Basic and Clinical
NEUROSCIENCE
September & October 2022 Volume 13, Number 5

Research Paper

Cannabidiol Modulating the Expression of Neurotrophin Signaling Pathways in Chronic Exposure to Methamphetamine in Rats During Abstinence Period

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Citation Razavi, Y., Najafi, M., Haghparast, A., Keyhanfar, F., Shabani, R, and Mehdizadeh, M. (2022). Cannabidiol Modulating the Expression of Neurotrophin Signaling Pathways in Chronic Exposure to Methamphetamine in Rats During Abstinence Period. *Basic and Clinical Neuroscience, 13*(5), 719-730. http://dx.doi.org/10.32598/bcn.2021.3059.1

http://dx.doi.org/10.32598/bcn.2021.3059.1

Article info:
Received: 28 Nov 2020
First Revision: 29 May 2021
Accepted: 09 Jun 2021
Available Online: 01 Sep 2022

Keywords:
Methamphetamine, Cannabidiol, Neurotrophin signaling pathway, Chronic exposure, Abstinence, Hippocampus

ABSTRACT

Introduction: Several neuropsychiatric disorders, such as addiction, have indicated variations in the levels of neurotrophic factors. As an extremely addictive stimulant, methamphetamine (METH) is associated with rising levels of abuse worldwide. We have recently demonstrated that repeated intracerebroventricular (ICV) of cannabidiol (CBD), the most important non-psychotomimetic compound, can lead to diminished impairing memory and hippocampal damage caused by chronic exposure to METH (CEM) in rats over the abstinence period. Furthermore, the results indicated a possible contribution of the neurotrophin signaling pathway (NSP) in regulating neurogenesis and survival. This study intends to evaluate whether these effects remained as measured in molecular pathways after the abstinence period.

Methods: The animals were given 2mg/kg METH twice a day for 10 days. Then, we adopted real-time polymerase chain reaction (PCR) throughout the 10-day abstinence period to assess the CBD's effect (10 and 50µg/5µL) on the levels of the mRNA expression of the NSP.

Results: The findings suggested that CEM, when compared to the control group in the hippocampus, downregulated mRNA expression of NSP. Moreover, a dosage of 50 µg/5µL CBD may possibly enhance the mRNA expression level of BDNF/TrkB and NGF/TrkA in the hippocampus. Besides, the expression of RAF-1 mRNA level could be reversed significantly by both doses of CBD.

Conclusion: According to our results, CBD may partly bring about neuroprotective effects by modulating the NSP. These findings set forth solid evidence demonstrating that CBD is a protective factor attributed to neuropsychiatric disorders, such as METH addiction.

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Highlights

- CEM decreased mRNA expression of NSP in the hippocampus.
- High-dosage CBD can increase the mRNA expression of BDNF/TrkB during abstinence.
- High-dosage CBD can increase the mRNA expression of NGF/TrkA during abstinence.
- Both dosages of CBD could boost the mRNA expression level of RAF1.

Plain Language Summary

Methamphetamine (METH) is an extremely addictive stimulant and is associated with increasing rates of abuse worldwide. This results suggest that CBD plays a role as a protective factor in preventing the development of a plastic transformation that triggers compulsive use of METH by modulating NSP.

1. Introduction

Methamphetamine (METH) is an extremely addictive stimulant causing serious health conditions, such as cognitive impairment, psychotic symptoms, and brain damage (Colfax et al., 2010). Despite the large population of METH users, no Food And Drug Administration (FDA)-approved pharmacotherapy is currently available for the treatment of METH abuse (Le Foll, 2016). Hippocampus-dependent cognitive impairments in METH-abusing patients can be associated with METH-induced transformations in the structural/functional plasticity of hippocampal neurons (Daumann et al., 2011; Morales et al., 2012; Nakama et al., 2011). Preclinical models of METH exposure have indicated behavioral deficits in METH addicts. For instance, these experimental schemes generate cognitive dysfunction and hippocampus-dependent memory impairments (Razavi et al., 2020). Moreover, the latest studies on Chronic Exposure to Methamphetamine (CEM) in adult rats revealed that METH can decrease dentate gyrus neurogenesis (Razavi et al., 2020). Withdrawal from METH administration can lead to compensatory changes in dentate gyrus neurogenesis and the survival rate of newly born neurons created after the withdrawal period will be strengthened. There is a hypothesis suggesting that such transformations in the hippocampus during withdrawal may regulate relapse to METH consumption (Razavi et al., 2020).

