European Journal of Pharmacology 885 (2020) 173527

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Full length article

The potential rewarding and reinforcing effects of the substituted benzofurans 2-EAPB and 5-EAPB in rodents

Leandro Val Sayson [a], Raly James Perez Custodio [a], Darlene Mae Ortiz [a], Hyun Jun Lee [a], Mikyung Kim [b], Youngdo Jeong [c], Yong Sup Lee [c], Hee Jin Kim [a,**], Jae Hoon Cheong [a,d,*]

[a] Uimyung Research Institute for Neuroscience, Department of Pharmacy, Sahmyook University, 815 Hwarang-ro, Nowon-gu, Seoul, 01795, Republic of Korea
[b] Department of Chemistry & Life Science, Sahmyook University, 815 Hwarang-ro, Nowon-gu, Seoul, 01795, Republic of Korea
[c] Medicinal Chemistry Laboratory, Department of Pharmacy & Department of Life and Nanopharmaceutical Sciences, College of Pharmacy, Kyung Hee University, 26 Kyungheedae-ro, Seoul, 02447, Republic of Korea
[d] School of Pharmacy, Jeonbuk National University, 567 Baekje-daero, Deokjin-gu, Jeonju-si, Jeollabuk-do, 54896, Republic of Korea

ARTICLE INFO

Keywords:
2-EAPB
5-EAPB
Conditioned place preference
Self-administration
Locomotor sensitization
Abuse potential

ABSTRACT

Accounts regarding the use of novel psychoactive substances continue to escalate annually. These include reports on substituted benzofurans (SBs), such as 1-(1-benzofuran-2-yl)-N-ethylpropan-2-amine (2-EAPB) and 1-(1-benzofuran-5-yl)-N-ethylpropan-2-amine (5-EAPB). Reports on the deaths and adverse consequences from the use of SBs warrant the investigation of their mechanism, possibly predicting the effects of similar compounds. Accordingly, we investigated the possible rewarding and reinforcing effects of 2-EAPB and 5-EAPB through conditioned place preference (CPP), self-administration, and locomotor sensitization tests. We also determined the possible influence of 2-EAPB and 5-EAPB administration on dopamine- and plasticity-related proteins in the nucleus accumbens and ventral tegmental area. 2-EAPB and 5-EAPB induced CPP at different doses and were self-administered by rats. Only 5-EAPB induced locomotor sensitization in mice. 2-EAPB and 5-EAPB did not alter the expressions of dopamine D1 and D2 receptors in the nucleus accumbens, nor changed tyrosine hydroxylase and dopamine transporter expressions in the ventral tegmental area. Both 2-EAPB and 5-EAPB enhanced deltaFosB, but not transcription factor cyclic AMP-response-element binding protein and brain-derived neurotrophic factor in the nucleus accumbens. Hence, the potential rewarding and reinforcing effects on rodents induced by 2-EAPB and 5-EAPB may possibly be associated with alterations in other neurotransmitter systems (besides mesolimbic) and/or neuro-plastic modifications.

1. Introduction

Globally, novel psychoactive substances constantly emerge from clandestine markets in order to circumvent the regulations prohibiting their use and distribution (Beltgens, 2017; Johnson et al., 2013; Smith and Garlich, 2013). As a result, the number of cases for the identification of these substances by drug monitoring agencies increases annually (Addiction, 2014; Welter-Luedeke and Maurer, 2016; UNODC, 2014). A contributing problem is the constant introduction of modifications to the structure of recognized illicit drugs (Kikura-Hanajiri et al., 2014; Weaver et al., 2015). This creates new compounds that may possibly be susceptible for recreational use; some of such compounds include substituted benzofurans (SBs) (Dolan et al., 2017; Eshleman et al., 2019). Considered as the third most prominent group of novel psychoactive substances that recently emerged in the past decade (Roque Bravo et al., 2019), these empathogens (heightens emotional state) were reported to function mainly on the serotonergic system (Eshleman et al., 2019), yet still generating downstream effects on dopaminergic proteins (Rickli et al., 2015). Their actions on these neurotransmitter networks enable addictive effects, as evidenced by previous studies (Adamowicz et al., 2014; Cha et al., 2016; Dawson et al., 2014; Fuwa et al., 2016), making several of these SBs illegal in some countries (Fuwa et al., 2016). Among these recent SBs are 1-(1-benzofuran-2-yl)-N-ethylpropan-2-amine (2-EAPB) and 1-(1-benzofuran-5-yl)-N-ethylpropan-2-amine

* Corresponding author. Uimyung Research Institute for Neuroscience, Department of Pharmacy, Sahmyook University, 815 Hwarang-ro, Nowon-gu, Seoul, 01795, Republic of Korea.
** Corresponding author.
E-mail addresses: hjkim@syu.ac.kr (H.J. Kim), cheongjh@syu.ac.kr (J.H. Cheong).

https://doi.org/10.1016/j.ejphar.2020.173527
Received 31 March 2020; Received in revised form 25 August 2020; Accepted 28 August 2020
Available online 29 August 2020

(5-EAPB) (Fuwa et al., 2016; King, 2014; Uchiyama et al., 2014).

Both 2-EAPB and 5-EAPB contain 2-ethylaminopropyl (EAP); however, the EAP (outlined in red) in 2-EAPB is located on R2 (Fig. 1C), whereas in 5-EAPB, it is on R5 (Fig. 1D). Both compounds are also structurally similar to methamphetamine (Fig. 1B) except for the presence of a furan ring (outlined in blue) and an additional methyl on the amine site (red arrow). Another SB similar to these compounds is 5-APB (an Ecstasy analog). Initially developed as a monoamine transport inhibitor (Dawson et al., 2014; Rickli et al., 2015), 5-APB was found to elicit conditioned place preference (CPP) (Cha et al., 2016). Other similar SBs are 6-MAPB, 5-MAPB, and 6-APB that have all been reported for recreational purposes due to their empathogenic and psycho-stimulating properties (Shimshoni et al., 2017). Several deaths from the chronic use of 6-APB and 5-APB have also been reported (Adamowicz et al., 2014; Nugteren-van Lonkhuyzen et al., 2015), with one death from 5-EAPB (Deville et al., 2019). These accounts strongly indicate the dangers and adverse consequences arising from their use, thus necessitating investigations on the neuronal mechanisms by which these drugs affect behavior. Indeed, such investigations are crucial for 2-EAPB and 5-EAPB, for which substantial relevant information is generally lacking (Deville et al., 2019; Uchiyama et al., 2014). Extensive studies on their pharmacodynamics could also generate significant information that would be applicable in predicting the likely effects of similarly structured compounds.

In this study, we explored the potential rewarding and reinforcing effects of 2-EAPB and 5-EAPB. In particular, we employed the CPP paradigm and the self-administration (SA) test in rats for any evidence of addictive effects. Furthermore, we also investigated the effects of these drugs on mice locomotor activity, since increased locomotor activity and sensitization are common characteristics of most addictive psychostimulants (Modi et al., 2006; Shimosato and Ohkuma, 2000). Since the neurochemistry of addiction is well-established to involve the mesolimbic dopamine system, we also examined the influence of 2-EAPB and 5-EAPB on various dopamine-related proteins, specifically dopamine D1 receptor, dopamine D2 receptor, tyrosine hydroxylase, and dopamine transporter. We also examined neuroplasticity-related proteins associated with addiction, namely phosphorylated (*p*-) cyclic AMP-response-element binding protein (CREB), deltaFosB, and brain-derived neurotrophic factor (BDNF). Additionally, all experiments were simultaneously duplicated using methamphetamine.

2. Materials and methods

2.1. Animals

All animals used in the study were purchased from Hanlim Animal Laboratory Co. (Hwasung, Korea) and were housed in a temperature- and humidity-controlled room (temperature: 22 ± 2 °C, relative humidity: 55 ± 5%) with a 12/12 h light/dark (07:00–19:00 light) cycle. Different cohorts of animals were used for each test. Only male subjects were used to avoid the confounding effects of hormonal changes in females (Fattore et al., 2008). Adolescent rodents were used since this stage of development was previously shown to increase novelty-seeking and exploratory behavior, and thus sensitivity to abuse studies (Zakharova et al., 2009). Sprague-Dawley rats (6 weeks old) were used in the CPP paradigm, SA test, and western blotting. For the SA test, rats were caged individually; for other tests, 4–6 rats were housed per cage. For locomotor sensitization test, five C57BL/6J mice (6 weeks old) were housed per cage. Animals were habituated in the animal room for 5 days prior to experiments. They had access to food and water, except when rats underwent lever training and the actual SA sessions. Animal use in this study was in accordance with the Principles of Laboratory Animal Care (NIH Publication No. 85-23, 1985 revision) and the Animal Care and Use Guidelines of Sahmyook University (SYUIACUC 2019-001).

