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Increased BDNF-TrkB signaling in the nucleus accumbens plays a role in the risk for psychosis after cannabis exposure during adolescence

Chao Dong[a,1], Zheng Tian[a,2], Kai Zhang[a], Lijia Chang[a], Youge Qu[a], Yaoyu Pu[a], Qian Ren[a], Yuko Fujita[a], Yuta Ohgi[b], Takashi Futamura[b], Kenji Hashimoto[a,*]

[a] Division of Clinical Neuroscience, Chiba University Center for Forensic Mental Health, Chiba, Japan
[b] Department of CNS Research, New Drug Research Division, Otsuka Pharmaceutical Co., Ltd., Tokushima, Japan

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ABSTRACT

Although epidemiological data suggest that repeated use of cannabis during adolescence may increase the risk for psychosis, its precise molecular mechanisms remain undetermined. In this study, we examined whether brain-derived neurotrophic factor (BDNF) and its receptor TrkB signaling plays a role in the risk for psychosis after exposure of cannabinoid (CB) receptor agonist during adolescence. Repeated administration of the CB receptor agonist WIN55,212-2 (2 mg/kg/day) during adolescence (P35 – P45) significantly increased methamphetamine (METH: 1 mg/kg)-induced hyperlocomotion in adulthood (P70 – P74) compared with vehicle-treated mice. Western blot analysis showed that BDNF-TrkB signaling in the nucleus accumbens (NAc) of WIN55,212-2-treated mice were significantly higher than that of vehicle-treated mice. Interestingly, an increase in the METH-induced locomotion in WIN55,212-2-treated mice was significantly attenuated by subsequent repeated administration of the TrkB antagonist ANA-12 (0.5 mg/kg/day from P70 to P83). Furthermore, increased BDNF-TrkB signaling in the NAc from WIN55,212-2-treated mice was also significantly attenuated after subsequent repeated administration of ANA-12. These findings suggest that increased BDNF-TrkB signaling in the NAc plays an important role in the increase in METH-induced locomotion in adulthood after repeated WIN55,212-2 administration during adolescence. Therefore, TrkB antagonists would be potential prophylactic and therapeutic drugs for psychosis in adult with cannabis use during adolescence.

1. Introduction

Cannabis is by far the most widely cultivated, trafficked and abused illicit drug. About 147 million people, 2.5% of the world population, consume cannabis (annual prevalence) compared with 0.2% consuming cocaine and 0.2% consuming opiates. Cannabis has become more closely linked to youth culture and the age of initiation is usually lower than for other drugs (WHO, 2018). Epidemiological data suggest that repeated cannabis use is a risk factor for the onset of psychosis or schizophrenia (Andréasson et al., 1987; Arseneault et al., 2002; Henquet et al., 2005; Moore et al., 2007). Certainly, cannabis users at a younger age tend to develop schizophrenia and suffer from more psychotic relapses (Linszen et al., 1994; Veen et al., 2004).

Emerging evidence suggests that adolescents may be particularly vulnerable to the adverse effects of cannabis use (Volkow, 2016). The use of exogenous cannabinoids during adolescence could disrupt normal brain development since adolescence represents a critical neurodevelopmental period characterized by marked synaptic pruning and increased myelination (Volkow, 2016; Lubman et al., 2015). However, the precise molecular mechanisms underlying the long-term consequences of adolescent exposure to synthetic cannabinoids remain undetermined.

The cannabinoid system involves two types of cannabinoid receptors (CB1 and CB2 receptors), and it acts a crucial neuromodulator in the central nervous system (CNS) (Colizzi et al., 2016; Curran et al., 2016; Leweke et al., 2016). Furthermore, synthetic cannabinoids have been related with psychosis and psychosis-like conditions (van Amsterdam et al., 2015), and exogenous cannabinoid agonists, administered during adolescence to rodents or nonhuman primates, produce a schizophrenia-like phenotype in adulthood (Rubino et al., 2009; Cass et al., 2014; Verrico et al., 2014; Aguilar et al., 2018). Collectively, it is possible that exposure of synthetic cannabinoids during

* Corresponding author at: Division of Clinical Neuroscience, Chiba University Center for Forensic Mental Health, 1-8-1 Inohana, Chiba 260-8670, Japan.
E-mail address: hashimoto@faculty.chiba-u.jp (K. Hashimoto).
[1] Current address: Chengde Central Hospital, Chengde, Hebei 067000, China.
[2] Current address: Histology and Embryology Division, Chengde Medical University, Chengde, China.

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adolescence can produce psychosis in adulthood although the detailed molecular mechanisms are currently unknown.

Abused drugs produce persistent restructuring of several neuronal cell types in the limbic regions of brain which are responsible for long-term behavioral plasticity driving addiction (Kauer and Malenka, 2007; Russo et al., 2009; Russo et al., 2010). Accumulating evidence suggests that brain-derived neurotrophic factor (BDNF) and its specific receptor, tropomyosin-related kinase (TrkB) signaling, plays an important role in the long-lasting alterations after repeated administration of abused drugs (Akbarian et al., 2002; Butovsky et al., 2005; Lobo et al., 2010; Pickens et al., 2011; Koo et al., 2012; Koo et al., 2015; Truitt et al., 2015; Ren et al., 2015; Anderson, 2017). Previously, we reported that increased BDNF-TrkB signaling in the nucleus accumbens (NAc) plays a key role in the methamphetamine (METH) withdrawal symptoms such as depression-like phenotype and behavioral sensitization (Ren et al., 2015). Furthermore, infusion of antibodies against BDNF or TrkB into NAc attenuates the stimulation of dopamine release and behavioral abnormalities after METH exposure (Narita et al., 2003). These all findings suggest that BDNF-TrkB signaling in the NAc plays a role in the behavioral abnormalities observed after repeated use of abused drugs.