During the treatment of drug abuse, effective pharmacological subjects are expected to diminish drug cravings. As a main non-psychotomimetic phytochemical, cannabidiol (CBD) can be found in the Cannabis sativa plant (Viudez-Martínez et al., 2018). Over the last decade, CBD has demonstrated a wide range of possible medicinal qualities in animal and human subjects, such as anxiolytic (Campos et al., 2012), antidepressant, neuroprotective and anti-inflammatory effects (Fernández-Ruiz et al., 2013). Despite such an impressive therapeutic profile in preclinical studies, there is little information about the molecular processes engaged in behavioral effects induced by CBD. According to the evidence, CBD can operate via various mechanisms capable of intensifying different behavioral effects (Campos et al., 2012). The latest findings suggest that CBD, administered whether acutely or repeatedly, can give rise to plastic transformations. For instance, CBD reduces the decrease in hippocampal neurogenesis. According to our recent report, CBD protects against memory impairments and hippocampal cell loss in rats undergoing CEM during the abstinence period (Razavi et al., 2020).

Neurotrophins are a family of growth factors involved in plasticity and neuronal survival (Blum & Konnerth, 2005), which protect neurons in the face of neurotoxic insults (Tabakman et al., 2004). The first neurotrophin, i.e. nerve growth factor (NGF), was unveiled during research on survival factors and adult neurogenesis regulation. It has been proven that in rat's dentate gyrus, NGF enhances cellular proliferation (Birch & Kelly, 2013) and immature neuron survival (Frielingsdorf et al., 2007). The second neurotrophin, i.e., Brain-Derived Neurotrophic Factor (BDNF), is a major regulator of neuronal survival, differentiation, and synaptic plasticity. Furthermore, for drug development in neuropsychiatric disorders, BDNF acts as a main molecular target. There is some specificity in neurotrophin for their interactions with the members of

this receptor family, where NGF activates tyrosine kinases receptors (TrkA), and BDNF activates TrkB (Rui, Herrington, & Carter-Su, 1999). Several TrkA-binding proteins, in addition to their downstream effector proteins, play a role in regulating survival and neuronal differentiation. SH2-B was detected as a binding receptor protein for NGF (TrkA) (Rui et al., 1999), which also contributes to neuronal survival. NGF stimulates the association of SH2-B with TrkA and the SH2 domain of SH2-B (Wang et al., 2004). Additionally, RAF-1 (proto-oncogene, serine/threonine kinase) activation of the MEK–ERK pathway has been attributed to apoptosis inhibition, bringing about cellular survival (Erhardt et al., 1999).

Boosted levels of BDNF in the dentate gyrus are positively correlated with a higher number of recently born neurons in the dentate gyrus under non-pathological conditions. Furthermore, the administration of METH is followed by the reduced expression of BDNF in the animal's hippocampus. The findings suggest that such transformations in the neurotrophic factors can be associated with cognitive deficits in METH-administered animals (Angelucci et al., 2007). The advantageous impacts of CBD on brain disorders have been attributed to the BDNF expression and interference with intracellular pathways (Campos et al., 2012; Fernández-Ruiz et al., 2013). Besides, CBD intensified the levels of BDNF in rat's hippocampus under an amphetamine-induced oxidative stress scheme, recommended to investigate mania (Valvassori et al., 2011). In the present study, we determine whether neurobiological alterations of CBD might change neurotrophin signaling pathway (NSP) expression in CEM rats after the abstinence period.

2. Materials and Methods

Study animals

This research involved a total of 62 mature male Wistar rats (220-280 g), each group containing 6 rats. The subjects were bought from the Pasteur Institute (Tehran, Iran). The animals were randomly held in groups of 3 in each cage under managed temperature and relative humidity (temperature 21°C±2°C; humidity 55%-60%). Access was provided to chow and faucet water in a 24 h light/dark cycle. Before stereotaxic surgery, the rats were acclimatized for 7 days. The entire experiments were carried out in line with the Guide for Care and Use of Laboratory Animals (National Institute of Health Publication No. 80-23, Revision 1996). Moreover, the Iran University of Medical Sciences (Research and Ethics Committee), Tehran, Iran (IR.IUMS.REC.1395.27882) approved the study procedures.