2.2. Drugs

2-EAPB hydrochloride was synthesized in five steps from benzofuran (Taniguchi et al., 2010). Briefly, benzofuran was treated with phosphorous oxychloride and *N,N*-dimethylformamide. The resulting 2-formylbenzofuran was condensed with nitroethane and then reduced with $LiAlH_4$ to give 1-(benzofuran-2-yl)propan-2-amine. This compound was acetylated using acetyl chloride and then reduced by $LiAlH_4$ to give 1-(benzofuran-2-yl)-*N*-ethylpropan-2-amine. The resulting amine was treated with hydrochloride to give 2-EAPB as a hydrochloride salt. Its structure was confirmed by the following spectroscopic analyses. H-NMR (400 MHz, D_2O) δ 7.57 (d, $J = 7.6$ Hz, 1H), 7.47 (d, $J = 7.6$ Hz, 1H), 7.28 (t, $J = 7.6$ Hz, 1H), 7.23 (t, $J = 7.6$ Hz, 1H), 6.70 (s, 1H), 3.68 (q, $J = 6.5$ Hz, 1H), 3.21-3.05 (m, 4H), 1.28 (d, $J = 6.5$ Hz, 3H), 1.22 (t, $J = 7.3$ Hz, 3H); ^{13}C NMR (100 MHz, D_2O) δ 153.84, 146.32, 130.34, 127.86, 125.58, 122.04, 111.56, 106.43, 55.16, 40.05, 38.68, 15.11,

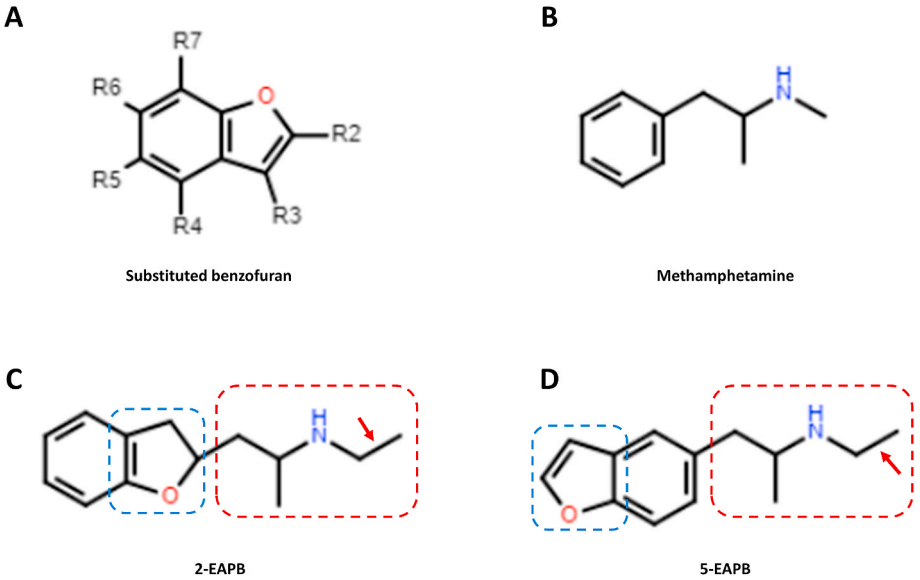

Fig. 1. Chemical structures of (A) a general substituted benzofuran, (B) methamphetamine (METH), (C) 2-EAPB, and (D) 5-EAPB. The furan ring is outlined in blue; the EAP group is outlined in red; an additional methyl on the amine site is indicated by the red arrow. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

10.60; HR-MS calculated for $C_{13}H_{18}NO$ $[M+H]^+$ 204.1383, found 204.1387.

5-EAPB hydrochloride was synthesized in four steps from benzofuran-5-carbaldehyde according to the procedure similar to that used for the synthesis of 2-EAPB hydrochloride (Taniguchi et al., 2010). Briefly, benzofuran-5-carbaldehyde was condensed with nitroethane and then reduced with $LiAlH_4$ to give 1-(benzofuran-5-yl) propan-2-amine; this compound was acetylated and then reduced by $LiAlH_4$ to give 1-(benzofuran-5-yl)-*N*-ethylpropan-2-amine. The resulting amine was treated with hydrochloride to give 5-EAPB as a hydrochloride salt. Its structure was confirmed by the following spectroscopic analyses. ^{1}H NMR (400 MHz, D_2O) δ 7.73 (1H, d, $J = 2.0$ Hz), 7.52 (1H, s), 7.51 (1H, d, $J = 8.4$ Hz), 7.19 (1H, dd, $J = 8.4$, 1.7 Hz), 6.83 (1H, d, $J = 2.0$ Hz), 3.54 (1H, m), 3.17-3.10 (2H, m), 3.10-2.86 (2H, m), 1.22-1.18 (6H, m). ^{13}C NMR (100 MHz, D_2O) δ 153.84, 146.32, 130.34, 127.86, 125.58, 122.04, 111.56, 106.43, 55.16, 40.05, 38.68, 15.11 10.60. HRMS calculated for $C_{13}H_{18}NO$ $[M+H]^+$ 204.1383, found 204.1389.

Methamphetamine was purchased from Sigma-Aldrich (St. Louis, Missouri, U.S.). All the drugs were dissolved in 0.9% sterile saline and administered intraperitoneally (i.p., for CPP, locomotor sensitization, treatment for western blotting) or intravenously (for SA). All dosages of drugs (2-EAPB, 5-EAPB, and methamphetamine) used in the present study were based on previous publications that evaluated the rewarding and reinforcing properties of amphetamine derivatives (Cain et al., 2008; Custodio et al., 2017; Marona-Lewicka et al., 1996).

2.3. Conditioned place preference test

2.3.1. Apparatus

The CPP apparatus consisted of two compartments measuring 47 × 47 × 47 cm^3. Each compartment provided distinct visual and tactile cues, one had black walls with smooth flooring, while the other compartment had black walls with white dots and rough black flooring. The compartments could be separated from each other by a detachable guillotine door. Illumination throughout the experiment was maintained at 12 lux. An automated system (Ethovison, Noldus, Netherlands) was utilized for the recording and analyses of animal movements.

2.3.2. Procedure

The protocol used was similar to that used in our previous studies (Custodio et al., 2020; Custodio et al., 2019), with some modifications. The test consisted of three phases: (1) habituation [3 days] and pre-conditioning [1 day], (2) conditioning [6 days], and (3) post-conditioning [1 day]. During the habituation phase, rats ($n = 8$) were given access to both compartments for 15 min on three consecutive days. The pre-conditioning phase began the following day during which the time spent in each compartment was recorded for 15 min. Rats were assigned to groups based on the pre-conditioning phase, specifically, their non-preferred side was designated as the drug-paired compartment. During the conditioning phase, the guillotine doors were closed. Rats received an i.p. injection of 2-EAPB (1, 3, and 10 mg/kg), 5-EAPB (0.3, 1, 3, and 10 mg/kg), methamphetamine (1 mg/kg), or saline, and were randomly placed in one of the compartments for 30 min. On alternate days, rats received saline injections and were confined in the compartment other than the drug-paired compartment. Immediately following the last conditioning day, the post-conditioning phase began wherein rats were drug-free and allowed to access both compartments as during the pre-conditioning phase.

2.4. Self-administration test

2.4.1. Apparatus

Standard operant chambers (Coulborn Instruments, Allentown, PA, USA) were kept inside sound-attenuating boxes with built-in ventilation fans. Each operant chamber included a pellet dispenser, left and right response levers (4.5 cm long), a stimulus-light source situated above the left lever, and a centrally located house light (2.5 W, 24 V) on top of the chamber. Downward pressure of 25 g on a lever resulted in an automated response. Adjacent to the operant chamber was a mechanically operated syringe pump that delivered solutions (0.01 mL/s) through Teflon tubes that were attached to the I.V. catheter of the rats. This was connected to a swivel system that allowed the free movement of rats. Built-in Graphic State Notation software (Coulborn Instruments) allowed automatic control over the experimental parameters and data collection.

2.4.2. Procedure

The entire protocol was based on our previous studies (Custodio et al., 2017; Custodio et al., 2019). Training for the drug-paired lever pressing was conducted for three consecutive days (30 min/session, 2 sessions/day) for a contingent food-pellet reward on a continuous schedule of reinforcement. Rats acquiring greater than 80 pellets on the last session of training were selected and prepared for surgery. Standard operating techniques were carried out as previously described (Custodio et al., 2019). The rats were left for 5 days to recover from surgery. After the recovery period, rats were given a consumable daily diet of pellets (approximately 20 g) and were subjected to a 2-h daily SA session under a fixed-ratio (FR) 1 schedule for 7 consecutive days, and a FR2 schedule for 3 days. During the SA test, both levers (right/left) were available to the rats. Pressing the left lever (active lever) resulted in a delivery of 0.1 mL of 2-EAPB (0.03, 0.1, or 0.3 mg/kg/infusion), 5-EAPB (0.03, 0.1, or 0.3 mg/kg/infusion), methamphetamine (0.1 mg/kg/infusion) or saline ($n = 7$). Simultaneously, the house light was switched off, and the stimulus light was illuminated and remained lit for 20 s after the end of the infusion (the time-out period). Lever presses during periods of "time-out" were recorded but had no effect. Right-lever presses (inactive lever) were recorded but not reinforced.

2.5. Locomotor sensitization

2.5.1. Apparatus

The locomotor activity of the mice was measured in a square, black-Plexiglas container with an open-field arena (42 × 42 × 42 cm^3). A computer system (Ethovison, Noldus, Netherlands) was utilized to record the total distance moved (cm) of each mouse.

2.5.2. Procedure

This test was based on our previous protocol (Custodio et al., 2017; Custodio et al., 2020), with few modifications. The behavioral assay consisted of four phases: habituation, drug treatment (T1-T7), drug abstinence (A1-A7), and drug challenge (Ch). For the first two days, mice were habituated to the apparatus for 30 min. On the third day (T0), locomotor activity was recorded and used as a baseline parameter. Thereafter, 2-EAPB (1, 3, and 10 mg/kg), 5-EAPB (1, 3, and 10 mg/kg), methamphetamine (1 mg/kg), or saline was administered to the mice ($n = 8$) for 7 days. Mice were then challenged with the same dose and drug after 7 days of abstinence. Locomotor activity was evaluated for 30 min immediately following the first, third, and seventh day of both drug or saline treatment and abstinence, as well as on the challenge day.