The present study was performed to determine whether exposure of CB receptor agonist during adolescence can affect the risk for psychosis in adulthood. WIN55,212-2 is a potent CB receptor agonist with Ki of 62.3 nM (human recombinant CB1) and 3.3 nM (human recombinant CB2) (Felder et al., 1995). First, we examined whether repeated exposure of WIN55,212-2 during adolescence (P35 – P45) can affect METH-induced hyperlocomotion in adulthood (P71 – P74). Next, we examined the role of BDNF-TrkB signaling in the brain regions in the marked increase of METH-induced locomotion in adulthood after repeated exposure of WIN55,212-2 during adolescence. Finally, we examined to study whether the TrkB antagonist ANA-12 (Cazorla et al., 2011) has prophylactic effects for psychosis after repeated exposure of WIN55,212-2 during adolescence.

2. Materials and methods

2.1. Animals

Male adult C57BL/6 mice (8 weeks old, body weight 20–25 g at the beginning of experiments; Japan SLC, Inc., Hamamatsu, Japan) were housed under controlled temperature and 12 h light/dark cycles (lights on between 07:00 and 19:00), with *ad libitum* food and water. All experiments were carried out in accordance with the Guideline for Animal Experimentation of Chiba University. This study was approved by the Chiba University Institutional Animal Care and Use Committee (permission number: 29-332 and 30-308).

2.2. Drugs

(*R*)-(+)-WIN55,212-2 mesylate salt (Sigma-Aldrich Corporation, Tokyo, Japan) was dissolved in physiological saline containing 1% dimethylsulfoxide (DMSO). ANA-12 (*N*-[2-[[(Hexahydro-2-oxo-1H-azepin-3-yl)amino] carbonyl]phenyl]-benzo[*b*]thiophene-2-carboxamide) (Maybridge, Ltd., Loughborough, Leicestershire, UK) was dissolved in phosphate-buffered saline containing 17% DMSO. Methamphetamine (METH) hydrochloride (1.0 mg/kg expressed as a hydrochloride salt, Dainippon Pharmaceutical Ltd., Osaka, Japan) was dissolved in physiological saline. Other chemicals were purchased from commercial sources. The dose of WIN55,212-2 (2 mg/kg) and ANA-12 (0.5 mg/kg) was selected as reported previously (Cass et al., 2014; Ren et al., 2015; Cazorla et al., 2011; Ma et al., 2016; Zhang et al., 2016).

2.3. Exposure of WIN during adolescence

Previously, Cass et al. (2014) reported that repeated WIN55,212-2 exposure during early (postnatal days - P35 – P40) or mid-(P40 – P45) adolescence, but not in late adolescence (P50 – P55) or adulthood (P75 – P80), is sufficient to yield a state of frequency-dependent prefrontal disinhibition in adulthood comparable to that seen in the juvenile mice. Therefore, we selected the early and mid-adolescence (P35 – P45) for repeated exposure of WIN55,212-2 in this study.

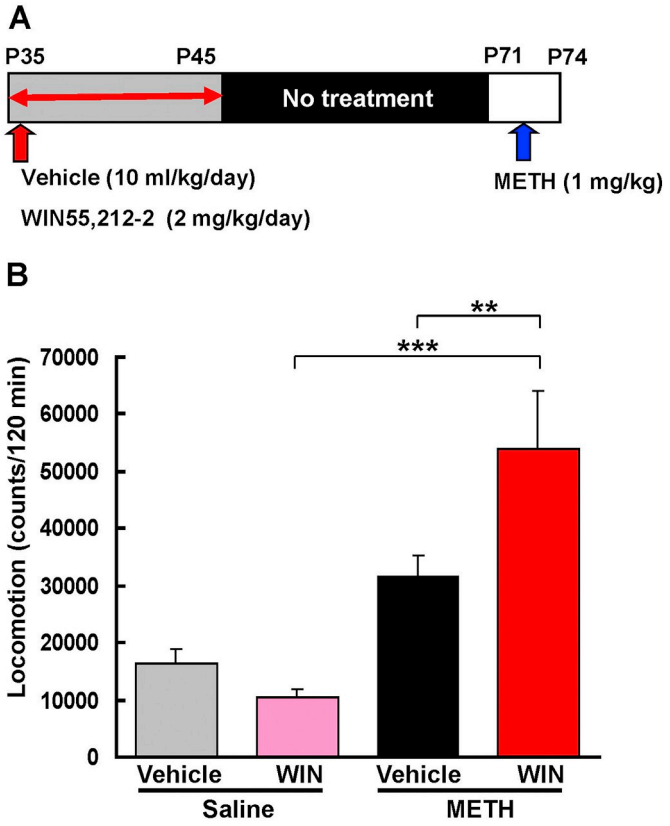

Fig. 1. Effect of WIN55,212-2 treatment during adolescence on METH-induced hyperlocomotion in adulthood.
A: Schedule of treatment and behavioral tests. Vehicle (10 ml/kg/day for 11 days) or WIN55,212-2 (2 mg/kg/day for 11 days) was injected i.p. into mice from P35 to P45. Behavioral tests after a single administration of METH (1 mg/kg, s.c.) were performed on P71-P74. B: The locomotor activity of the WIN55,212-2-treated mice was significantly higher than that of vehicle-treated mice after a single administration of METH (1 mg/kg). There was no change between the vehicle-treated mice and WIN55,212–2-treated mice after a single administration of saline. Each value is the mean ± S.E.M. (n = 10). **P < 0.01 as compared with control (vehicle-treated) group.

2.4. METH-induced hyperlocomotion

Vehicle (10 ml/kg/day for 11 days) or WIN55,212–2 (2 mg/kg/day for 11 days) was intraperitoneally (i.p.) administered into mice (n = 10) from P35 to P45 continuously (Cass et al., 2014). Following, a single dose of METH (1 mg/kg) was injected subcutaneously (s.c.) into mice from P71 to P74 when performing the locomotion test (Fig. 1A). METH (1 mg/kg) was used to examine the potentiating effects of WIN55,212-2 as reported previously (Ren et al., 2015). Mice were placed in experimental cages (length × width × height: 450 × 450 × 295 mm). Locomotor activity was counted by the SCANET MV-40 (MELQUEST Co., Ltd., Toyama, Japan), and cumulative exercise was recorded for 180 min. At the point of 60 min (habituation), mice were given a low dose of METH (1 mg/kg, s.c.), as reported previously (Ren et al., 2015; Horio et al., 2012). Cages were cleaned between testing sessions.