Study drugs

Laboratory of Medicinal Chemistry, School of Pharmacy, Baghiyatallah University of Medical Sciences (Tehran, Iran) synthesized and analyzed the METH hydrochloride. The dilution of fresh METH was done in normal saline, and then it was administered subcutaneously (SC) as much as 2 mg/kg. Subsequently, the dissolution of CBD (Tocris Bioscience, Missouri, USA) was done in a mixture containing dimethyl sulfoxide (DMSO) 10% and phosphate-buffer saline solution (PBS) 90%. Next, a 5-µL Hamilton syringe was used to inject the mixture intracerebroventricularly (ICV) into the lateral cerebral ventricle (10 and 50µg/5µL) in the subjects (Karimi-Haghighi & Haghparast, 2018; Razavi et al., 2020).

Methamphetamine administration procedure

Before every administration, we dissolved fresh METH in a 0.9% saline solution. The study animals (n=62) were repeatedly injected with the same dose (2mg/kg; SC, twice every day, for 10 days continually). Similarly, 1mL/kg SC injections of saline were done for the control group twice a day, for 10 days. Injection timing was 09:00 and 15:00. However, the dose of METH that was applied in this scheme was determined based on previous experiments, where the dosage was found to cause significant changes in the locomotor activity and behavioral tests (Razavi et al., 2020).

Procedures of microinjection and surgery

The rats were put inside a stereotaxic device (Stoelting, USA) and were then numbed with 10 mg/kg xylazine and 100 mg/kg ketamine. Next, the cannula was placed in the lateral ventricle of the cerebrum of the brain. According to the rat brain atlas (Paxinos and Watson, 2007), we specified the implant zone as 4.2 mm deep from the dura, 1.6 mm lateral, and 0.5 mm posterior to the bregma. Using small screws and dental acrylic, the cannula was fixed. Once the surgical process was completed, the mice were isolated and left to get well from the surgical process for 7 days before the experiments. The unit of injection comprised a polyethylene tube (PE-20) linked to a 5-µL Hamilton syringe plus a 30-gauge needle of the 11-mm tip. The medicines were injected into the brain cerebrum lateral ventricle completed in a 2-min interval. The needle of infusion was held in the cannula for 1 min for the drug to distribute thoroughly from the tip and ultimately avoid drug backflow. In the end, the obturator was inserted back into the guide cannula.

Experimental design

We initially examined the repetitive CBD administration effects in the NSP expression in the CEM mice over the abstinence period (Figure 1). This scheme involved the experimental groups (5 groups) injected with METH for 10 days (i.e., phase of chronic exposure). During the initial 10 days of the chronic exposure phase, one group was given METH only. Four other groups of the subjects experienced the abstinence period in the following procedure: 2 groups separately received 10 or 50 µg/ 5 µL of CBD for the second 10-day period; the vehicle control group and one sham group took DMSO only in place of CBD as a vehicle for the period of the abstinence protocol. The other 2 control groups were given merely saline as METH solvent and none (i.e., naïve) for the duration of the induction and abstinence phases.

cDNA Synthesis, RNA extraction, and real-time polymerase chain reaction

On the 20th day of the experiments, 6 mice from each group were sacrificed for the gene transcription analysis. Given the materials and criteria, the entire RNA was cleansed from the hippocampus tissue via homogenization in 1 mL of TRIzol (Yektatajhiz Azma, Tehran, Iran #YT9063). The purity and concentration of samples with extracted RNA were all analyzed spectrophotometrically through NanoDrop 2000 (Thermo Scientific, USA). Subsequently, the RNA samples went under reverse transcription into cDNA via the Prime Script RT reagent Kit (Takara, Shiga, Japan #RR037A). Then, we evaluated each gene's relative mRNA expression through a real-time polymerase chain reaction (real-time PCR). Using the ABI System (USA), this was completed with SYBR Green Real-Time PCR Master Mix (Takara, Shiga, Japan) reagents. AlleleID v. 7.5 (PREMIER Biosoft, Palo Alto, CA) was employed to design the primer sequences (Table 1). We conducted PCR in a final volume of 20 µL, which was subsequently warmed to 95 °C for a 10-min interval before heating it by 95°C for 30 s in 40 cycles, 62°C for 30 s, and 72°C for 30 s. We normalized all genes to the β-actin level (internal control) and analyzed the qPCR data through the Ct method (Karimi-Haghighi et al., 2020).

Study statistics

The data were entirely represented as mean ± standard error of the mean. Furthermore, the data were processed through GraphPad Prism 6.0, a commercially available software. We examined the normal distribution through the Kolmogorov-Smirnov normality test. Moreover, we carried out the 1-Way Analysis Of Variance (ANOVA) for comparing the groups in terms of statistically significant levels. In the next stage, the Tukey test was conducted for comparing multiple groups against one another. The P values less than 0.05 were considered statistically significant ($P<0.05$).