2.6. Western blotting

The procedure for obtaining protein, gel preparation, and blot analysis was based on our previous studies (Custodio et al., 2019; Kim et al., 2019), with some modifications. Rats ($n = 6$) were treated with 2-EAPB (10 mg/kg), 5-EAPB (1 mg/kg), methamphetamine (1 mg/kg), or saline using the treatment schedule employed in CPP in order to associate possible modified protein levels with the probable induction of CPP behavior. Treatment was done in the CPP experiment room, but the test was not conducted. The rats were killed by decapitation 24 h after the last drug administration and used for protein extraction. Brains were

rapidly and carefully removed and placed in ice-cold saline to prevent damage to the brain. The nucleus accumbens and ventral tegmental area were sliced into sections using a rat-brain matrix. The regions were then isolated from the slices and immediately frozen at −80 °C until further use. Tissues were lysed with 500 μL homogenization buffer (RIPA buffer [Biosesang Inc., Seongnam, Korea], cOmplete™ ULTRA protease inhibitor cocktail tablets [05892791001; Sigma-Aldrich], and PhosSTOP™ phosphatase inhibitor cocktail tablets [04906845001, Sigma-Aldrich]). Tissue extracts were centrifuged at 16000 *g* at 4 °C for 20 min. Samples were heated at 95 °C for 5 min 20 μg of protein lysates were then loaded onto 10% sodium-dodecyl sulfate/polyacrylamide gel electrophoresis (SDS/PAGE) gels, separated, and transferred to nitrocellulose membranes. Blots were blocked with 5% bovine serum albumin in Tris-buffered saline in 0.1% Tween-20 (TBST) solution for 1 h and incubated with specific primary antibodies (Mouse Monoclonal Anti-Dopamine D1 receptor Antibody [MA1-46024 Invitrogen, (Salyer et al., 2011); Rabbit Polyclonal Anti-Dopamine D_2 receptor Antibody [D2R-201AP FabGennix International, Inc., (Rentesi et al., 2013)]; Rabbit Polyclonal Anti-Tyrosine hydroxylase Antibody [AB152; Sigma-Aldrich, (Kawahata et al., 2009)]; Rabbit Monoclonal Anti-Dopamine transporter [ab184451 Abcam]; Rabbit Monoclonal Anti-CREB Antibody [#9197S Cell Signaling Technology]; Rabbit Monoclonal Anti-Phospho-CREB Antibody [#9198S Cell Signaling Technology]; Rabbit Monoclonal Anti-FosB Antibody [ab184938 Abcam]; Rabbit Monoclonal Anti-BDNF Antibody [ab108319 Abcam]; Mouse Monoclonal Anti-Beta-actin (β-actin) Antibody [A5441 Sigma-Aldrich]) overnight at 4 °C. The next day, blots were washed three times in TBST and incubated with horseradish peroxidase-conjugated anti-rabbit (1:3000) or anti-mouse secondary antibodies (1:5000) for 1 h. After three washes with TBST, the blots were visualized using enhanced chemiluminescence (Clarity Western ECL; Bio-Rad Laboratories, Hercules, CA, USA) and ChemiDoc Imaging System (Image Lab software, version 6.0; Bio-Rad). Values for phosphorylation-independent protein levels were normalized to β-actin. Phosphorylated proteins were normalized to their phosphorylation-independent form. Fold change was determined by normalizing to the values of the saline group.

2.7. Data analysis

All values were expressed as mean ± standard error of the mean (S.E.M.). Results from CPP, western blotting, and mean number of infusions in SA were analyzed by one-way analysis of variance (ANOVA), followed by Tukey's post-test. Data from the active-lever responses and the number of infusions during SA test under the FR schedule of reinforcement were analyzed using two-way repeated measures ANOVA with treatments as the between-subject factor and SA days as the within-subject factor (note that data from each FR schedule were analyzed separately). Tukey's or Bonferroni's post-test was utilized for further comparison. Comparison of the mean number of infusions between saline and methamphetamine was analyzed using unpaired *t*-test. Data from locomotor sensitization were analyzed using a two-way repeated measures ANOVA with treatments as between-subject factor and days as within-subject factor, with Tukey's or Bonferroni's test for post-hoc analyses. Values of *P* less than 0.05 was the criterion for statistical significance. All statistical analyses were performed using GraphPad Prism software v. 8.01 (San Diego, CA, USA).

3. Results

3.1. 2-EAPB and 5-EAPB induce CPP in rats at different doses

Fig. 2 illustrates the CPP scores of rats treated with 2-EAPB EAPB (1, 3, and 10 mg/kg), 5-EAPB (0.3, 1, 3, and 10 mg/kg), methamphetamine (1 mg/kg) or saline. One-way ANOVA indicated a significant difference among the treatment groups [$F(8, 63) = 6.01$, $P < 0.001$]. Furthermore, Tukey's post-test indicated that rats treated with 2-EAPB (10 mg/kg), 5-EAPB (1 mg/kg), and methamphetamine had significantly higher CPP scores compared to the saline-treated group.

3.2. 2-EAPB and 5-EAPB are self-administered by rats

A two-way ANOVA in Fig. 3A exhibits significant differences between treatment groups [$F(3, 24) = 38.4$, $P < 0.001$], SA days [$F(6, 144) = 21.5$, $P < 0.001$], and an interaction between treatment and days [$F(18, 144) = 3.48$, $P < 0.001$] under FR1, and also significant differences between treatment groups [$F(3, 24) = 102$, $P < 0.001$] and an interaction between treatment and SA days [$F(6, 48) = 13.0$, $P < 0.001$] under FR2. Tukey's post-test showed significantly increased lever pressing in rats that self-administered 2-EAPB (0.1 and 0.3 mg/kg/infusion). Two-way ANOVA of Fig. 3B shows significant differences between treatment groups [$F(3, 24) = 120$, $P < 0.001$], SA days [$F(4.35, 104) = 4.29$, $P < 0.01$], and an interaction of the two [$F(18, 144) = 4.06$, $P < 0.001$] under FR1, and also between treatment groups [$F(3, 24) = 120$, $P < 0.001$], SA days [$F(2.00, 48.0) = 19.8$, $P < 0.001$], and an interaction of treatment and days [$F(6, 48) = 3.78$, $P < 0.01$] under FR2. Tukey's post-test showed significantly increased lever pressing in rats that self-administered 5-EAPB at all doses. Fig. 3C two-way ANOVA displays a significant difference only between treatment groups [$F(1, 12) = 442$, $P < 0.001$] under FR1, and between treatment groups [$F(1, 12) = 880$, $P < 0.001$] under FR2. Bonferroni's post-test exhibited significantly increased lever pressing in rats that self-administered methamphetamine. Two-way ANOVA of Fig. 3D displays a significant difference between treatments [$F(3, 24) = 27.9$, $P < 0.001$], SA days [$F(3.97, 95.3) = 13.5$, $P < 0.001$], and an interaction between the two [$F(18, 144) = 2.33$, $P < 0.01$] under FR1, and significant differences between treatments [$F(3, 24) = 18.4$, $P < 0.001$] and an interaction between treatment and SA days [$F(6, 48) = 3.73$, $P < 0.01$] under FR2. Tukey's post-test showed significantly greater number of infusions acquired by rats that self-administered 2-EAPB at all doses. Fig. 3E two-way ANOVA exhibits significant differences between treatments [$F(3, 24) = 36.6$, $P < 0.001$], SA days [$F(4.55, 109) = 5.35$, $P < 0.001$], and an interaction between treatment and days [$F(18, 144) = 1.74$, $P < 0.05$] under FR1, and significant differences between treatments [$F(3, 24) = 27.6$, $P < 0.001$] and SA days [$F(1.90, 45.7) = 4.69$, $P < 0.05$] only under FR2. Tukey's post-test presented significantly greater number of infusions acquired by rats that self-administered 5-EAPB at all doses. Two-way ANOVA of Fig. 3F exhibits significant differences between treatments only [$F(1, 12) = 152$, $P < 0.001$] under FR1, but with significant differences between treatments [$F(1, 12) = 443$, $P < 0.001$] and SA days [$F(1.98, 23.8) = 4.13$, $P < 0.05$] under FR2. Bonferroni's post-test revealed significantly greater number of infusions acquired by rats that self-administered methamphetamine. One-way ANOVA of

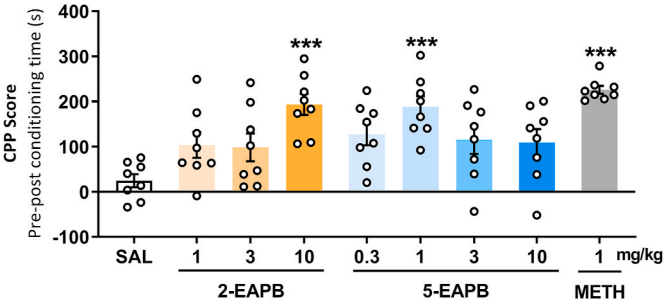

Fig. 2. The effects of 2-EAPB and 5-EAPB on rat place preference. Rats were conditioned with 2-EAPB (1, 3, and 10 mg/kg) and 5-EAPB (0.3, 1, 3, and 10 mg/kg), METH (1 mg/kg), or saline (SAL). 2-EAPB and 5-EAPB induced CPP in rats, suggesting their rewarding effects similar to METH. Data are presented as means ± S.E.M. $n = 8$. ***$P < 0.001$ significantly different from the SAL group (One-way ANOVA, Tukey's post-test).

