium. According to Henry's law, the concentrations of gaseous materials in a liquid depend on partial pressure and temperature. Therefore, when the gas pressure and temperature are kept constant, the concentration of gaseous materials in the liquid should also stay constant. This is also applicable for volatile organic compounds. The VOC exposure system for sliced brain samples that we developed is based on this principal. In this system, not only the O_2/CO_2 but also the 1-BP concentrations in the bottle should stay constant even when the amount of solution decreases with time, because
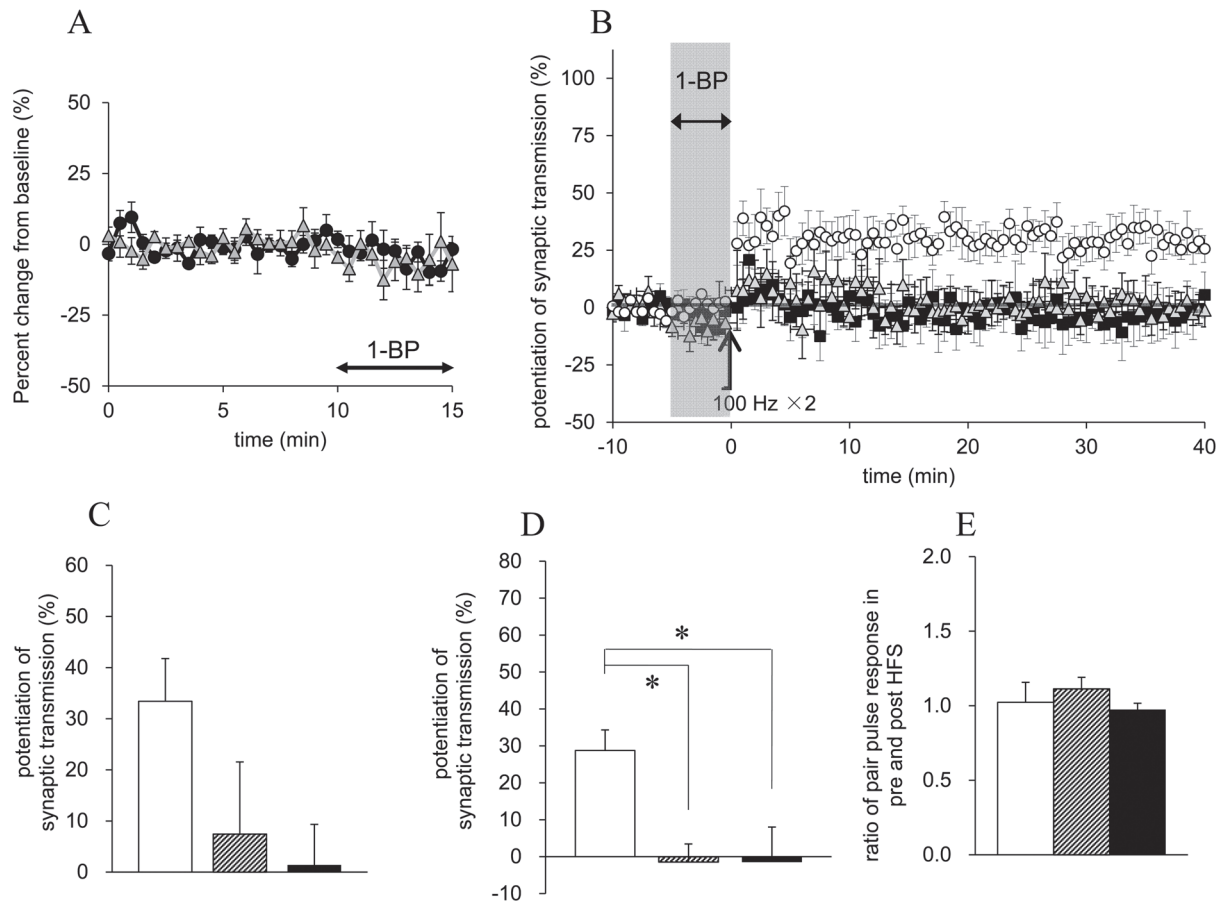
Fig. 5.   Direct effects of 1-BP on field excitatory postsynaptic potentials (fEPSPs) recorded in the rat dentate gyrus using the VOC exposure system. Concentration of the 1-BP in the chamber, estimated from retention concentration when 3.33 and 10 mM 1-BP solutions were perfused (2.2 and 6.4 mM). A: 1-BP did not change the efficacy of synaptic transmission (slope/volley) of fEPSP evoked in the dentate gyrus of rats. B: Potentiation of synaptic transmission lasted for 40 min in the control group, while in the 1-BP-exposed group, the suppressive effects of synaptic transmission induced by 1-BP continued stably for 40 min. C: Potentiation of synaptic transmission at 2 min after high frequency stimulation (HFS) tended to be reduced by 1-BP. D: At 35–40 min after HFS, the potentiation was clearly suppressed by 1-BP. E: 1-BP did not change the paired-pulse ratio of the synaptic transmission in the interpulse interval 100 ms recorded at the pre- and post-HFS. In A and B, white circles, gray triangles and black circles represent control, 2.2 mM and 6.4 mM. In C, D and E, white, gray and black columns represent the data of control, 2.2 mM and 6.4 mM. Values represent means ± SEs. Note significant difference compared with control. $*p < 0.05$.

the evaporation of 1-BP during the experiment is so small that changes in the 1-BP concentration in the liquid can be neglected. The validity of this consideration is proved in Fig. 3.