Results

The ICV injection of 10 and 50 µg/ 5 µL CBD prevented impairing the memory recognition following CEM in rats for the period of abstinence (Razavi et al., 2020). We intended to identify whether the expression of NSP in the hippocampus is affected by CBD.

The effect of cannabidiol on BDNF mRNA expression in the hippocampus during abstinence period in chronic exposure to methamphetamine in mice

To clarify the CBD's effect at high and low dosages (10 and 50 µg / 5 µL) on changes in the expression of BDNF in the hippocampus of CEM animals, CBD was administered ICV in the CEM group for the period of abstinence. As illustrated in Figure 2A, the 1-way ANOVA test, and the Tukey post hoc analysis ($F_{6, 7}=75.21$, $P<0.001$) revealed that BDNF expression levels significantly decreased in the chronic METH-treatment group (black bar) compared to the saline and control groups ($P<0.001$). mRNA expression of BDNF was risen in the sham group compared to the METH-treatment animals ($P<0.001$). In addition, the 1-way ANOVA test and the Tukey post hoc test established that the BDNF expression level in rats that received 50 µg / 5 µL CBD for the period of abstinence was significantly reversed ($P=0.003$). Nevertheless, in the case of the low dose of CBD (10 µg / 5 µL) compared to the CEM control group, this effect was not significant. In addition, differences between the two doses of CBD ($P<0.05$ and $P<0.01$, respectively) were significant compared to the control group (DMSO). These results show that CBD will possibly inverse BDNF expression level for the phase of abstinence.

Cannabidiol effect on TrkB mRNA expression in the hippocampus for the period of abstinence in chronic exposure to methamphetamine in rats

We investigated the role of CBD in the METH effect on TrkB mRNA expression. As displayed in Figure 2B right panel, the 1-way ANOVA test and the Tukey comparison test ($F_{6,7}=13.65$, $P=0.015$) demonstrated a significant decrease in the TrkB expression level in the CEM group

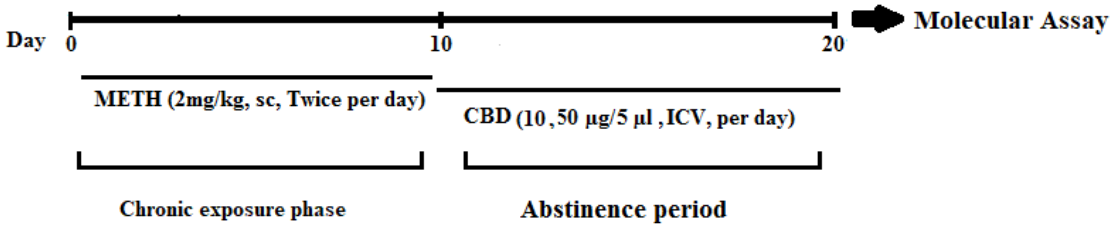

Figure 1. Schematic illustration of the experimental protocol

Rats were treated with methamphetamine (2mg/kg twice a day) or its vehicle (saline) for 10 days. that, the effects of intracerebroventricular administration of cannabidiol (10, 50 µg/5 µL) or vehicle (dimethyl sulfoxide) during the 10–20-day time of abstinence. After the accomplishment of the experiment, the rats were sacrificed and their hippocampus tissues were removed for molecular assay.

(black bar) compared to the control and saline groups. Then again, the 1-way ANOVA test and the Tukey comparison test revealed that only one dose of 50µg/5µL of CBD could increase the TrkB expression level induced by METH in the phase of chronic exposure and lower the dose of CBD (10µg/5µL) as the phase of the abstinence that did not show any significant increase in the expression level of TrkB compared to the CEM group. However, there was a significant difference between the expression level in the sham group compared to the CEM group ($P<0.05$).

Cannabidiol effects on NGF mRNA expression in the hippocampus during abstinence period in chronic exposure to methamphetamine in rats

As shown in Figure 2C, the 1-way ANOVA test and the Tukey post hoc test ($F_{6,7}=13.33$, $P=0.016$) demonstrated a significant decrease in the NGF mRNA expression level between the CEM group compared to the saline group. Moreover, the 1-way ANOVA test and the Tukey multiple comparison tests revealed a significant reduction in the NGF expression level in the CEM group compared to the high dose of CBD-treated groups ($P<0.01$). Additionally, there were significant differences between the high dose of CBD ($P<0.05$) compared to the sham group. However, no significant differences existed between the two doses of CBD compared to the control group (DMSO). These data suggest that CBD with a high dose (50µg/5µL) could reverse NGF expression levels during the abstinence phase.