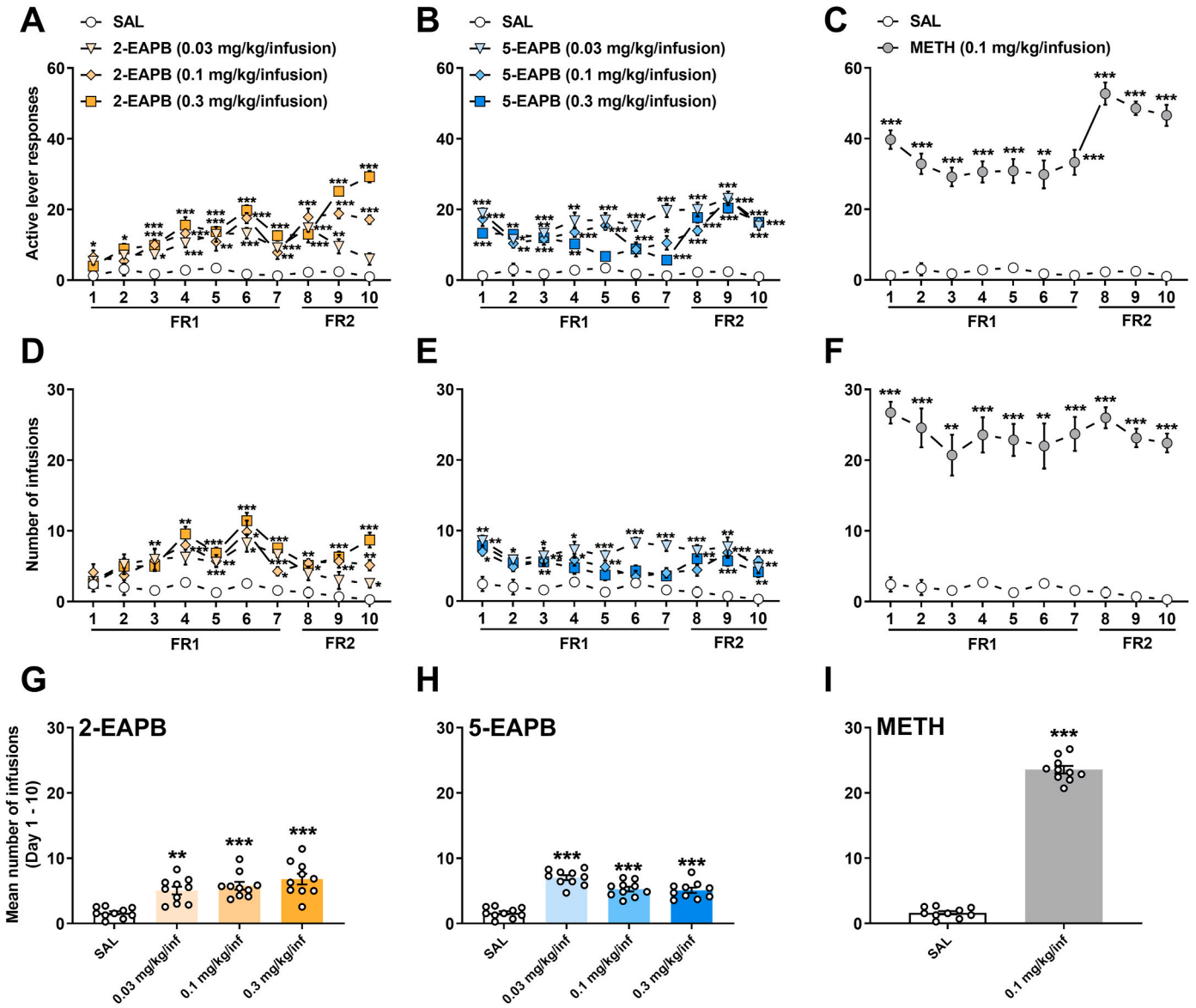

Fig. 3. The effects of 2-EAPB and 5-EAPB on rat SA (A, B, C): The total active-lever responses; (D, E, F): total number of infusions; (G, H, I): mean number of infusions for each treatment group during the 2-h, 10-day SA experiment in rats. 2-EAPB and 5-EAPB were modestly self-administered by rats, obtaining a higher number of active lever responses and infusions as compared with the SAL group, indicating its reinforcing effects. However, 2-EAPB and 5-EAPB have weaker reinforcing effects as compared to METH. Values are mean ± S.E.M. $n = 7$. $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ compared with the SAL group (active lever responses and number of infusions [Two-way RM-ANOVA, Tukey's or Bonferonni's post-test]; mean number of infusions [One-way ANOVA, Tukey's post-test; Unpaired *t*-test (METH)]).

Fig. 3G shows a significant difference between treatment groups [F (3, 36) = 13.9, $P < 0.001$], with a Tukey's post-hoc analysis showing an increased mean number of infusions of 2-EAPB at all doses. In Fig. 3H, one-way ANOVA shows a significant difference between treatment groups [F (3, 36) = 40.0, $P < 0.001$]; a Tukey's post-test exhibited an increased mean number of infusions of 5-EAPB at all doses. Unpaired *t*-test of Fig. 3I shows a significantly higher mean number of infusions acquired by rats that self-administered methamphetamine [$t = 35.1$, df = 18, $P < 0.001$] compared to saline.

3.3. 5-EAPB but not 2-EAPB induces locomotor sensitization in mice

Fig. 4 exhibits the locomotor response of mice on the first, third, and seventh days of treatment following 2-EAPB and 5-EAPB (1, 3, 10 mg/kg), methamphetamine (1 mg/kg) or saline administration for 7 days, and the comparison between the distance moved of mice between the first day of treatment and the challenge day. In Fig. 4A, a two-way ANOVA shows a significant difference between treatment groups [F (4, 35) = 9.18, $P < 0.001$], treatment days [F (7, 245) = 21.1, $P < 0.001$], and an interaction between the two [F (28, 245) = 5.91, $P < 0.001$]. Tukey's post-test showed that only methamphetamine enhanced locomotor activity on the first, third, and seventh days of treatment compared to saline. In Fig. 4B, a two-way ANOVA reveals a significant difference between treatment groups [F (4, 35) = 10.4, $P < 0.001$], treatment days [F (7, 245) = 33.5, $P < 0.001$], and an interaction between the two [F (28, 245) = 7.64, $P < 0.001$]. A Tukey's post-hoc analysis showed that 5-EAPB (3 and 10 mg/kg) and methamphetamine enhanced mice locomotor activity on the first, third, and seventh days of treatment and on the challenge day compared to saline. Fig. 4C displays a two-way ANOVA indicating a significant difference between treatments [F (4, 35) = 19.2, $P < 0.001$], between days [F (1, 35) = 69.4, $P < 0.001$], and an interaction of treatment and days [F (4, 35) = 5.57, $P = 0.001$], with Bonferroni's post-test showing only methamphetamine treatment having a significant difference between the first day of

