Biochemical and Biophysical Research Communications 584 (2021) 46–52

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Long-term efavirenz exposure induced neuroinflammation and cognitive deficits in C57BL/6 mice

Runji Zhang [a, 1], Jian Bao [b, 1], Jialu Qiao [b], Wenshuang Li [b], Feng Qian [c], Kanghong Hu [a, **], Binlian Sun [b, *]

[a] Hubei Provincial Key Laboratory of Industrial Microbiology, School of Bioengineering and Food Science, Sino-German Biomedical Center, National 111 Center for Cellular Regulation and Molecular Pharmaceutics, Hubei University of Technology, Hubei Province, Wuhan, 430068, China
[b] Wuhan Institute of Biomedical Sciences, School of Medicine, Jianghan University, Wuhan, 430056, China
[c] Division of HIV/AIDS, The Second Affiliated Hospital of Soochow University, No. 1055 Sanxiang Road, Suzhou, 215004, China

ARTICLE INFO

Article history:
Received 2 September 2021
Accepted 3 November 2021
Available online 6 November 2021

Keywords:
Efavirenz (EFV)
Cognitive deficits
Spatial learning
Synaptic dysfunction
Pro-inflammatory cytokines

ABSTRACT

Efavirenz (EFV) is a non-nucleoside reverse transcriptase inhibitor (NNRTI), which is widely used for anti-HIV-1. Evidences revealed that several central nervous system side effects could be observed in mice and patients with administration of EFV. However, the detailed mechanisms are still unknown. In this study, we investigated the effects of long-term EFV treatment on cognitive functions and the potential underlying mechanisms in mice. We maintained C57BL/6 mice aged 2 months with treatment containing 40 or 80 mg/kg/day EFV for 5 months, while control group treated with saline. The cognitive functions were evaluated by novel object recognition test, Barnes maze test and Morris water maze. The results showed significant short-term memory impairment in 40 and 80 mg/kg groups, and notable spatial learning and memory impairments in 80 mg/kg group, without any spontaneous activity alteration. Moreover, EFV induced impairments in dendritic integrity and synaptic plasticity in hippocampus. Furthermore, Significant increases were observed in the expression levels of pro-IL-1β, a similar tendency of TNF-α and phosphorylation of p65 of the 80 mg/kg group compared with control group. These results imply that long-term EFV treatment causes synaptic dysfunction resulting in cognitive deficits, which might be induced by the enhanced pro-inflammatory cytokines IL-1β and TNF-α via activating NF-κB pathway.

1. Introduction

Efavirenz (EFV) is a non-nucleoside reverse transcriptase inhibitor (NNRTI), can cause misalignment of template-primer to the catalytic site, thereby preventing incoming dNTP from being incorporated [1,2]. EFV was recommended to be the first-line anti-HIV-1 drug in 2013. After several years of application, central nervous system (CNS) side effects were frequently reported. However, because of the high antiviral effect, low pill burden and a relatively stable plasma concentration [3], there are still millions of patients receiving EFV, especially in the developing countries [4]. Hence, it is becoming a nonnegligible clinical concern to investigate the potential side effect of EFV to CNS [3,5–7].

Although some side-effects would gradually disappear in a month, most of the patients who receive EFV treatment, still suffer mild and chronic EFV-related CNS side-effects [4,8]. It was reported that over 50% of the EFV-treated patients suffer from neuropsychiatric side effects, including vivid dreams, dizziness, insomnia, decreased concentration, anxiety and depression [8,9].

It was also reported that EFV is somehow relevant to poorest cognitive performances in some cohort studies, but in some other trials, EFV is unrelated [10–15]. Besides, research revealed that EFV could cause higher prevalence of neurocognitive disorders among asymptomatic patients, suggesting potential neurotoxicity and causing cognitive deficits [11].

The number of newly reported HIV-1 infected individuals in adolescents and young adults are rising [16,17], but it is not well known whether the long-term EFV treatment to the young adult has CNS side effects.

Pro-inflammatory cytokines might be one reason of

* Corresponding author.
** Corresponding author.
E-mail addresses: hukh@hbut.edu.cn (K. Hu), binlian17@jhun.edu.cn (B. Sun).
[1] These authors contribute equally to the present study.

https://doi.org/10.1016/j.bbrc.2021.11.015
0006-291X/

neurodegeneration [18], the activation of NF-κB pathway result in the expression of pro-inflammatory cytokines [19,20]. In this study, we tried to investigate that the behavioral changes and potential mechanisms of 5-month-EFV treated mice, equal to around 10 years of human [21]. We detected biochemically the indicators of cognition function and studied the potential underlying mechanism. The results will provide a reference of EFV for young HIV-infected individuals.