Cannabidiol effects on TrkA mRNA expression in the hippocampus during abstinence period in chronic exposure to methamphetamine in rats

As shown in Figure 2D, the 1-way ANOVA test and the Tukey comparison test ($F_{6,7}=235.7$, $P<0.001$) for changes in the TrkA mRNA expression depicted that CEM group animals decreased TrkA expression compared to the control and saline groups. Considerably, the statistical analysis exposed a significant rise in the expression level of TrkA in both high dose (50µg/5µL, $P<0.001$) and low dose (10µg/5µL, $P<0.01$) of CBD following ICV administration during the abstinence period compared to the CEM group. While both dosages of CBD showed significant differences in the TrkA mRNA expression compared to the DMSO group, supplementary analyses showed that the greater doses of CBD were more efficient than the lesser doses ($P<0.001$). Furthermore, remarkable differences existed in the TrkA mRNA expression between the sham and the CBD (10µg/5µL, $P=0.066$; 50µg/5µL, $P=0.004$) treated groups.

Cannabidiol effects on RAF1 mRNA expression in the hippocampus during abstinence period in chronic exposure to methamphetamine in rats

One-way ANOVA and the Tukey post hoc test ($F_{6,7}=69.52$, $P<0.001$) demonstrated a significant difference in the RAF1 mRNA expression between the CEM and control groups (Figure 2E, $P<0.001$). Additionally, the 1-way ANOVA test and the Tukey comparison test showed a significant decrease in the RAF1 mRNA expression in the CEM group compared to the group treated with both doses of CBD. Statistical analyses discovered that low dosages of CBD (10µg/5µL) could improve the expression level of RAF1 microinjected during the 10-day abstinence period in the CEM animals ($P<0.05$, Figure 2E). Besides, the high dose of CBD (50µg/5µL) affects/reverses the RAF1 mRNA expression in CEM rats ($P<0.001$). Considerably, the statistical analyses showed that both low and high doses of CBD ($P<0.05$ and $P<0.001$, respectively) had significant differences during the 10-day abstinence period in CEM animals compared to the control group (DMSO). Moreover, the