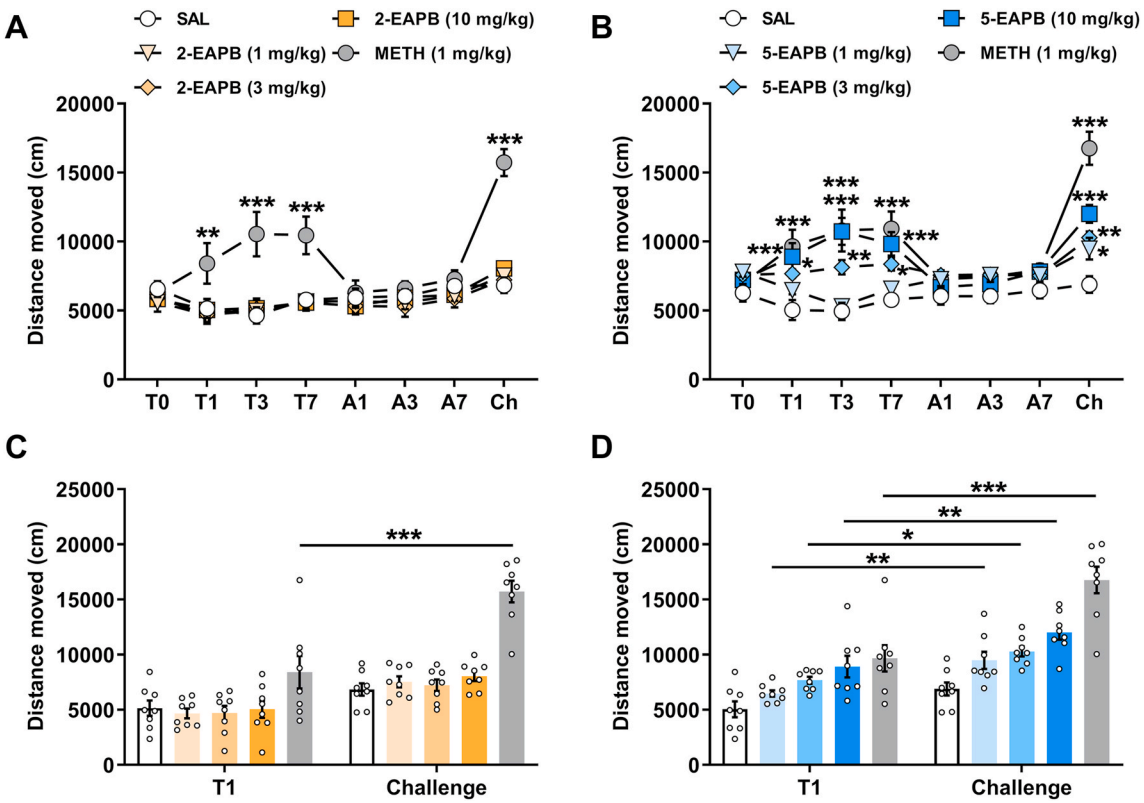

Fig. 4. Effects of 2-EAPB and 5-EAPB on mice locomotor sensitization. (A, B): The locomotor activity of mice (distance moved, cm) before drug treatment (T0), on the first (T1), third (T3), and seventh (T7) days of treatment, on the first (A1), third (A3), and seventh (A7) days of drug abstinence, and challenge day (Ch). Only 5-EAPB and METH increased the locomotor activity of mice supported by the significant difference in their distance moved compared to SAL. Values are mean ± S.E.M. $n = 8$. *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ significantly different from the SAL group (Two-way ANOVA, Tukey's post-test). (C, D): Comparison of locomotor activity during the first day of drug treatment versus drug challenge. Only 5-EAPB and METH induced locomotor sensitization in mice confirmed by the significant difference in their distance moved between T1 and Ch. Values are mean ± S.E.M. *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ significantly different from T1 (Two-way ANOVA, Bonferroni's post-test).

treatment and the challenge day. Fig. 4D shows the results of a two-way ANOVA indicating significant differences between treatment groups [$F(4, 35) = 17.8$, $P < 0.001$], between days [$F(1, 35) = 81.0$, $P < 0.001$], and an interaction of the two [$F(4, 35) = 5.47$, $P = 0.002$]. Bonferroni's post-hoc analysis showed that treatment with 5-EAPB (at all doses) and methamphetamine produced a significantly higher distance moved between the first day of treatment and the challenge day.

3.4. 5-EAPB and 2-EAPB showed no alterations in dopamine-related proteins in the nucleus accumbens and ventral tegmental area of rats

Fig. 5 illustrates the effects on dopamine D1 receptor and dopamine D2 receptor expression in the nucleus accumbens, and tyrosine hydroxylase and dopamine transporter expression in the ventral tegmental area of rats treated with 2-EAPB (10 mg/kg), 5-EAPB (1 mg/kg), methamphetamine (1 mg/kg), or saline. In Fig.s 5B, 5C, and 5F, a one-way ANOVA showed no significant differences in dopamine D1 receptor [$F(3, 20) = 2.25$, $P < 0.114$], dopamine D2 receptor [$F(3, 20) = 2.23$, $P < 0.116$], and dopamine transporter [$F(3, 20) = 1.83$, $P < 0.174$] expressions in rat brain after the administration of 2-EAPB or 5-EAPB. A one-way ANOVA of Fig. 5E exhibited a significant difference among groups [$F(3, 20) = 10.6$, $P < 0.001$], with a Tukey's post-hoc analysis revealing a significant increase in tyrosine hydroxylase expression after methamphetamine administration only.

3.5. 2-EAPB and 5-EAPB alter deltaFosB expression in the nucleus accumbens

Fig. 6 demonstrates the effects on *p*-CREB, deltaFosB, and BDNF expression in the nucleus accumbens of rats treated with 2-EAPB (10 mg/kg), 5-EAPB (1 mg/kg), methamphetamine (1 mg/kg), or saline. A one-way ANOVA in Fig. 6C exhibited a significant difference among groups [$F(3, 20) = 4.84$, $P < 0.05$]. Tukey's post-hoc analysis revealed increased deltaFosB expressions after 2-EAPB and 5-EAPB administration. No significant changes in *p*-CREB or BDNF expressions were exhibited between treatment groups in Fig. 6B [$F(3, 20) = 1.38$, $P < 0.277$], and 6D [$F(3, 20) = 0.688$, $P < 0.570$].

4. Discussion

Treatment with specific doses of 2-EAPB (10 mg/kg) and 5-EAPB (1 mg/kg), along with METH (1 mg/kg), generated place preference in rats. These results are similar to the previously reported data for CPP induced by the SB 5-APB (1 and 2 mg/kg) (Cha et al., 2016). This suggests that 2-EAPB and 5-EAPB may possess potential rewarding effects similar to methamphetamine (Pandy et al., 2018). Interestingly, 2-EAPB required a higher dose for CPP induction than 5-EAPB. This observation might suggest a greater tendency for 5-EAPB abuse given its ability to elicit a reward-like effect at a lower dose, which is comparable to methamphetamine, than 2-EAPB. The two compounds were also modestly self-administered by rats at certain doses, as shown by the higher active-lever responses and number of infusions across the SA days (FR1 and FR2 schedules) compared to saline. This indicates that they may also possibly possess potential reinforcing properties, although at lower strengths compared to methamphetamine (dela Peña et al., 2013). Remarkably, 5-EAPB induced greater SA responses at lower doses than 2-EAPB, probably implying a stronger inclination for self-administering 5-EAPB. Given that agents with increased serotonin-releasing efficacy

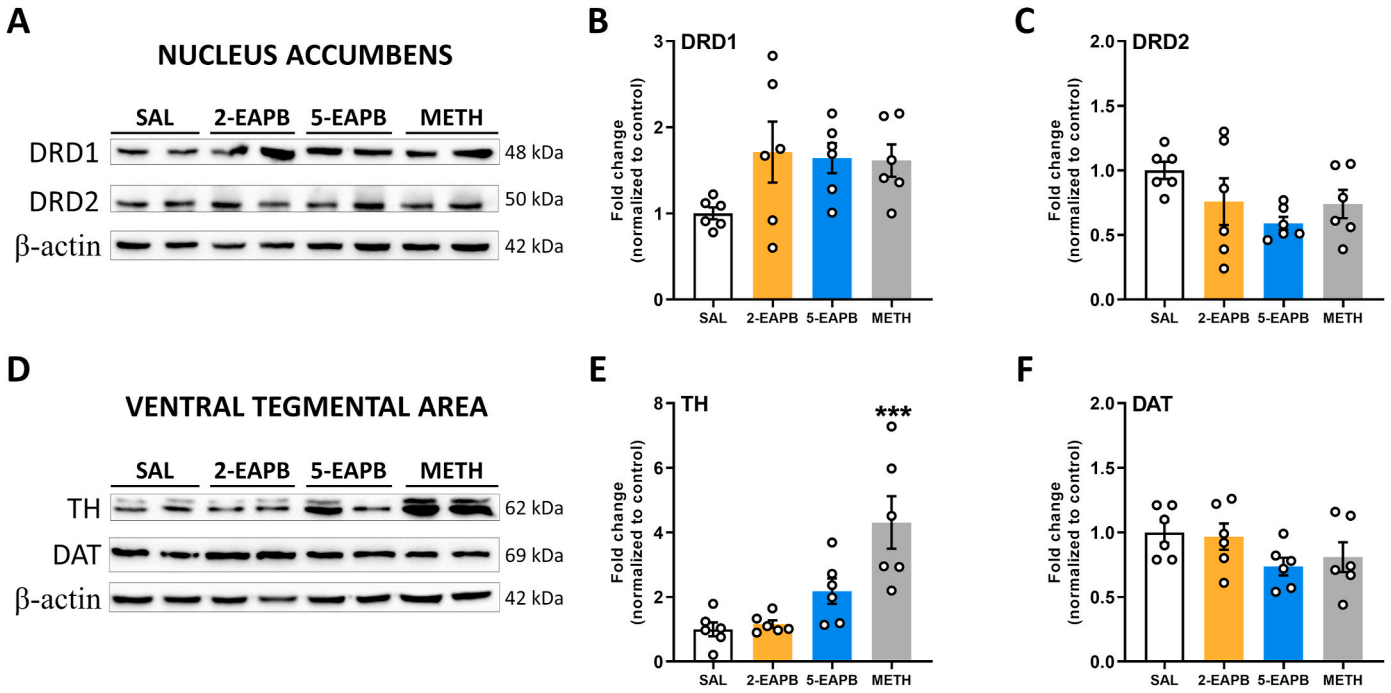

Fig. 5. Effects of 2-EAPB (10 mg/kg), 5-EAPB (1 mg/kg), METH (1 mg/kg), or SAL on dopamine-related protein levels. (A): Representative blots of the target proteins in the nucleus accumbens. Protein levels of (B) dopamine D1 receptor (DRD1) and (C) dopamine D2 receptor (DRD2). (D): Representative blots of the target proteins in the ventral tegmental area. Protein levels of (E) tyrosine hydroxylase (TH) and (F) dopamine transporter (DAT). Only METH significantly increased TH expression. Values are mean ± S.E.M. $n = 6$. $^{***}P < 0.001$ significantly different from the SAL group (One-way ANOVA, Tukey's post-test).