In this study, we prepared and perfused the 1-BP solution in an airtight condition without bubbling in order to prevent any change in the concentration of the 1-BP, and saturated O_2 and CO_2 levels in the 1-BP solution. In addition, we used Teflon tubes to avoid the adsorption of 1-BP in all the perfusion paths. As a result, our system could perfuse the 1-BP solution with little fluctuation in the concentration and with only a small coefficient of variation of the mean values (about 6%). Furthermore, we were able to record stable responses from hippocampal slice prepara-

tions without bubbling of the O_2 / CO_2 mixed gas.

The 1-BP could be supplied for 2 h with little fluctuation of the concentration in the chamber by our new system, whereas it could not be done by the conventional method, so it can be said that the new system is suitable for long-term direct application of 1-BP. When perfusing the 3.33 mM and 10 mM 1-BP solutions, the 1-BP concentrations in the slice chamber were not different from 3 min to 2 h later in the new system (Fig. 3), even though in the conventional method the 1-BP concentration in the bottle exponentially reduced with time in 2 h (Fig. 2), because the 1-BP was purged by bubbling in the original solution.

Because of the significant losses of 1-BP concentration while perfusing the 1-BP original solution of 3.33

and 10 mM, we determined the retention ratio of 1-BP as shown by the following formula in this system, that is, the ratio of the initial 1-BP concentration in the bottle and the final one in the slice chamber.

$$Retention\ ratio = \frac{concentration\ in\ the\ slice\ chamber}{1-BP\ concnetration\ in\ the\ bottle} \times 100\,(\%)$$

In our new system, the retention ratios stabilized at between 66 and 72% of their original 1-BP solution in the 3.33 and 10 mM, respectively, at 2 h. With respect to 0 min, the concentrations in both methods were 35 to 43%. The reason why the 0 min was relatively lower than that of 3 and 5 min and 2 h may have been caused by the partial replacement of the solution.

In the concentration range of 10–0.01 mM, we had the higher retention ratio of 66–72% than that in the previous study[7], in which the concentration of toluene in the slice chamber was 20% of the bottle concentration.

1-BP can be easily dispelled from the slice chamber, because it was an open system and its temperature was kept at 30°C. Although, emission from the slice chamber might be the main factor of the loss of 1-BP, vapor-liquid equilibrium in the drip chamber after switching from ACSF to 1-BP solution, volume of 1-BP solution in the drip chamber can be considered factors that affect the retention ratio at the early period of 1-BP perfusion.

It was also confirmed that the coefficient of variation of the mean values in the new system was smaller than that in the conventional system. In the other previous study[8], the toluene solution, which has lower volatility (its saturated pressure is 2.93 kPa at 20°C) than 1-BP, were prepared by bubbling with O_2/CO_2 gas and toluene to ACSF. And the coefficients of variation of the mean values in the concentration of the solution were below 20%. On the other hand, that in the new system was below 6% from a few minutes to some hours after reaching the 1-BP solution.

As described above, we can recognize that the new system using Teflon tubing in the route of the system and perfusing the VOC solution in an airtight condition allow for the perfusing of VOCs with a small coefficient of variation of the mean values. This allows us to perfuse the VOC solution stably with little loss of the VOC.

In the conventional method, the bubbling of an O_2/CO_2 mixed gas to the perfusion stock ACSF solution in the bottle is needed to maintain the slice. However, we elucidated that slices can be maintained by perfusing the ACSF solution saturated with the O_2/CO_2 mixed gas in an airtight condition without bubbling the mixed gas.

Furthermore, we could evaluate the direct effects of VOCs by using this system. For instance, we demonstrated that 1-BP suppressed the LTP in DG, although there were no significant effects of 1-BP on PPF by using our system. These results suggest that the 1-BP affects the receptors or second messengers related to LTP, such as *N*-methyl-D-aspartate receptors and calcium-calmodulin-dependent protein kinase II[14, 15], but not presynaptic components. In this way, we could record the response and evaluate the neurotoxicity of VOCs in extracellular recording from hippocampal slice preparations by using the new system.

According to our previous study[18], in which blood sample was obtained after decapitation of rats, 1-BP concentration in blood after exposure to 1,500 ppm of 1-BP for 3 weeks was calculated about 0.1 mM. Therefore we decreased the concentration to 0.01 mM and obtained 69% of the retention ratio.

We have also demonstrated the possibility that this new system can be adapted to the model of 0.01–10 mM of exposures in slice preparations. In this study, we assumed the acute high-level exposures, so we perfused relatively high concentrations of 1-BP to hippocampal slices in a short term. However, this system may also be adaptable to 0.01–10 mM of exposures for hours, because it has stability of concentration in two hours of application.

Conclusions

We developed a direct application system of 1-BP for slice preparations. This system is applicable to VOCs, so elucidation of a part of the mechanisms of the effects of VOCs effects on the central nervous system in the *in vitro* assay is close to an *in vivo* one. This direct application system of VOCs can be suitably applied to assays such as whole cell clamp and patch clamp. The direct application system is useful in assessing the central neurotoxicity of VOCs, which will help in the risk assessment of VOCs.

Acknowledgements

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