2. Materials and methods

2.1. Animals

Two-month-old male C57BL/6 mice were randomly divided into 3 groups, 20 mice for each: (a) vehicle control group; (b) low-dose group, received 40 mg/kg/day of EFV; (c) clinical-dose group, received 80 mg/kg/day of EFV (equal to 600 mg per day for adult human). Each animal were sacrificed at 7-month-old after finishing behavioral tests. Mice were housed and maintained in Medicine Animal Center of Jianghan University and all experiments were approved by the Medical Ethics Committee of Jianghan University.

2.2. EFV treatment

The gavage volume is 0.2 mL for each mouse, the concentration of EFV is 0, 40 or 80 mg/kg/day. The treatment started when mice were 2-month-old and ended up at 7-month-old.

2.3. Novel object recognition test

This test was performed in an open box (50 × 50 × 40 cm XR-XZ301, Xinruan, China). Two identical objects were placed. Each animal was allowed to explore the box for 10 min at day 1. Replace one object into a different object, then repeat the procedure at day 2. Discrimination index was calculated as time exploring at novel-object quadrant minus time in familiar-object quadrant, then divide total time spent in two quadrants. The whole protocol was based on Lueptow [22].

2.4. Barnes maze test

The Barnes maze contains a circular platform which is 91 cm in diameter. 20 circular holes around the perimeter of the platform. A light source was placed over the center of the platform. Name each hole after 1–20, under the hole1 was a box. Then place 3 different plastic cards above the platform. The movements of animals were registered automatically (XR-XB108, Xinruan, China) and stored on a computer.

Day 1 was for adaptation, put each animal on the center of the platform, let them to explore, limited with 60 s. If the animal successfully entered the box, then time 90 s; if the animal failed to enter the box, then guide the animal enter the box, then time 90 s. Two trails per day, trails were at least 30 min apart.

Day 2-day 5 was for acquisition. Change the explore time into 180 s, and the in-box time was 30 s. Two trails per day, trails were at least 30 min apart.

Day 6 was for probe test. Allow animals explore for 60 s without the box, record the first time each animal successfully find the hole1 area; if failed, record the time as 60 s. The whole protocol was based Pitts [23].

2.5. Morris water maze

The Morris water maze (MWM) contains a circular pool, surrounded by decorations for abundant spatial cues. Divide the pool into 4 quadrants. The platform was submerged underneath the surface of the 23 °C water. The movements of animals were registered automatically (XR-XM101, Xinruan, China) and stored on a computer.

The animals were placed facing the wall. If the animal failed then it was guided to the platform and stayed for 20 s; if the animal successfully reached the platform within 60 s, let the animal stayed for 15 s. All animals were given 8 trails/day during 5 acquisition days, 15 min between subsequent trails. The day 6 was probe test day, during the trail platform was removed, time for each animal to enter the quadrant and the platform-area was recorded with a maximum of 60 s [24].

2.6. Western blot analysis

After transcardially perfused, tissue samples were homogenized in RIPA strong lysis buffer with PMSF and phosphatase inhibitor cocktail (Beyotime, China). Supernatants were separated, concentration of protein was measured and separated using polyacrylamide gel electrophoresis (PAGE) and transferred to nitrocellulose membranes. Membranes were blocked with 5% non-fat milk for 1 h at room temperature and incubated overnight at 4 °C with primary antibodies. Blots were incubated with secondary antibodies (1:3000; Beyotime, China) for 1 h at room temperature. Immune bands were visualized using ECL reagents (Beyotime, China), followed by using Image J (2x, USA) for quantitatively analysis. Primary antibodies were used as follow: β-actin (1:3000; #58169), Synapsin-1 (1:1000; #5297), Synaptophysin (1:1000; #36406), Synaptotagmin-1 (1:1000; #14558), PSD95 (1:1000; #3409), (Cell Signaling, USA); NF-κB P65 (1:1000; #AF5243), Phospho-NF-κB P65 (Ser536) (1:1000; #AF5881), (Beyotime, China); TNF-α (1:500; #17590-1-AP), (Proteintech, China); IL-1β (1:3000; #A16288), (Abclonal, China).