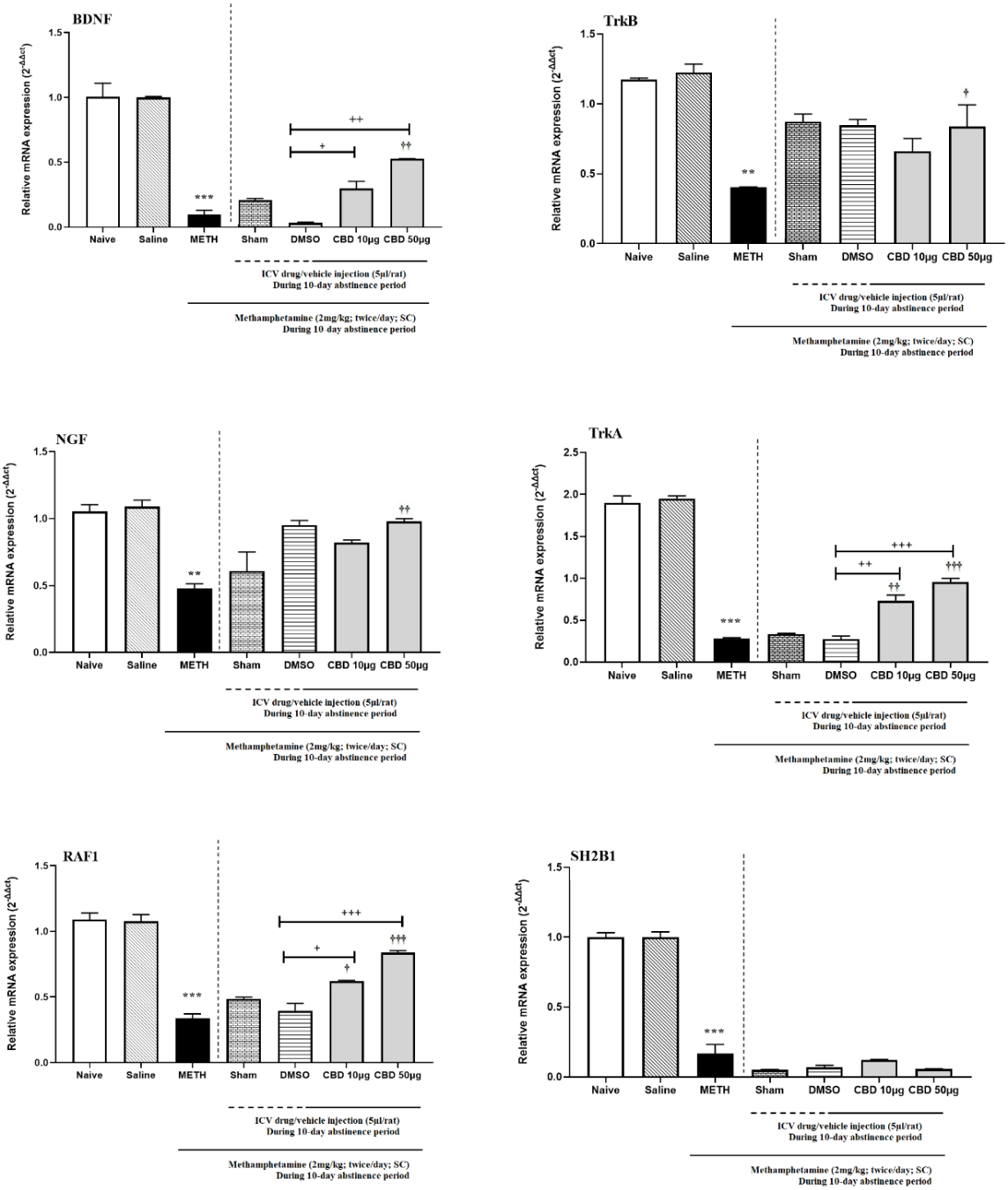

Figure 2. Effects of intracerebroventricular administration of cannabidiol on neurotrophic signaling pathway at mrna level during abstinence period

Quantitative polymerase chain reaction data analysis of A: BDNF, B: TrkB, C: NGF, D: TrkA, E: RAF1, and F: SH2B1 in the hippocampus of the control, saline, METH, Sham, DMSO, and CBD (10,50µg/kg) groups. The data are described as the Mean±SEM. n=6 rats and the differences between groups were established by the analysis of variance and the Tukey test.

** P<0.01 and *** P<0.001 different from the control group.

† P<0.05, †† P<0.01, and ††† P<0.001 different from the METH group.

+ P<0.05, ++ P<0.01, and +++ P<0.001 different from the DMSO group.

Table 1. Primer sequences (5′–3′) applied in real-time polymerase chain reaction

Gene	Forward	Reverse
BDNF	TTCTGTAATCGCCAAGGT	TGGTCATCACTCTTCTCAC
TrkB	CTTGACTTGTCTGACCTGAT	GTATTCTTGCTGCTCTCATTG
NGF	CATCGCTCTCCTTCACAG	TAGAACAACATGGACATTACG
TrkA	GAAGCCTAACCATCGTGAA	AAAGCATTGGAGGAGAGATT
RAF1	GTGAGGTGATGCTGTCTAC	TGAAGTTGCTCTGGAGTTG
SH2B1	TAGCCAGGATCTTCTTCT	AAGCAGTTCCATTGAGTC
β-actin	TCTATCCTGGCCTCACTGTC	AACGCAGCTCAGTAACAGTCC

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1-way ANOVA and the Tukey comparison test demonstrated no significant difference in the mRNA expression in the CEM group compared to the sham group.

Cannabidiol effects on SH2B1 mRNA expression in the hippocampus during abstinence period in chronic exposure to methamphetamine in rats

As shown in Figure 2F, the 1-way ANOVA test and the Tukey comparison test ($F_{6,7}$=193.6, P<0.001) for calculating changes in the SH2B mRNA expression in the hippocampus demonstrated that CEM animals (2mg/kg; SC) significantly decreased the SH2B expression level compared to the control and saline groups (P<0.001). Notably, the statistical analyses revealed no significant difference in SH2B mRNA expression in the CEM group compared to both CBD-treated groups.

4. Discussion

In this paper, we attempted to investigate the effects of ICV administration of CBD on the levels of NSP in the hippocampus in CEM for the 10 days of abstinence. The main findings can be summarized as follows: 1) CEM decreased mRNA expression of NSP in the hippocampus; 2) high-dosage CBD can increase the mRNA expression levels of BDNF/TrkB and NGF/TrkA in the hippocampus during abstinence; 3) both dosages of CBD (10, 50 µg/5 µL) could boost the mRNA expression level of RAF1.