Next, vehicle (10 ml/kg/day for 11 days) or WIN55,212-2 (2 mg/kg/day for 11 days) was administered i.p. into mice (n = 9) from P35 to

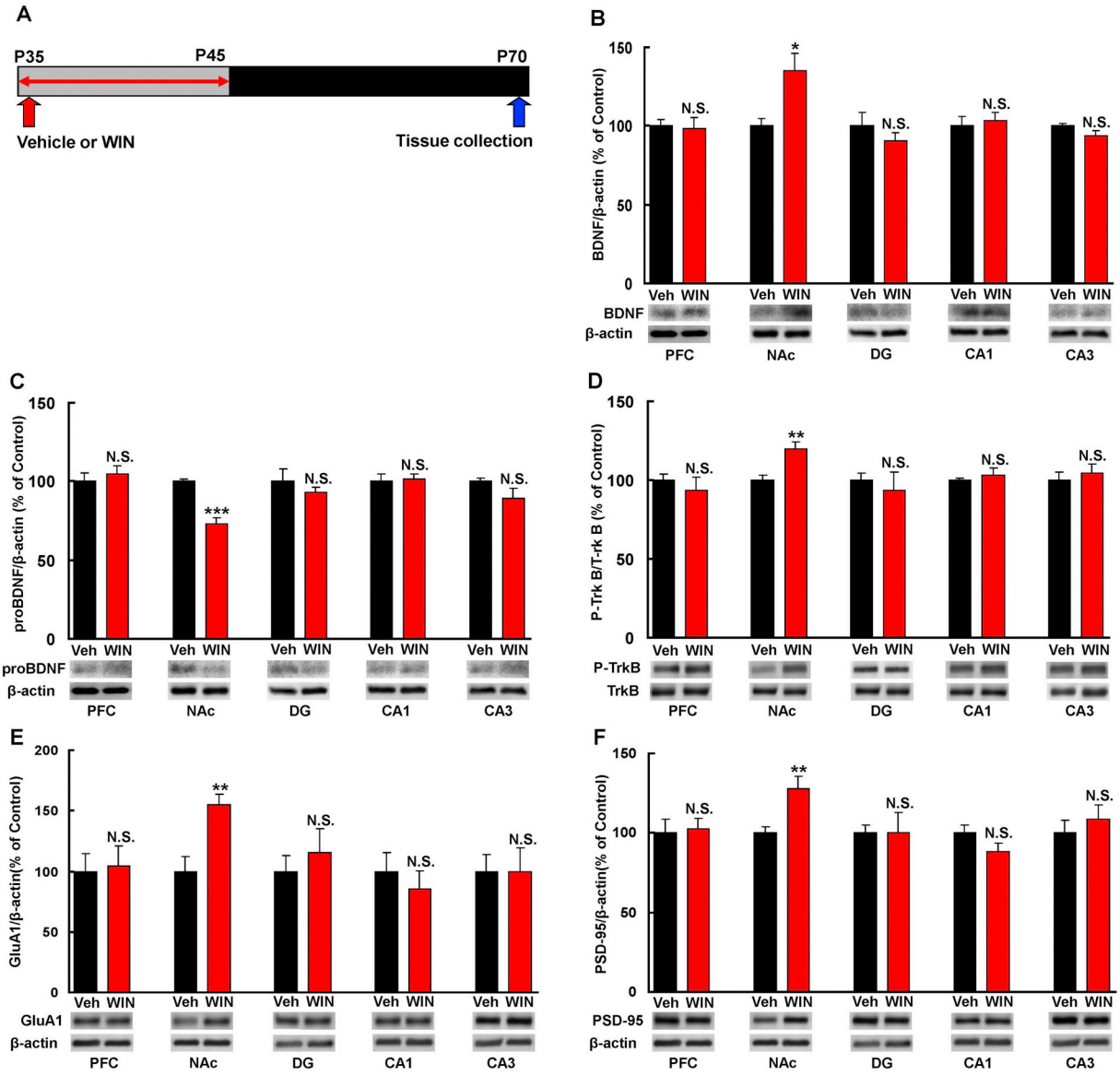

Fig. 2. Role of BDNF-TrkB signaling in the brain regions after repeated WIN55,212-2 administration.
A: Schedule of treatment and brain tissues collection. Vehicle (10 ml/kg/day for 11 days) or WIN55,212-2 (2 mg/kg/day for 11 days) was injected i.p. into mice. Sample correction for Western blot analysis was performed at P70. B: BDNF. The level of BDNF protein in the NAc of WIN55,212-2-treated mice was significantly higher than that of vehicle-treated mice. There were no differences between the two groups in the PFC, DG, CA1, and CA3. C: proBDNF. The level of proBDNF protein in the NAc of WIN55,212-2-treated mice was significantly lower than that of vehicle-treated mice. There were no differences between the two groups in the PFC, DG, CA1, and CA3. D: p-TrkB/TrkB ratio. The ratio of p-TrkB/TrkB in the NAc of WIN55,212-2-treated mice was significantly higher than that of control mice. There were no differences between the two groups in the PFC, DG, CA1 and CA3. E: GluA1. The level of GluA1 protein in the NAc of WIN55,212-2-treated mice was significantly higher than that of vehicle-treated mice. There were no differences between the two groups in the PFC, DG, CA1, and CA3. F: PSD-95. The level of PSD-95 protein in the NAc of WIN55,212-2-treated mice was significantly higher than that of vehicle-treated mice. There were no differences between the two groups in the PFC, DG, CA1, and CA3. Each value is the mean ± S.E.M. (n = 6 per group). *P < 0.05, **P < 0.01, ***P < 0.001 as compared with vehicle-treated group. Veh: Vehicle, WIN: WIN55,212-2. N.S.: not significant.