2.7. Golgi staining and dendritic complexity analysis

Perfused brains were transferred into Golgi-Cox solution, which was refreshed every 2 days. 14 days later, all brains were transferred into 30% sucrose solution till sank. Then brains were sliced at 70 μm using vibratome (7000smz-2, Campden Instruments LTD, Britain). Slices were stained as follows: ddH_2O (1 min), ammonium hydroxide (60 min, in the dark), ddH_2O (1 min), Kodak Fix solution (30 min, in the dark) and ddH_2O (1 min), followed by dehydration through alcohol with ascending concentrations (50%, 70%, 95%, 100%) and then cleared by chloroform, alcohol and xylene. All images were taken by a light microscope (Olympus, Germany). Images of neurons were taken for Z-stack procedure by using Image J (2x, USA).

2.8. Immunofluorescence

After paraformaldehyde fixation, brains were sliced at 30 μm. Slices were washed by PBS and blocked in PBS containing 0.1% Triton and 3% BSA for 1 h at room temperature, then all sections were incubated at 4 °C with primary antibody MAP2 (1:200; #8707), (Cell Signaling, USA) overnight. Day 2 after washed by PBS, sections were incubated with Alexa Flour 488 (1:200; Invitrogen, USA) conjugated secondary antibody at 37 °C for 1 h. After washed by PBS, sections were dyed with Hoechst (1:1000; Invitrogen, USA) for 10 min. Images were acquired using a confocal microscope (TCS sp8, Leica, Germany).

2.9. Statistical analysis

All data are represented as mean ± SEM. All statistical tests were

performed using GraphPad Prism 8 (GraphPad Software, USA). Two-tailed unpaired *t*-test were used for representing intergroup differences. *P* value < 0.05 was considered statistically significant.

3. Results

3.1. Long-term EFV exposure causes impairments in short-term memory and spatial learning and memory of C57BL/6 mice

After 5-month EFV treatment, cognitive performances were measured. In the NOR test, there was a significant decreased discrimination index between control and 40 mg/kg group, meanwhile compared with control group, 80 mg/kg group, showed a similar tendency of decreased discrimination index. Both EFV-treated group showed decreased discrimination index suggesting short-term memory deficits (Fig. 1A).

During acquisition phase of Barnes maze, neither EFV-treated groups showed significant increased primary latency compared with control group (Fig. 1B). During probe test, primary latency showed significantly increased in 80 mg/kg group compared with control group, which indicated a deficit in spatial learning and memory (Fig. 1C). Meanwhile, the average speed showed no significant difference (Fig. 1D).

MWM was employed to further confirm the results. During acquisition phase, 80 mg/kg group showed a significantly increased primary latency compared with control group, indicating impairments in spatial learning and memory (Fig. 1E). During probe test, primary latency, quadrant occupancy rate, average speed and platform crossing showed no significant alternations (Fig. 1F, G, H and I). Altogether, these results strongly suggested that long-term EFV exposure caused short-term memory loss, impairments in spatial learning and memory of C57BL/6 mice.