Neurotrophic factors and their receptors are frequently expressed in the hippocampus (Masi & Brovedani, 2011). In recent studies, researchers have attempted to specify the molecular properties of the neural mechanisms contributing to the behavioral results of drug abuse in various zones of the neurotrophin pathway. It is well-accepted that neurotrophic factors are very involved in the survival and differentiation of neurons during the development controlled by neural plasticity among adults (Vilar & Mira, 2016). Nonetheless, no reports have been released on the possible changes of neurotrophic factors in the hippocampus by the CBD treatment following the behaviors of the CEM group.

Generated in the hippocampus, NGF is moved back to the basal forebrain and imposes a trophic action on cholinergic neurons. These crucial neurons must continue to function for memory processes (Fuji et al., 1993). Our behavioral investigation is consistent with reports from other authors discovering that METH can lead to hippocampus damage and memory performance impairment (Razavi et al., 2020; Thompson et al., 2004). According to these data, the way memory processes are affected by METH may be linked to mitigated trophic support of NGF. Furthermore, it has been demonstrated that cholinergic denervation and transection of the septohippocampal pathway (Balse et al., 1999) can give rise to the hyperactivity of the dopaminergic mesolimbic system and assist the locomotor activity. Therefore, the METH-induced hyperlocomotion might be assisted by curtailing NGF synthesis in the hippocampus in CEM rats. This can eventually impact the activity of cholinergic neurons (Campos et al., 2012).

BDNF is known as a trophic factor contributing to survival and differentiation of dopaminergic neurons and can affect the neurotransmission of dopamine and serotonin. As demonstrated by previous experiments on rats, amphetamine may lead to a failure of dopaminergic neurons. Moreover, these neurons can be protected from amphetamine neurotoxicity BDNF (Matsuzaki et al., 2004). These findings suggest that BDNF synthesis can be mitigated by amphetamine-induced neurotoxicity on

dopaminergic neurons. Furthermore, other studies have revealed that amphetamine decreased NGF levels in the hypothalamus, hippocampus, and occipital cortex, along with BDNF in the hypothalamus and occipital cortex in rat brains (Angelucci et al., 2007). Meanwhile, another research has mentioned a rise of BDNF mRNA in rats' brains in the aftermath of repeated amphetamine administration (Meredith et al., 2002). Even though that study involved a different dose of 5 mg/kg of amphetamine for 5 consecutive days, increased BDNF mRNA level could possibly reflect a compensatory mechanism for mitigating BDNF protein. In one more explanation, the distinctions found between levels and our consequences on BDNF are related to dosage and also time. In a similar report, there has heightened BDNF levels of plasma among human subjects following over a 30-month administration of amphetamine (Kim et al., 2005). Nonetheless, individuals engaged in that experiment were kept abstinent for 1 month or longer. Therefore, the elevated levels of BDNF could justifiably be a representative of a neuroadaptive response to the toxicity induced by amphetamine. In this paper, we discovered that METH in the hippocampus downregulates neurotrophic pathways. Other research papers have indicated transformations in BDNF and NGF following lithium therapy and antipsychotic treatments, suggesting that this area contributes to the functional mechanism of psychoactive medicines (Angelucci et al., 2004). Additionally, the expression style of neurotrophin receptors in the adult rats' subventricular zone, as pinpointed by RT-PCR (Galvão et al., 2008), reveals that the most abundant receptor is TrkB, which was mitigated by CEM rats in our study. Furthermore, the general criticality of BDNF/TrkB in adult hippocampal neurogenesis has been clearly documented. For instance, BDNF knockdown in the dentate gyrus mitigates neurogenesis through lentiviral-mediated RNAi (Taliaz et al., 2010) but increases that in response to BDNF injection exogenously. Normal proliferation and neurogenesis in the subgranular zone require TrkB. Therefore, it can be suggested that the reduction of neurotrophic factors may be very involved in decreasing the effects of neurogenesis during the abstinence period in CEM rats.

In this paper, we revealed the 10-day abstinence of METH has heightened the expression levels of the BDNF/TrkB, and NGF/TrkA mRNA in the rats treated with the METH. Consistent with our study, BDNF levels are higher in METH-addicted humans during early withdrawal (for 1 to 7 days) and lower during the first month of abstinence (Ren et al., 2016). In another human-participated study, Chen et al. discovered that serum BDNF levels were dramatically lower among METH users during early withdrawal (subjects were kept abstinent for 21 days) compared to healthy controls, which was consistent with our results at baseline. The BDNF mRNA expression in this paper was significantly lower than the control at baseline. Such discrepancies could be associated with a variety of factors, including the number of abstinence days and the sample size. Accordingly, it can be argued that BDNF may play an important role in METH withdrawal and may serve as a potential biomarker for METH withdrawal.