P45 continuously (Cass et al., 2014). There was no treatment from P46 to P69. Subsequently, vehicle (10 ml/kg/day for 14 days) or ANA-12 (0.5 mg/kg/day for 14 days) was administered i.p. into mice (n = 9) from P70 to P83 continuously (Ren et al., 2015). Following, a single dose of METH (1 mg/kg) was injected s.c. into mice from P85 to P88 when performing the locomotion test (Fig. 3A). Mice were placed in experimental cages (length × width × height: 450 × 450 × 295 mm). Locomotor activity was counted by the SCANET MV-40 (MELQUEST Co., Ltd., Toyama, Japan) as described above.

2.5. Western blot analysis

Western blot analysis was performed as previously reported (Ma et al., 2016; Zhang et al., 2016; Zhang et al., 2015; Yang et al., 2015; Dong et al., 2017). Vehicle (10 ml/kg/day for 11 days) or WIN55,212-2 (2 mg/kg/day for 11 days) was administered i.p. into mice from P35 to P45. On day P70 (Fig. 2A) or day P85 (Fig. 4A), mice were killed by sacrifice under 5% isoflurane. Prefrontal cortex (PFC), CA1, CA3 and dentate gyrus (DG) of hippocampus, and NAc were dissected on ice using a Leica microscope S9E (Leica Microsystems, Tokyo, Japan), and stored at −80 °C. Tissue samples were homogenized in Laemmli lysis

buffer. Equal amount of proteins (10–20 μg) for each sample were measured by DC protein assay kit (Bio-Rad, Hercules, CA), and incubated for 5 min at 95 °C with protein buffer (125 mM Tris/HCl (pH 6.8), 20% glycerol, 0.1% bromophenol blue, 10% β-mercaptoethanol, 4% sodium dodecyl sulfate). The protein samples were loaded into AnykD mini-gels (Mini-PROTEAN® TGX™ Precast Gel; Bio-Rad) for electrophoresis. Polyvinylidene difluoride (PVDF) membranes with transferred proteins were blocked with 2% BSA plus 5% nonfat dry milk in TBST (TBS + 0.1% Tween-20) (for BDNF and proBDNF) or 2% BSA in PBST (PBS + 0.1% Tween-20) (for GluA1) for 1 h and kept with primary antibodies overnight at 4 °C. The following primary antibody was used: proBDNF (1:400, #ANT-006: Alomone Labs Ltd., Jerusalem, Israel), BDNF (H-117) (1:1000, sc-20981: Santa Cruz Biotechnology, Inc., CA), phosphorylated-TrkB (Tyr 706) (1:200, sc-135645: Santa Cruz Biotechnology, Inc., CA), TrkB (80E3) (1:1000, #4606: Cell Signaling Technology, MA, USA), AMPA glutamate receptor 1 (GluA1) (1 μg/ml, ab31232: Abcam, Cambridge, UK), postsynaptic density protein 95 (PSD-95) (1 μg/ml, 51–6900: Invitrogen, Carlsbad, CA, USA) and β-actin (1:10000, A5441: Sigma-Aldrich Co., Ltd., St Louis, MO). The next day, blots were washed three times in TBST and incubated with horseradish peroxidase conjugated anti-rabbit antibody or anti-mouse antibody for 1 h. After final three washes with TBST, bands were detected using enhanced chemiluminescence (ECL) plus the Western Blotting Detection system (GE healthcare Bioscience) and the images were captured with Fuji LAS3000-mini imaging system (Fujifilm, Tokyo, Japan), and immunoreactive bands were quantified.

2.6. Statistical analysis

Data were presented as the mean ± standard error of the mean (S.E.M.). The data were analyzed by Student *t*-test or two-way analysis of variance (ANOVA), followed *post hoc* the Fisher's Least Significant Difference (LSD) test. The supplemental data of time course of locomotion were analyzed by repeated-measured Two-way ANOVA. The P values of < 0.05 were considered statistically significant.

3. Results

3.1. Effect of WIN55,212-2 exposure during adolescence on METH-induced hyperlocomotion in adulthood

First, we examined whether repeated administration of WIN55,212-2 (2 mg/kg/day for 11 days from P35 to P45) can affect METH-induced hyperlocomotion in adulthood (P71 – P74) (Fig. 1A). Two-way ANOVA revealed statistical differences among the four groups [WIN: $F_{(1,39)} = 2.229$, P = 0.144, METH: $F_{(1,39)} = 27.973$, $P < 0.001$, interaction (WIN × METH): $F_{(1,39)} = 6.543$, P = 0.015] (Fig. 1B). The time course of METH-induced locomotion was shown in the Fig. S1. The locomotor activity of WIN55,212-2-treated mice after a single METH (1 mg/kg) administration was significantly higher than that of vehicle-treated mice. There was no change of between two groups after a single administration of saline. These data showed that repeated administration of WIN55,212-2 during adolescence (P35 – P45) could enhance METH-induced locomotion in adulthood (P71 – P74), suggesting a higher risk for psychosis in adulthood after repeated WIN55,212-2 exposure during adolescence.