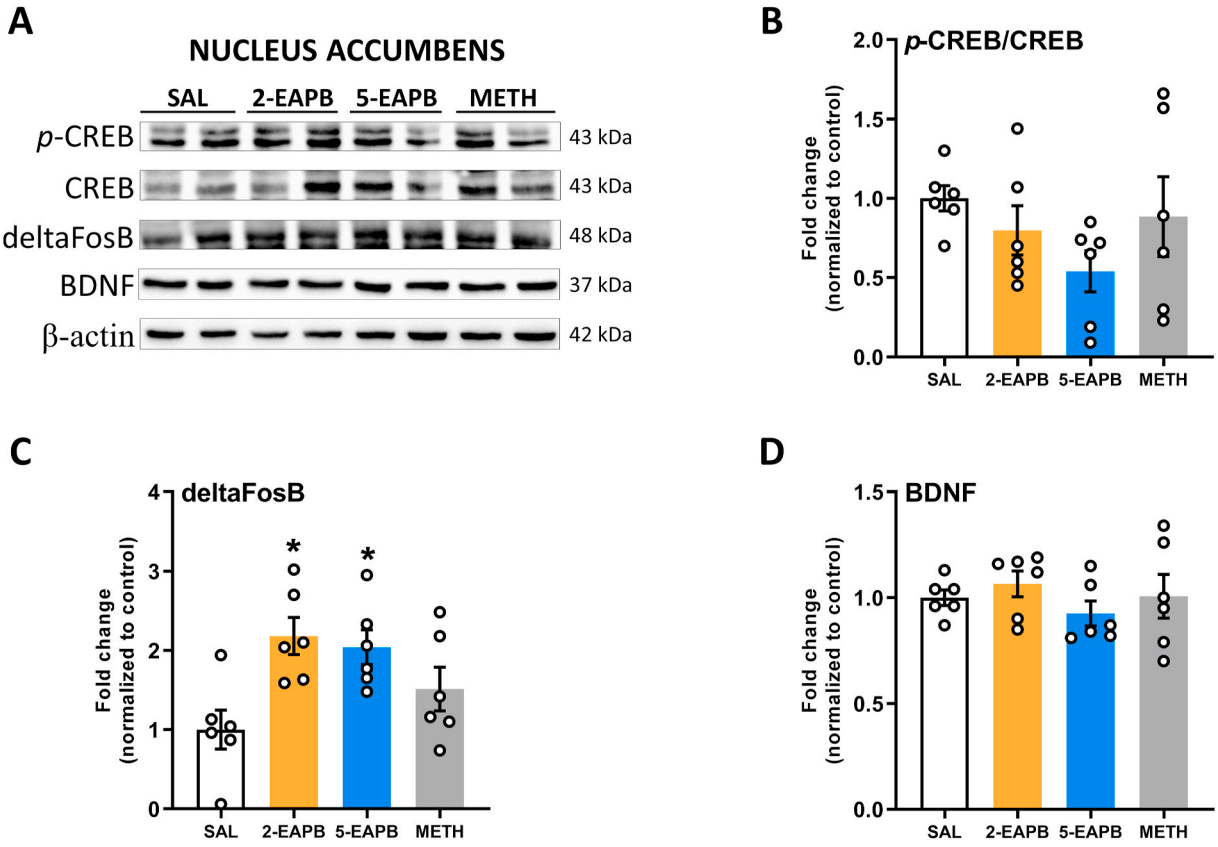

Fig. 6. Effects of 2-EAPB (10 mg/kg), 5-EAPB (1 mg/kg), METH (1 mg/kg), or SAL on plasticity-related protein levels. (A) Representative blots of the target proteins in the nucleus accumbens. Protein levels of (B) *p*-CREB, (C) deltaFosB, and (D) BDNF. Both 2-EAPB and 5-EAPB significantly increased deltaFosB levels in the nucleus accumbens. Values are mean ± S.E.M. $n = 6$. $^{*}P < 0.05$ significantly different from the SAL group (One-way ANOVA, Tukey's post-test).

relative to dopamine (such as SBs) normally decrease reinforcement (Gomila et al., 2017; Rickli et al., 2015), it may seem peculiar how 2-EAPB and 5-EAPB still induced SA responses, although lower than methamphetamine. 5-EAPB possesses relatively higher affinity for the dopamine transporter (IC_{50} = 4.9 μM) than other SBs (Rickli et al., 2015); hence, it may be plausible that the capability of the two compounds to induce SA might be associated with a combination of dopaminergic and serotonergic mediation. Further investigations are needed to elucidate this, particularly since there are no binding-affinity data available for 2-EAPB to our knowledge. In addition, only 5-EAPB (1, 3, and 10 mg/kg) and methamphetamine (1 mg/kg) were capable of inducing locomotor sensitization in mice, highlighting yet another pharmacological difference between the two SBs. Their different effects on mouse locomotor activity may again be attributed to the relatively higher dopamine transporter affinity of 5-EAPB because other SBs such as 5-APB (IC_{50} = 6.1 μM (Rickli et al., 2015)) and 6-APDB (IC_{50} = 33 μM (Rickli et al., 2015)) also induced dose-dependent horizontal stimulation at similar doses (Roque Bravo et al., 2019). Supplementary experiments to determine the dopamine transporter affinity of 2-EAPB may be necessary to elucidate its lack of stimulant effects. It is of note that 5-EAPB elicited sensitization at the CPP-inducing dose (1 mg/kg) and at higher doses (3 and 10 mg/kg), similar to other addictive drugs (Jing et al., 2014; Shimosato and Ohkuma, 2000). Overall, our behavioral assessments moderately suggest that the alterations engendered by 2-EAPB are comparable to the effects of some cannabinoids (Tampus et al., 2015) and amphetamine derivatives (Custodio et al., 2017), whereas the elicited changes by 5-EAPB are similar to the effects of opioid-analgesic treatments in adolescent C57BL/6 mice (Niikura et al., 2013). The variation between the behaviors induced by the two compounds may possibly stem from their conformational and potential binding-affinity differences, since structure-activity relationships influence the addictive (and other) effects of drugs (Glennon and Dukat, 2016; Wiley et al., 2016). Taken together, our results suggest that 2-EAPB and 5-EAPB, although with varying potencies, may hold a significant likelihood for abuse due to their potential rewarding and reinforcing effects.

We also determined the influence of 2-EAPB and 5-EAPB on dopamine-related proteins in the nucleus accumbens and ventral tegmental area, brain regions that constitute the mesolimbic dopamine system. Drugs of abuse have been recognized to modify the expression of receptors and other proteins in this system, which have all been implicated in the manifestation of addictive-like behaviors in rodents, such as CPP and SA (Abiero et al., 2019; Botanas et al., 2017; Custodio et al., 2019). Peculiarly, none of these drugs were able to elicit significant changes to the expressions of dopamine-related proteins, except for methamphetamine, which significantly increased tyrosine hydroxylase in the ventral tegmental area. Increased tyrosine hydroxylase could correspond to an upregulation in the release of dopamine in the nucleus accumbens (Abiero et al., 2019), which was also reported to contribute in facilitating addiction-like behaviors (Custodio et al., 2019). A possible rationale for the lack of consistent statistical significant changes in expressions of the other proteins might be due to methodological dissimilarities (e.g. exposure duration, treatment frequency, effective dose, euthanizing time after last treatment) between our lab and previous studies (Abiero et al., 2019; Krasnova et al., 2013). Despite these differences, our results could still be suggestive of the involvement of other neurotransmitter systems that might have influenced the development of drug-seeking behaviors in rodents after 2-EAPB and 5-EAPB exposure (Lanteri et al., 2008; Pierce and Kumaresan, 2006). In fact, the anticonvulsant drug pregabalin was capable of eliciting CPP in mice in the same manner as cocaine, but was unable to alter extracellular dopamine levels in the nucleus accumbens, suggesting the involvement of additional receptor mechanisms besides dopamine that could mediate its rewarding effects (Coutens et al., 2019). Likewise, the participation of dopaminergic receptors may not be entirely disregarded as a mechanism for the potential abuse liabilities of these SBs, unless the contribution of dopamine D1 and D2 receptors are determined (through competitive receptor antagonism) or actual dopamine levels in the mesolimbic system are characterized. Further studies are underway for the determination of other potential neuromodulators that may be implicated in the rewarding effects of 2-EAPB and 5-EAPB.

Neuronal adaptations have been exhibited during the expression of addictive behaviors after chronic exposure to abused drugs (Russo et al., 2010). These changes are generally accompanied by modifications in gene expression, as evidenced by the varying expression levels of specific transcriptional regulators in the brain after repeated drug exposure. One of these transcription factors is CREB that is normally activated by the cAMP pathway. Altered *p*-CREB has been shown in the nucleus accumbens after repeated exposure to addictive drugs (McClung and Nestler, 2003). Another transcription factor, deltaFosB (Fos family), was previously identified to accumulate in the nucleus accumbens after repeated treatment of abused substances (McClung and Nestler, 2003). Protein expression results exhibited significantly increased deltaFosB levels in the nucleus accumbens of rats after 2-EAPB and 5-EAPB treatments. These are consistent with previous reports of deltaFosB induction by morphine and cocaine (McClung and Nestler, 2003; Zachariou et al., 2006). The overall effect of enhanced deltaFosB increases medium spiny-neuron density in the nucleus accumbens, thereby leading to sensitized behavioral responses to drugs of abuse (Russo et al., 2010). This suggests that deltaFosB induction in the nucleus accumbens may have also contributed to the development of the potential rewarding effects by 2-EAPB and 5-EAPB. However, none of the drugs (including methamphetamine) were able to modify *p*-CREB expression, unlike in previous reports of abused substances (Pluzarev and Pandey, 2004). The lack of significance in *p*-CREB/CREB ratio after methamphetamine treatment might be because its effect on *p*-CREB expression is a significant reduction only after a long period of withdrawal following continuous exposure (McDaid et al., 2006). The lack of significant changes in deltaFosB expression after methamphetamine administration may not be due to prolonged euthanizing time after last treatment, but probably due to intermittent treatment frequency, as studies that do report increased deltaFosB expression exposed their subjects to methamphetamine continuously over several days (Custodio et al., 2019; Wen et al., 2020). Furthermore, one of the gene targets of CREB is BDNF, a neurotrophic factor that contributes to neuronal growth and synaptic differentiation, and has been well-implicated in drug-reward mechanisms (Li and Wolf, 2015). In contrast to previous reports (Koo et al., 2012; Ren et al., 2015), 2-EAPB, 5-EAPB, and methamphetamine treatments did not alter BDNF expression in the nucleus accumbens, despite their induction of potential rewarding effects. However, these results are also consistent with a previous study showing the normalization of increased BDNF 24 h after cocaine SA (Graham et al., 2007). Since the brain extraction in our study was performed 24 h after the last drug administration, we may not have detected the possible transient increase in BDNF. Overall, the modification in the expression of deltaFosB may possibly suggest a facilitating role in the induction of the potential rewarding effects of 2-EAPB and 5-EAPB.