In this study, we intended to find the NSP changes in ICV administration of CBD-treated, METH-consuming rats. The suppression of hippocampal neurogenic proliferation seems to be a communal consequence of METH exposure. Studies have lately outlined the CBD's capacity to minimize the behavioral and histological reflections of maladaptive neuroplasticity underlying METH abuse. We have recently unveiled that CBD treatment can prevent memory impairment during abstinence while reinforcing neurogenesis levels in CEM rats (Razavi et al., 2020). In this paper, we found that CBD administration boosts neurotrophin factors, including NGF and BDNF as well as their receptors in the hippocampus during abstinence in rats. Consistent with our data, CBD could reverse the amphetamine-induced damage and escalate BDNF expression in an animal-based scheme of mania (El-Remessy et al., 2003). Most recently, Campos et al. (Campos et al., 2015) indicated the attribution of the neuroprotective effects of CBD in a murine scheme of cerebral malaria to its anti-inflammatory activities and capacities for up-regulating BDNF expression in the hippocampus. Following CBD treatment on CEM rats during abstinence, we can observe the upward trend of NGF in the hippocampus (Figure 2C). The NGF criticality in neuroplastic events (Levi-Montalcini et al., 1996) reflects the fact that the participation of this neurotrophin in the neuroprotective effects of CBD may not be assumed improbable.

Similar to the evaluation of adult hippocampal neurogenesis in CEM rats treated with CBD, during abstinence in previous research, we discovered the frequently observed mitigation in CBD-amplified hippocampal neural proliferation. A dramatic increase of Ki67/DCX staining was found in the dentate gyrus of CBD-treated, METH-consuming mice. It is reported in studies that CBD can enhance adult hippocampal neurogenesis possibly by boosting neurotransmission of CB1R in the hippocampus (Campos et al., 2013). According to this assumption, it is argued that CBD significantly raised the expression of CB1R in the hippocampus, a receptor adequately regulating adult

neurogenesis in this region (Prenderville et al., 2015). Although it is not possible to clarify how CBD caused such an increase, previous studies have noted comparable alterations in the expression of the CB1R following the administration of the CBD (Viudez-Martínez et al., 2018). Furthermore, other results demonstrated elevated ERK1/2 (MAPK) and CREB phosphorylation and enhanced BDNF expression in CBD-treated mice (Luján et al., 2018). In the meantime, the MAPK-CREB pathway phosphorylation is induced by the activation of CB1R (Mallipeddi et al., 2017). This subsequently assists gene expression influencing in cell proliferation (Ortega-Martínez, 2015) and BDNF activity. Our study showed that the administration of CBD intensified the expression of neurotrophic factors, such as BDNF and NGF in the hippocampus following CEM rats during abstinence. Consistent with this evidence, it can be proposed that CBD can enhance the expression of CB1R in the hippocampus resulting in upregulating the downstream MAPK/CREB pathway.

The previous data have demonstrated that activated RAF-1 can lead to the differentiation of the hippocampal neuronal cells. RAF-1 is a critical component of the different signaling pathways induced by the growth factor. In addition, RAF-1 kinase leads to the activation of the downstream RAF-1 mediated MAPK signaling pathway (Charntikov et al., 2015). Our data suggest a protective mechanism by which cell survival is promoted by RAF-1. In line with this notion, RAF-1 has shown that it can inhibit death signaling (Chen et al., 2001). According to this evidence and our results in this study, we can propose that mRNA expression of RAF-1 in the CEM animals is through the enhancement in the death signaling pathway in the hippocampus.

5. Conclusion

In conclusion, our findings demonstrate that CBD-strengthened neurotrophic factors may have a role in preventing the development of plastic transformation, inducing the compulsive METH intake. In any case, new investigations are required to identify the specific mechanisms involved in the CBD effects.

Ethical Considerations

Compliance with ethical guidelines

All experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals (National Institute of Health Publication No. 80-23, 1996 revision). In addition, the Research and Ethics Committee of Iran University of Medical Sciences, Tehran, Iran (IR.IUMS. REC.1395.27882) approved the study procedures.

Funding

This project was supported by the Vice-Chancellor for Research & Technology of Iran University of Medical Sciences (Grant No. 95011127882) and was funded by the Iran National Science Foundation (INSF).

Authors' contributions

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Conflict of interest

The authors declared no conflicts of interest

Acknowledgments

The authors would like to thank the Neuroscience Research Center, School of Medicine, Shahid Beheshti University of Medical Sciences.

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