3.2. BDNF-TrkB signaling in the brain regions after repeated WIN55,212-2 exposure during adolescence

Previously, we reported that BDNF-TrkB signaling in the NAc plays a key role in METH-induced withdrawal symptoms, such as depression and behavioral sensitization (Ren et al., 2015). Therefore, we performed Western blot analysis of BDNF, its precursor proBDNF, TrkB, phosphorylated TrkB (p-TrkB), synaptic markers [α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor type 1 (GluA1) and postsynaptic density protein 95 (PSD-95)] in selected brain regions (PFC, NAc, DG, CA1 and CA3) of mice. The levels of BDNF, p-TrkB/TrkB, GluA1 and PSD-95 in the NAc of WIN55,212-2-treated mice were significantly higher than that of vehicle-treated mice [BDNF: $F_{(1,11)} = 3.580$, $t = -2.819$, P = 0.018, p-TrkB/TrkB: $F_{(1,11)} = 0.757$, $t = -3.602$, P = 0.005, GluA1: $F_{(1,11)} = 1.771$, $t = -3.543$, P = 0.005, PSD-95: $F_{(1,11)} = 5.936$, $t = -3.241$, P = 0.009] (Fig. 2B, D–F). In contrast, the levels of proBDNF in the NAc of WIN55,212-2-treated mice were significantly lower than that of vehicle-treated mice [$F_{(1,11)} = 5.055$, $t = 6.844$, $P < 0.001$] (Fig. 2C). There were no differences of all proteins in the PFC and hippocampus between two groups [BDNF: PFC, $F_{(1,11)} = 1.719$, t = 0.228, P = 0.824, DG, $F_{(1,11)} = 0.945$, t = 0.903, P = 0.388, CA1, $F_{(1,11)} = 0.547$, t = −0.439, P = 0.670, CA3, $F_{(1,11)} = 4.167$, t = 0.068, P = 0.116, proBDNF: PFC, $F_{(1,11)} = 0.072$, t = −0.619, P = 0.550, DG, $F_{(1,11)} = 2.420$, t = 0.151, P = 0.435, CA1, $F_{(1,11)} = 0.777$, t = 0.399, P = 0.788, CA3, $F_{(1,11)} = 5.903$, t = 1.577, P = 0.146, p-TrkB/TrkB: PFC, $F_{(1,11)} = 2.272$, t = 0.711, P = 0.493, DG, $F_{(1,11)} = 9.203$, t = 0.534, P = 0.605, CA1, $F_{(1,11)} = 1.421$, t = −0.775, P = 0.456, CA3, $F_{(1,11)} = 0.126$, t = −0.556, P = 0.590, GluA1: PFC, $F_{(1,11)} = 0.502$, t = −0.198, P = 0.847, DG, $F_{(1,11)} = 1.974$, t = −0.636, P = 0.539, CA1, $F_{(1,11)} = 0.092$, t = 0.682, P = 0.510, CA3, $F_{(1,11)} = 2.438$, t = 0.027, P = 0.979, PSD-95: PFC, $F_{(1,11)} = 0.053$, t = −0.229, P = 0.824, DG, $F_{(1,11)} = 6.863$, t = −0.014, P = 0.989, CA1, $F_{(1,11)} = 0.103$, t = 1.615, P = 0.137, CA3, $F_{(1,11)} = 0.101$, t = −0.740, P = 0.476] (Fig. 2B–F). These data showed that increased BDNF-TrkB signaling and synaptogenesis in the NAc after repeated WIN55,212-2 exposure during adolescence play a role in the marked increases of METH-induced locomotion in adulthood.

3.3. Effects of ANA-12 on METH-induced hyperlocomotion in adulthood after WIN55,212-2 exposure during adolescence

Previously, we reported that repeated administration of TrkB antagonist ANA-12 could prevent METH-induced withdrawal symptoms, such as depression and behavioral sensitization (Ren et al., 2015). Therefore, we examined whether repeated administration of ANA-12 could affect METH-induced hyperlocomotion in adult mice after repeated exposure of WIN55,212-2 during adolescence. Vehicle (10 ml/kg/day for 11 days from P35 to P45) or WIN55,212-2 (2 mg/kg/day for 11 days from P35 to P45) was administered i.p. into mice. Subsequently, vehicle (10 ml/kg/day for 14 days, from P70 to P83) or ANA-12 (0.5 mg/kg/day for 14 days, from P70 to P83) was administered into mice. Behavioral tests after a single administration of METH were performed 2–5 days (P85 – P88) after the end of the treatment (Fig. 3A). Two-way ANOVA revealed statistical differences among the four groups [WIN: $F_{(1,35)} = 13.987$, P = 0.001, ANA-12: $F_{(1,35)} = 8.744$, P = 0.006, interaction (WIN × ANA-12), $F_{(1,35)} = 16.143$, $P < 0.001$] (Fig. 3B). The time course of METH-induced locomotion was shown in the Fig. S2. Repeated administration of ANA-12 significantly attenuated METH-induced increase of locomotion in WIN55,212-2-treated mice compared with vehicle-treated mice (Fig. 3B).

3.4. Effects of ANA-12 on BDNF-TrkB signaling and synaptogenesis in the NAc

Since BDNF-TrkB signaling pathways in the NAc play a key role in the increase of METH-induced locomotion after WIN55,212-2 exposure during adolescence, we measured the levels of BDNF, proBDNF, TrkB, p-TrkB, GluA1 and PSD-95 in the NAc. Two-way ANOVA revealed statistical differences among the four groups [BDNF: WIN, $F_{(1,23)} = 28.266$, $P < 0.001$, ANA-12, $F_{(1,23)} = 0.345$, P = 0.563, interaction (WIN × ANA-12), $F_{(1,23)} = 0.329$, P = 0.573, proBDNF: WIN, $F_{(1,23)} = 31.723$, $P < 0.001$, ANA-12, $F_{(1,23)} = 1.578$, P = 0.223, interaction (WIN × ANA-12), $F_{(1,23)} = 0.002$, P = 0.967] (Fig. 4B and C).