To summarize, 2-EAPB and 5-EAPB induced CPP at different dosages and were also modestly self-administered by rats, indicating their potential rewarding and reinforcing effects. Only 5-EAPB was able to induce locomotor sensitization in mice at a dose-dependent manner, suggesting a possible greater tendency for 5-EAPB drug tolerance and withdrawal compared to 2-EAPB. The variations in the CPP-inducing doses and the absence/presence of locomotor alterations could suggest the involvement of other diverse receptor mechanisms that can influence behavior. The elicited addictive phenotypes by 2-EAPB and 5-EAPB treatments may possibly be associated with deltaFosB induction in the nucleus accumbens, although dopaminergic mediation may not be completely disregarded. Our investigation has generated preliminary evidence that 2-EAPB and 5-EAPB possess great potential for abuse; consequently, we recommend that the legal status of their availability to

the public should be rapidly rationalized.

Role of funding source

This work was supported by the Ministry of Food and Drug Safety (19182MFDS410) and the National Research Foundation of Korea (2017R1D1A1A02018695).

CRediT authorship contribution statement

Leandro Val Sayson: Investigation, Data curation, Formal analysis, Writing - original draft. **Raly James Perez Custodio:** Investigation, Formal analysis. **Darlene Mae Ortiz:** Investigation, Formal analysis. **Hyun Jun Lee:** Investigation, Formal analysis. **Mikyung Kim:** Investigation, Formal analysis. **Youngdo Jeong:** Investigation, Formal analysis. **Yong Sup Lee:** Investigation, Formal analysis. **Hee Jin Kim:** Conceptualization, Methodology, Project administration, Validation. **Jae Hoon Cheong:** Conceptualization, Methodology, Project administration, Validation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

Abiero, A., Botanas, C.J., Custodio, R.J., Sayson, L.V., Kim, M., Lee, H.J., Kim, H.J., Lee, K.W., Jeong, Y., Seo, J.-W., 2019. 4-MeO-PCP and 3-MeO-PCMo, new dissociative drugs, produce rewarding and reinforcing effects through activation of mesolimbic dopamine pathway and alteration of accumbal CREB, deltaFosB, and BDNF levels. Psychopharmacology 1–16.

Adamowicz, P., Zuba, D., Byrska, B., 2014. Fatal intoxication with 3-methyl-N-methylcathinone (3-MMC) and 5-(2-aminopropyl) benzofuran (5-APB). Forensic Sci. Int. 245, 126–132.

Addiction, E., 2014. European Drug Report: Trends and Developments.

Beltgens, M.T., 2017. Legislative Measures' Impact on the New Psychoactive Substances Market, Dual Markets. Springer, pp. 171–180.

Botanas, C.J., Yoon, S.S., De La Pena, J.B., dela Peña, I.J., Kim, M., Woo, T., Seo, J.-W., Jang, C.-G., Park, K.-T., Lee, Y.H., 2017. The abuse potential of α-piperidinopropiophenone (PIPP) and α-piperidinopentiothiophenone (PIVT), two new synthetic cathinones with piperidine ring substituent. Biomolecules & therapeutics 25, 122.

Cain, M.E., Denehy, E.D., Bardo, M.T., 2008. Individual differences in amphetamine self-administration: the role of the central nucleus of the amygdala. Neuropsychopharmacology 33, 1149–1161.

Cha, H.J., Lee, K.-W., Eom, J.-H., Kim, Y.-H., Shin, J., Yun, J., Han, K., Kim, H.S., 2016. 5-(2-Aminopropyl) benzofuran and phenazepam demonstrate the possibility of dependence by increasing dopamine levels in the brain. Pharmacol. Biochem. Behav. 149, 17–22.

Coutens, B., Mouledous, L., Stella, M., Rampon, C., Lapeyre-Mestre, M., Roussin, A., Guiard, B.P., Jouanjus, E., 2019. Lack of correlation between the activity of the mesolimbic dopaminergic system and the rewarding properties of pregabalin in mouse. Psychopharmacology 236, 2069–2082.

Custodio, R.J.P., Botanas, C.J., Yoon, S.S., De La Pena, J.B., dela Peña, I.J., Kim, M., Woo, T., Seo, J.-W., Jang, C.-G., Kwon, Y.H., 2017. Evaluation of the abuse potential of novel amphetamine derivatives with modifications on the amine (NBNA) and phenyl (EDA, PMEA, 2-APN) sites. Biomolecules & therapeutics 25, 578.

Custodio, R.J.P., Sayson, L.V., Botanas, C.J., Abiero, A., You, K.Y., Kim, M., Lee, H.J., Yoo, S.Y., Lee, K.W., Lee, Y.S., 2019. 25B-NBOMe, a novel N-2-methoxybenzyl-phenethylamine (NBOMe) derivative, may induce rewarding and reinforcing effects via a dopaminergic mechanism: Evidence of abuse potential. Addiction Biol., e12850

Custodio, R.J.P., Sayson, L.V., Botanas, C.J., Abiero, A., Kim, M., Lee, H.J., Ryu, H.W., Lee, Y.S., Kim, H.J., Cheong, J.H., 2020. Two newly-emerging substituted phenethylamines MAL and BOD induce differential psychopharmacological effects in rodents. J. Psychopharmacol., 0269881120936458

Dawson, P., Opacka-Juffry, J., Moffatt, J.D., Daniju, Y., Dutta, N., Ramsey, J., Davidson, C., 2014. The effects of benzofury (5-APB) on the dopamine transporter and 5-HT2-dependent vasoconstriction in the rat. Prog. Neuro Psychopharmacol. Biol. Psychiatr. 48, 57–63.

dela Peña, I., Jeon, S.J., Lee, E., Ryu, J.H., Shin, C.Y., Noh, M., Cheong, J.H., 2013. Neuronal development genes are key elements mediating the reinforcing effects of methamphetamine, amphetamine, and methylphenidate. Psychopharmacology 230, 399–413.

Deville, M., Dubois, N., Cieckiewicz, E., De Tullio, P., Lemaire, E., Charlier, C., 2019. Death following consumption of MDAI and 5-EAPB. Forensic Sci. Int. 299, 89–94.

Dolan, S.B., Forster, M.J., Gatch, M.B., 2017. Discriminative stimulus and locomotor effects of para-substituted and benzofuran analogs of amphetamine. Drug Alcohol Depend. 180, 39–45.

Eshleman, A.J., Nagarajan, S., Wolfrum, K.M., Reed, J.F., Swanson, T.L., Nilsen, A., Janowsky, A., 2019. Structure-activity relationships of bath salt components: substituted cathinones and benzofurans at biogenic amine transporters. Psychopharmacology 236, 939–952.

Fattore, L., Altea, S., Fratta, W., 2008. Sex differences in drug addiction: a review of animal and human studies. Wom. Health 4, 51–65.

Fuwa, T., Suzuki, J., Tanaka, T., Inomata, A., Honda, Y., Kodama, T., 2016. Novel psychoactive benzofurans strongly increase extracellular serotonin level in mouse corpus striatum. J. Toxicol. Sci. 41, 329–337.

Glennon, R.A., Dukat, M., 2016. Structure-activity Relationships of Synthetic Cathinones, Neuropharmacology of New Psychoactive Substances (NPS). Springer, pp. 19–47.

Gomila, I., Moranta, C., Quesada, L., Pastor, Y., Dastis, M., Torrents, A., Elorza, M.Á., Busardò, F.P., Barceló, B., 2017. Cross-reactivity of selected benzofurans with commercial amphetamine and ecstasy immunoassays in urine. Bioanalysis 9, 1771–1785.

Graham, D.L., Edwards, S., Bachtell, R.K., DiLeone, R.J., Rios, M., Self, D.W., 2007. Dynamic BDNF activity in nucleus accumbens with cocaine use increases self-administration and relapse. Nat. Neurosci. 10, 1029–1037.

Jing, L., Zhang, M., Li, J.-X., Huang, P., Liu, Q., Li, Y.-L., Liang, H., Liang, J.-H., 2014. Comparison of single versus repeated methamphetamine injection induced behavioral sensitization in mice. Neurosci. Lett. 560, 103–106.

Johnson, L.A., Johnson, R.L., Portier, R.-B., 2013. Current "legal highs". J. Emerg. Med. 44, 1108–1115.