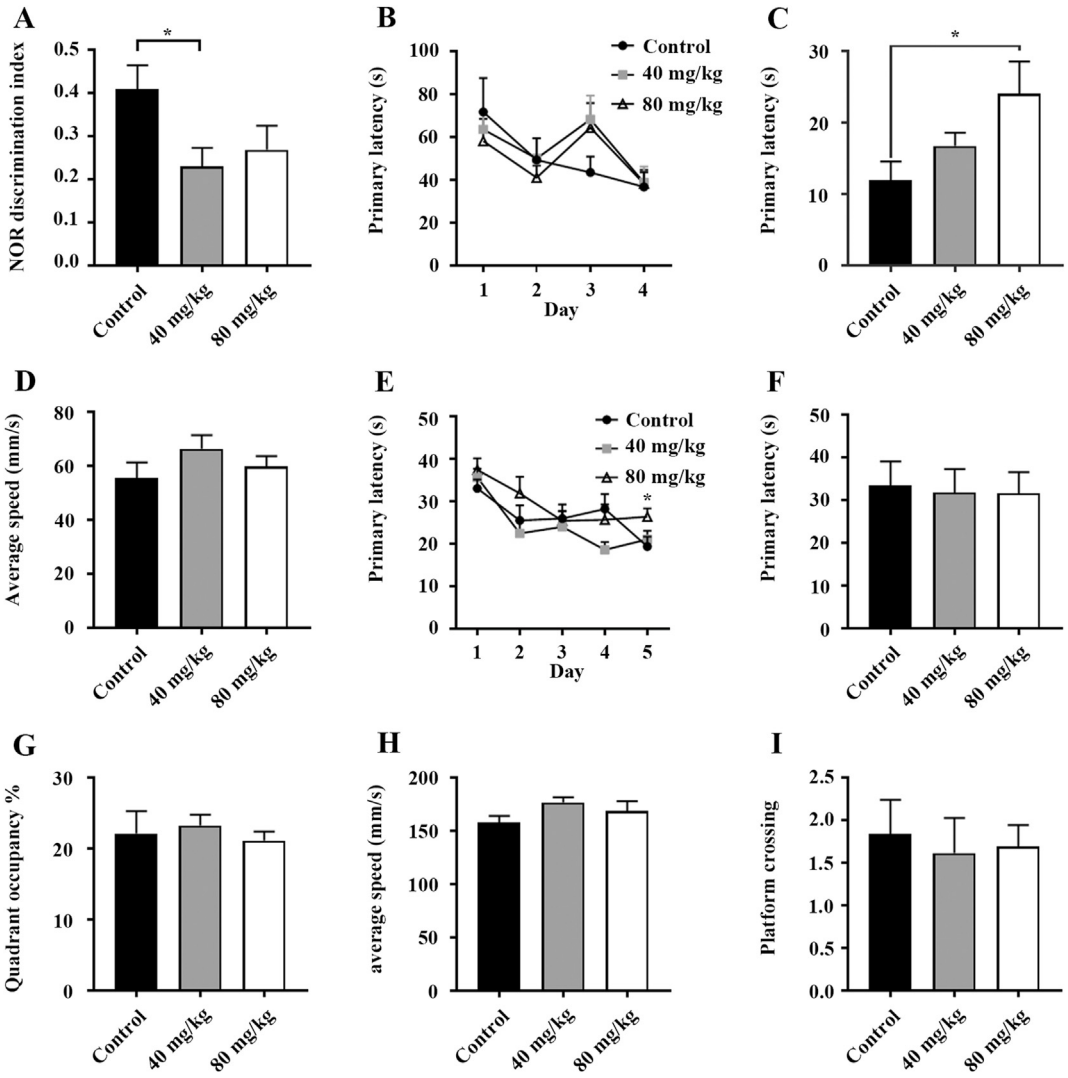

Fig. 1. Long-term administration of efavirenz (40 and 80 mg/kg/day) impaired cognitive performance.
(A) Results on NOR discrimination index in mice subjected to NOR test. (Control, n = 14; 40 mg/kg, n = 20; 80 mg/kg, n = 20). Unpaired *t*-test was performed. Error bars indicate SEM. Asterisks indicate significant differences (*, $P < 0.05$). (B) Primary latency in mice subjected to the acquisition phase of Barnes maze test. (C) Primary latency and (D) average speed in mice subjected to the probe test of Barnes maze test. (Control, n = 12; 40 mg/kg, n = 20; 80 mg/kg, n = 18). Unpaired *t*-test was performed. Error bars indicate SEM. Asterisks indicate significant differences (*, $P < 0.05$). (E) Primary latency and (H) average speed in mice subjected to the acquisition phase of MWM. (F) Primary latency, (G) quadrant occupancy and (I) platform crossing in mice subjected to the probe test of MWM. (Control, n = 14; 40 mg/kg, n = 20; 80 mg/kg, n = 20). Unpaired *t*-test was performed. Error bars indicate SEM. Asterisks indicate significant differences (*, $P < 0.05$).

3.2. Long-term EFV exposure alters synaptic morphology and expression levels of synapse-associated proteins

To investigate influences of long-term EFV exposure on synapses, we performed immunofluorescence (IF) using MAP2 antibody as dendritic marker (Fig. 2A) in hippocampus, since the hippocampus plays a critical role in spatial learning memory and cognition [25]. Results showed decreased tendencies of dendritic density in CA1 and DG of both EFV treated group comparing with control group (Fig. 2B and D), and no change in CA3 (Fig. 2C).

To evaluate the alterations in dendritic morphology, we performed Sholl analysis using ImageJ after Golgi staining (Fig. 3A) and measured the dendritic spines (Fig. 3C). Results indicating that dendritic complexity (Fig. 3B) and synaptic plasticity (Fig. 3D) were decreased in CA1 of EFV-treated groups when compared with control group.

To further investigate potential molecular mechanisms underlying the cognitive deficits, several synapse-associated proteins were analyzed (Fig. 2E). In hippocampus, the expression level of PSD95 was reduced of 40 mg/kg group compared with control group; expression level of Synapsin-1 showed a significant reduction of 80 mg/kg group compared with control group (Fig. 2F). Additionally, there was a similar tendency of synaptic expression levels in the prefrontal cortex (Supplementary Fig. 1A), which are associated with response control, impulse control, attention, rule representation and strategy shifting [26–29]. However, there was no difference in cortex (Supplementary Fig. 1B).