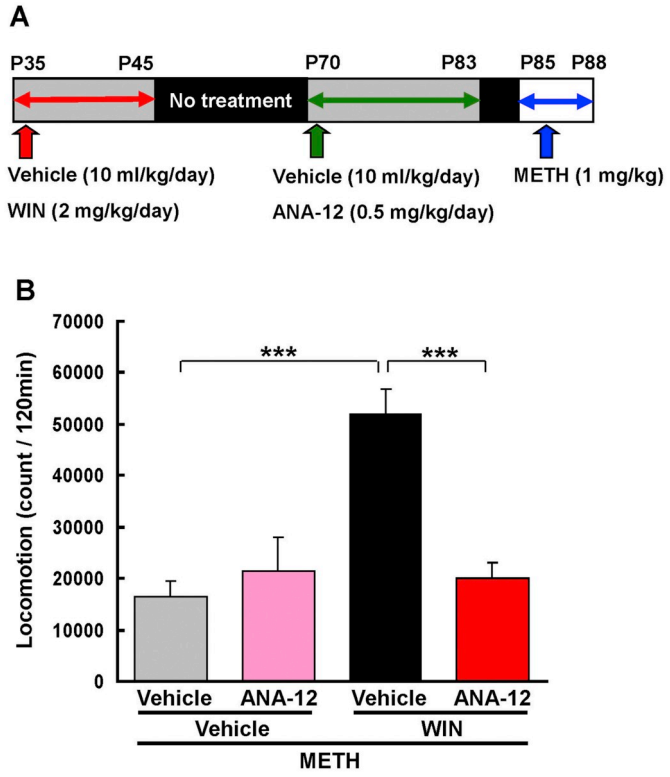

Fig. 3. Effects of ANA-12 on an increase of METH-induced locomotion after repeated WIN55,212-2 exposure during adolescence.
A: Schedule of treatment and behavioral tests. Vehicle (10 ml/kg/day for 11 days) or WIN55,212-2 (2 mg/kg/day for 11 days) was injected i.p. into mice from P35 to P45. Subsequently, vehicle (10 ml/kg/day for 14 days) or ANA-12 (0.5 mg/kg/day for 14 days) was injected i.p. into mice from P70 to P83. Behavioral tests after a single administration of METH (1 mg/kg, s.c.) were performed on P85-P88. B: Repeated administration of ANA-12 significantly attenuated the increased of METH-induced locomotion after WIN55,212-2 exposure during adolescence. There was no change between the vehicle-treated mice and ANA-12-treated mice after a single administration of saline. Each value is the mean ± SEM (n = 9 per group). ***P < 0.001 as compared with control (vehicle-treated) group. METH: methamphetamine, WIN: WIN55,212-2.

Repeated administration of ANA-12 did not affect alterations in the BDNF and proBDNF in the NAc from WIN55,212-2-treated mice.

Two-way ANOVA revealed statistical differences among the four groups [p-TrkB/TrkB: WIN, $F_{(1,23)} = 5.619$, $P = 0.028$, ANA-12, $F_{(1,23)} = 3.873$, $P = 0.063$, interaction (WIN ×ANA-12), $F_{(1,23)} = 7.756$, $P = 0.011$, GluA1: WIN, $F_{(1,23)} = 19.423$, $P < 0.001$, ANA-12, $F_{(1,23)} = 5.404$, $P = 0.031$, interaction (WIN ×ANA-12), $F_{(1,23)} = 18.423$, $P < 0.001$, PSD-95: WIN, $F_{(1,23)} = 8.572$, $P = 0.008$, ANA-12, $F_{(1,23)} = 2.618$, $P = 0.121$, interaction (WIN ×ANA-12), $F_{(1,23)} = 3.435$, $P = 0.079$] (Fig. 4D–F). These data suggest that repeated administration of ANA-12 could attenuate marked increases in the p-TrkB/TrkB and GluA1 in the NAc from WIN55,212–2-treated mice.

3.5. Effects of repeated WIN55,212-2 exposure during adulthood on METH-induced hyperlocomotion

Finally, we examined whether repeated exposure of WIN55,212-2 (2 mg/kg/day for 11 days from P70 to P80) during adulthood can affect METH (1 mg/kg)-induced hyperlocomotion in mice. METH (1 mg/kg) was injected into mice from P110 to P113 (Fig. 5A). METH-induced locomotor activity of the WIN55,212-2-treated mice was significantly higher than that of vehicle-treated mice. There was no change between the vehicle-treated mice and WIN55,212-2-treated mice after a single administration of saline. Two-way ANOVA revealed statistical differences among the four groups [WIN: $F_{(1,39)} = 6.116$, $P = 0.018$, METH: $F_{(1,39)} = 23.904$, $P < 0.001$, interaction (WIN × METH), $F_{(1,39)} = 5.194$, $P = 0.029$] (Fig. 5B). The time course of METH-induced locomotion was shown in the Fig. S3. These data suggest that repeated administration of WIN55,212-2 during adulthood can increase risk for psychosis in adulthood.

4. Discussion

The major findings of the present study are as follows: First, increased BDNF-TrkB signaling and synaptogenesis in the NAc after repeated WIN55,212-2 exposure during adolescence plays a key role in the marked increases of METH-induced locomotion in adulthood. Second, marked increases of METH-induced locomotion after repeated WIN55,212-2 exposure during adolescence are attenuated after subsequent repeated administration of ANA-12. Furthermore, increased p-TrkB/TrkB ratio and increased synaptogenesis (GluA1 and PSD-95) in the NAc of WIN55,212-2-treated mice during adolescence are also attenuated after subsequent repeated administration of ANA-12. These findings are consistent with the previous report of METH withdrawal symptoms (Ren et al., 2015). Collectively, increased BDNF-TrkB signaling in the NAc plays a key role in the long-lasting behavioral abnormalities in adulthood after CB receptor stimulation during adolescence. Taken together, it is likely that TrkB antagonist would be potential prophylactic and therapeutic drugs for psychosis in adulthood after repeated use of cannabis during adolescence.

In this study, we found that repeated administration of WIN55,212-2 during adolescence caused a marked increase of BDNF protein and p-TrkB/TrkB ratio as well as synaptic proteins (GluA1 and PSD-95) in the NAc, resulting in the long-lasting increase of METH-induced locomotion in adulthood. It is reported that repeated METH administration during adulthood caused a marked increase of BDNF-TrkB signaling and synaptogenesis in the NAc, resulting in the long-lasting depression-like phenotype and behavioral sensitization after METH withdrawal (Ren et al., 2015). In addition, several studies suggest that BDNF-TrkB signaling in the NAc plays a causal role in the plasticity observed in other abused drugs, including cocaine and morphine (Lobo et al., 2010; Graham et al., 2009). Taken all together, it is likely that BDNF-TrkB signaling in the NAc plays a key role in the long-lasting behavioral abnormalities after repeated use of abused drugs, including cannabis. Importantly, these findings identify TrkB antagonists as promising prophylactic or therapeutic drugs for a number of behavioral abnormalities associated with abused drugs in humans.