Kawahata, I., Tokuoka, H., Parvez, H., Ichinose, H., 2009. Accumulation of phosphorylated tyrosine hydroxylase into insoluble protein aggregates by inhibition of an ubiquitin–proteasome system in PC12D cells. J. Neural. Transm. 116, 1571.

Kikura-Hanajiri, R., Kawamura, N.U., Maiko, Goda, Y., 2014. Changes in the prevalence of new psychoactive substances before and after the introduction of the generic scheduling of synthetic cannabinoids in Japan. Drug Test. Anal. 6, 832–839.

Kim, M., Custodio, R.J., Botanas, C.J., de la Peña, J.B., Sayson, L.V., Abiero, A., Ryoo, Z. Y., Cheong, J.H., Kim, H.J., 2019. The circadian gene, Per 2, influences methamphetamine sensitization and reward through the dopaminergic system in the striatum of mice. Addiction Biol. 24, 946–957.

King, L., 2014. New phenethylamines in Europe. Drug Test. Anal. 6, 808–818.

Koo, J.W., Mazei-Robison, M.S., Chaudhury, D., Juarez, B., LaPlant, Q., Ferguson, D., Feng, J., Sun, H., Scobie, K.N., Damez-Werno, D., 2012. BDNF is a negative modulator of morphine action. Science 338, 124–128.

Krasnova, I.N., Chiflikyan, M., Justinova, Z., McCoy, M.T., Ladenheim, B., Jayanthi, S., Quintero, C., Brannock, C., Barnes, C., Adair, J.E., 2013. CREB phosphorylation regulates striatal transcriptional responses in the self-administration model of methamphetamine addiction in the rat. Neurobiol. Dis. 58, 132–143.

Lanteri, C., Salomon, L., Torrens, Y., Glowinski, J., Tassin, J.-P., 2008. Drugs of abuse specifically sensitize noradrenergic and serotonergic neurons via a non-dopaminergic mechanism. Neuropsychopharmacology 33, 1724–1734.

Li, X., Wolf, M.E., 2015. Multiple faces of BDNF in cocaine addiction. Behav. Brain Res. 279, 240–254.

Marona-Lewicka, D., Rhee, G.-S., Sprague, J.E., Nichols, D.E., 1996. Reinforcing effects of certain serotonin-releasing amphetamine derivatives. Pharmacol. Biochem. Behav. 53, 99–105.

McClung, C.A., Nestler, E.J., 2003. Regulation of gene expression and cocaine reward by CREB and ΔFosB. Nat. Neurosci. 6, 1208–1215.

McDaid, J., Graham, M.P., Napier, T.C., 2006. Methamphetamine-induced sensitization differentially alters pCREB and ΔFosB throughout the limbic circuit of the mammalian brain. Mol. Pharmacol. 70, 2064–2074.

Modi, G.M., Yang, P.B., Swann, A.C., Dafny, N., 2006. Chronic exposure to MDMA (Ecstasy) elicits behavioral sensitization in rats but fails to induce cross-sensitization to other psychostimulants. Behav. Brain Funct. 2, 1.

Niikura, K., Ho, A., Kreek, M.J., Zhang, Y., 2013. Oxycodone-induced conditioned place preference and sensitization of locomotor activity in adolescent and adult mice. Pharmacol. Biochem. Behav. 110, 112–116.

Nugteren-van Lonkhuyzen, J.J., van Riel, A.J., Brunt, T.M., Hondebrink, L., 2015. Pharmacokinetics, pharmacodynamics and toxicology of new psychoactive substances (NPS): 2C-B, 4-fluoroamphetamine and benzofurans. Drug Alcohol Depend. 157, 18–27.

Pandy, V., Wai, Y.C., Roslan, N.F.A., Sajat, A., Jallb, A.H.A., Vijeepallam, K., 2018. Methanolic extract of Morinda citrifolia Linn. unripe fruit attenuates methamphetamine-induced conditioned place preferences in mice. Biomed. Pharmacother. 107, 368–373.

Pierce, R.C., Kumaresan, V., 2006. The mesolimbic dopamine system: the final common pathway for the reinforcing effect of drugs of abuse? Neurosci. Biobehav. Rev. 30, 215–238.

Pluzarev, O., Pandey, S.C., 2004. Modulation of CREB expression and phosphorylation in the rat nucleus accumbens during nicotine exposure and withdrawal. J. Neurosci. Res. 77, 884–891.

Ren, Q., Ma, M., Yang, C., Zhang, J., Yao, W., Hashimoto, K., 2015. BDNF–TrkB signaling in the nucleus accumbens shell of mice has key role in methamphetamine withdrawal symptoms. Transl. Psychiatry 5, e666.

Rentesi, G., Antoniou, K., Marselos, M., Syrrou, M., Papadopoulou-Daifoti, Z., Konstandi, M., 2013. Early maternal deprivation-induced modifications in the neurobiological, neurochemical and behavioral profile of adult rats. Behav. Brain Res. 244, 29–37.

Rickli, A., Kopf, S., Hoener, M.C., Liechti, M.E., 2015. Pharmacological profile of novel psychoactive benzofurans. Br. J. Pharmacol. 172, 3412–3425.

Roque Bravo, R., Carmo, H., Carvalho, F., Bastos, M.d.L., Dias da Silva, D., 2019. Benzo fury: A new trend in the drug misuse scene. J. Appl. Toxicol. 39, 1083–1095.
Russo, S.J., Dietz, D.M., Dumitriu, D., Morrison, J.H., Malenka, R.C., Nestler, E.J., 2010. The addicted synapse: mechanisms of synaptic and structural plasticity in nucleus accumbens. Trends Neurosci. 33, 267–276.
Salyer, S., Lesousky, N., Weinman, E.J., Clark, B.J., Lederer, E.D., Khundmiri, S.J., 2011. Dopamine regulation of Na+-K+-ATPase requires the PDZ-2 domain of sodium hydrogen regulatory factor-1 (NHERF-1) in opossum kidney cells. Am. J. Physiol. Cell Physiol. 300, C425–C434.
Shimosato, K., Ohkuma, S., 2000. Simultaneous monitoring of conditioned place preference and locomotor sensitization following repeated administration of cocaine and methamphetamine. Pharmacol. Biochem. Behav. 66, 285–292.
Shimshoni, J.A., Winkler, I., Golan, E., Nutt, D., 2017. Neurochemical binding profiles of novel indole and benzofuran MDMA analogues. N. Schmied. Arch. Pharmacol. 390, 15–24.
Smith, S.W., Garlich, F.M., 2013. Availability and Supply of Novel Psychoactive Substances, Novel Psychoactive Substances. Elsevier, pp. 55–77.
Tampus, R., Yoon, S.S., De La Pena, J.B., Botanas, C.J., Kim, H.J., Seo, J.-W., Jeong, E.J., Jang, C.G., Cheong, J.H., 2015. Assessment of the abuse liability of synthetic cannabinoid agonists JWH-030, JWH-175, and JWH-176. Biomolecules & therapeutics 23, 590.
Taniguchi, M., Yamamoto, Y., Nishi, K., 2010. A technique combining trifluoroacetyl derivatization and gas chromatography–mass spectrometry to distinguish methamphetamine and its 4-substituted analogs. J. Mass Spectrom. 45, 1473–1476.
Uchiyama, N., Shimokawa, Y., Kawamura, M., Kikura-Hanajiri, R., Hakamatsuka, T., 2014. Chemical analysis of a benzofuran derivative, 2-(2-ethylaminopropyl) benzofuran (2-EAPB), eight synthetic cannabinoids, five cathinone derivatives, and five other designer drugs newly detected in illegal products. Forensic Toxicol. 32, 266–281.
United Nations Office on Drugs and Crime (UNODC), 2014. World drug rep. 2014. Published june 26. Available at: http://www.unodc.org/documents/data-and-analysis/WDR2014/World_Drug_Report_2014_web.pdf. Accessed March 19, 2020.
Weaver, M.F., Hopper, J.A., Gunderson, E.W., 2015. Designer drugs 2015: assessment and management. Addiction Sci. Clin. Pract. 10, 8.
Welter-Luedeke, J., Maurer, H.H., 2016. New psychoactive substances: chemistry, pharmacology, metabolism, and detectability of amphetamine derivatives with modified ring systems. Ther. Drug Monit. 38, 4–11.
Wen, D., Hui, R., Liu, Y., Luo, Y., Wang, J., Shen, X., Xie, B., Yu, F., Cong, B., Ma, C., 2020. Molecular hydrogen attenuates methamphetamine-induced behavioral sensitization and activation of ERK-ΔFosB signaling in the mouse nucleus accumbens. Prog. Neuro Psychopharmacol. Biol. Psychiatr. 97, 109781.
Wiley, J.L., Marusich, J.A., Thomas, B.F., 2016. Combination Chemistry: Structure–Activity Relationships of Novel Psychoactive Cannabinoids, Neuropharmacology of New Psychoactive Substances (NPS). Springer, pp. 231–248.
Zachariou, V., Bolanos, C.A., Selley, D.E., Theobald, D., Cassidy, M.P., Kelz, M.B., Shaw-Lutchman, T., Berton, O., Sim-Selley, L.J., Dileone, R.J., 2006. An essential role for ΔFosB in the nucleus accumbens in morphine action. Nat. Neurosci. 9, 205–211.
Zakharova, E., Leoni, G., Kichko, I., Izenwasser, S., 2009. Differential effects of methamphetamine and cocaine on conditioned place preference and locomotor activity in adult and adolescent male rats. Behav. Brain Res. 198, 45–50.