Repeated exposure to WIN55,212-2 during adolescence results in a progressively enhanced and enduring behavioral response to METH, a phenomenon known as behavioral sensitization. A number of behavioral, neurochemical, biochemical, and molecular studies have shown that the initiation of this complex process involves the interaction of several neurotransmitters, neuropeptides, neurotrophic factors, and their associated receptor signaling pathways (Robinson and Becker, 1986; Pierce and Kalivas, 1997). Dopamine release and dopamine-induced behavioral abnormalities by METH have been reported to be significantly suppressed by pretreatment with intra-NAc injections of TrkB antibodies (Narita et al., 2003). Interestingly, a single bilateral infusion of ANA-12 into NAc showed rapid and long-lasting therapeutic effects in METH withdrawal symptoms in mice (Ren et al., 2015). In this study, we found that an increase of METH-induced locomotion after repeated WIN55,212-2 administration during adolescence is improved after subsequent repeated administration of ANA-12. These findings suggest that increased BDNF-TrkB signaling in the NAc plays a key role in WIN55,212-2-induced behavioral sensitization in rodents. Therefore, it is likely that blocking TrkB signaling in the NAc by a TrkB antagonist can attenuate an increase of METH-induced locomotion after repeated WIN55,212-2 administration during adolescence.

It is reported that repeated treatment of WIN55,212-2 during early

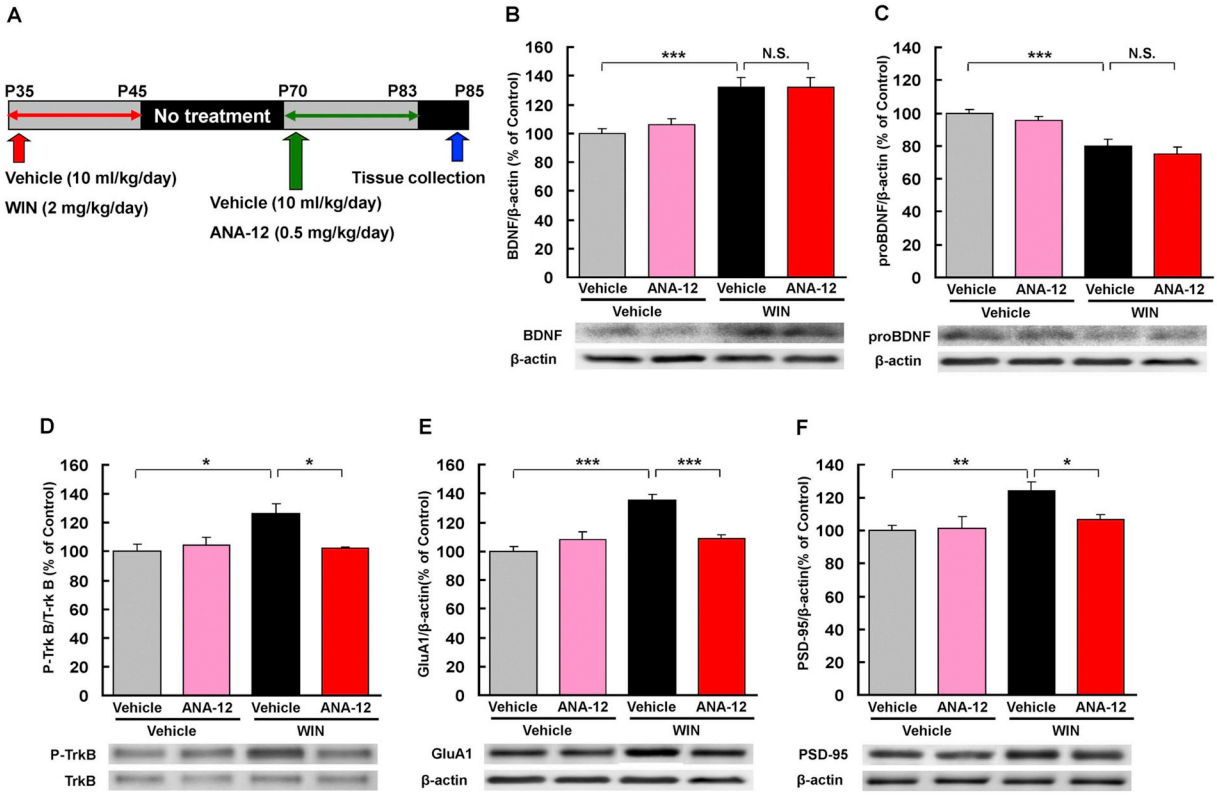

Fig. 4. Role of BDNF-TrkB signaling in the NAc on the effects of ANA-12 in the METH-induced hyperlocomotion after repeated exposure of WIN55,212-2 during adolescence.

A: Schedule of treatment and brain tissues collection. Vehicle (10 ml/kg/day for 11 days) or WIN55,212-2 (2 mg/kg/day for 11 days) was injected i.p. into mice from P35 to P45. Subsequently, vehicle (10 ml/kg/day for 14 days) or ANA-12 (0.5 mg/kg/day for 14 days) was injected i.p. into mice from P70 to P83. Sample correction for Western blot analysis was performed on P85. B: BDNF. Repeated administration of ANA-12 did not alter increased levels of BDNF protein in the NAc of WIN55,212-2-treated mice. C: proBDNF. Repeated administration of ANA-12 did not alter decreased levels of proBDNF protein in the NAc of WIN55,212-2-treated mice. D: p-TrkB/TrkB ratio. Repeated administration of ANA-12 significantly attenuated increased ratio of p-TrkB/TrkB in the NAc of WIN55,212-2-treated mice. E: GluA1. Repeated administration of ANA-12 significantly attenuated increased levels of GluA1 in the NAc of WIN55,212-2-treated mice. F: PSD-95. Repeated administration of ANA-12 attenuated increased levels of PSD-95 in the NAc of WIN55,212-2-treated mice. Each value is the mean ± S.E.M. (n = 6 per group). *P < 0.05, **P < 0.01, ***P < 0.001 as compared with vehicle-treated group. N.S.: not significant. WIN: WIN55,212-2.

(P35 – P40) and mid- (P40 – P45) adolescence can trigger an enduring state of frequency-dependent prefrontal disinhibition in adulthood (Cass et al., 2014). This study suggests that early and mid-adolescence constitutes a critical period during which repeated CB receptor stimulation is sufficient to elicit an enduring state of PFC disinhibition resulting from developmental impairment of local prefrontal GABAergic transmission (Cass et al., 2014). Unfortunately, they did not examine the region NAc. In this study, we did not find any change of BDNF-TrkB signaling and synaptic proteins in the PFC after repeated WIN55,212-2 exposure during adolescence. Further detailed study in the both regions is needed. Finally, this paper has limitation. In this study, we used non-selective CB receptor agonist WIN55,212-2, but not cannabis. Therefore, further study using cannabis (or marijuana) is needed.

In addition, we also found that repeated administration of WIN55,212-2 during adulthood could increase METH-induced locomotion in adulthood, although WIN55,212-2 exposure during adulthood were less potent compared with adolescence (Figs. S1 and S3). It seems that increased BDNF-TrkB signaling in the NAc may play a role in METH-induced hyperlocomotion after WIN55,212-2 exposure during adulthood. Furthermore, this study suggests that the use of cannabis during adulthood could increase the risk for psychosis later in life. The data support the epidemiological data showing that repeated cannabis use is a risk factor for the onset of psychosis or schizophrenia (Andréasson et al., 1987; Arseneault et al., 2002; Henquet et al., 2005; Moore et al., 2007). Preclinical studies suggest that, relative to adults, adolescent rodents are more susceptible to the chronic treatment of CB1 agonists (Schneider and Koch, 2003; Raver et al., 2013; Renard et al., 2013), although little is known about the molecular mechanisms underlying the age-dependent and long-lasting behavioral effects of chronic exposure of CB1 agonists. A recent cross-sectional study demonstrated that marijuana use is increasing among middle-aged (age 50–64) and older adults (> 65) in the USA (Han and Palamar, 2018). Collectively, it is likely that the use of cannabis may increase the risk for psychosis in all ages although the further study is needed.

In conclusion, the present study demonstrated that exposure of WIN55,212-2 during adolescence could increase the risk for psychosis in adulthood, and that increased BDNF-TrkB signaling in the NAc plays a key role in the long-lasting behavioral changes after the repeated exposure to WIN55,212-2 during adolescence. Therefore, TrkB antagonist would be potential prophylactic or therapeutic drugs for psychosis in young adult after repeated use of cannabis during adolescence.

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Conflicts of interests

Dr. Kenji Hashimoto is an inventor on a field patent application on “The use of TrkB antagonists in the treatment of substance abuse” by Chiba University. Dr. Ohgi, and Dr. Futamura are employee of Otsuka

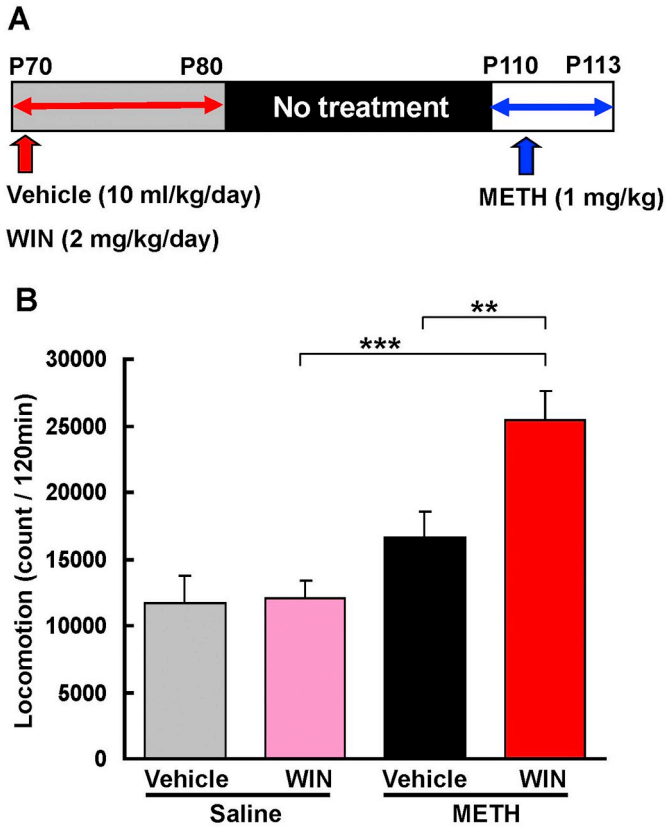

Fig. 5. Effect of repeated WIN55,212-2 exposure during adult on METH-induced hyperlocomotion.
A: Schedule of treatment and behavioral tests. Vehicle (10 ml/kg/day for 11 days) or WIN55,212-2 (2 mg/kg/day for 11 days) was injected i.p. into mice from P70 to P80. Behavioral tests after a single administration of METH (1 mg/kg, s.c.) were performed on P110-P113. B: The locomotor activity of the WIN55,212-2-treated mice was significantly higher than that of vehicle-treated mice after a single administration of METH (1 mg/kg). There was no change between the vehicle-treated mice and WIN55,212-2-treated mice after a single administration of saline. Each value is the mean ± S.E.M. (n = 10 per group). **P < 0.01, ***P < 0.001 as compared with control (saline-treated) group. METH: methamphetamine, WIN: WIN55,212-2.

Pharmaceutical Co., Ltd. (Tokyo, Japan). The other authors declare no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.pbb.2019.01.002.

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