Taken together, all data suggest that long-term EFV exposure causes synaptic impairments in hippocampus that might be the reason of worsen cognition in C57BL/6 mice.

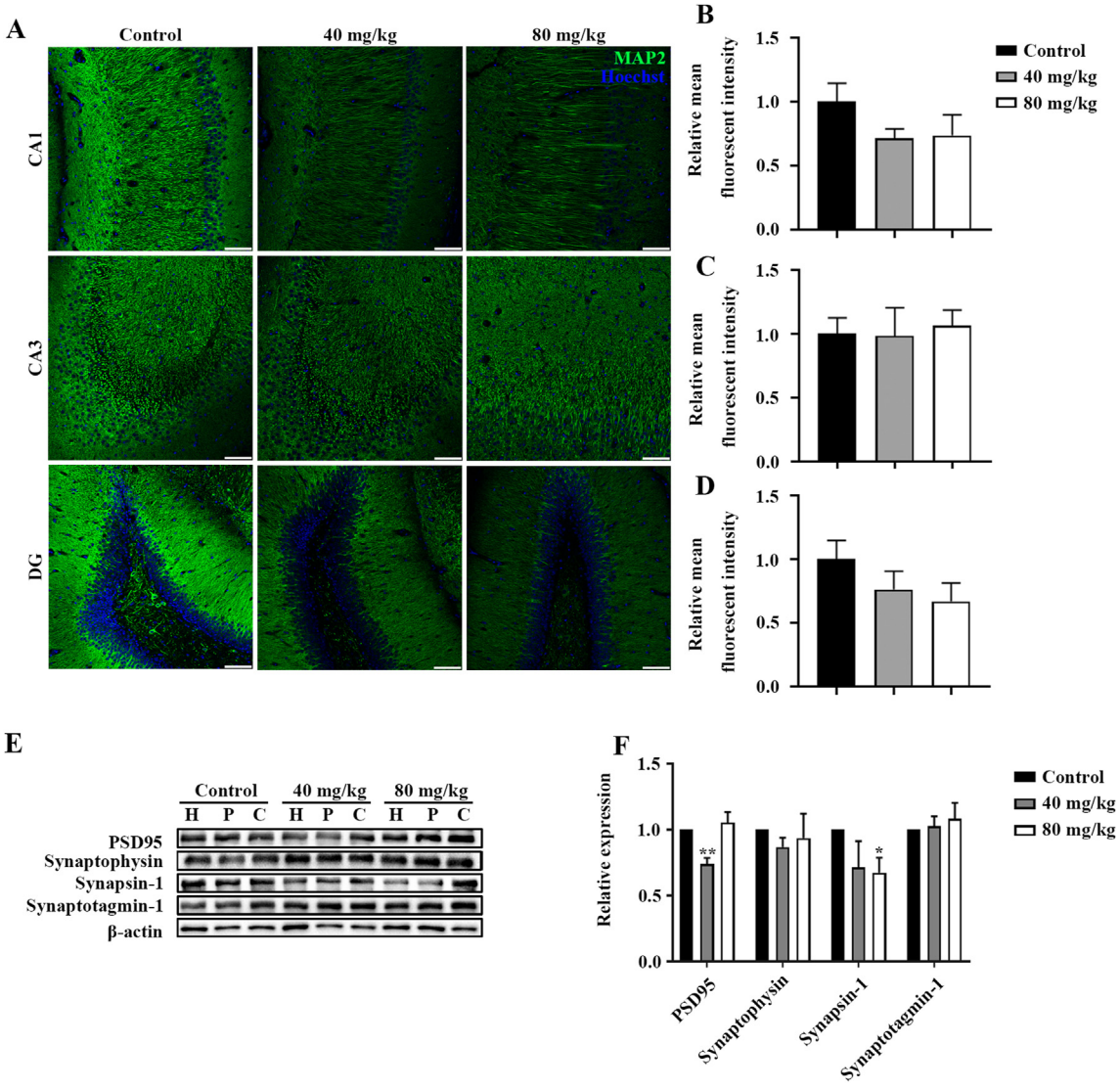

Fig. 2. Effects of long-term administration of efavirenz (40 and 80 mg/kg/day) on dendritic density and expression levels of synapse-associated proteins of hippocampus. (A) Representative immunofluorescent images of dendrite stained with MAP2 (green) and Hoechst labeling of cell nuclei (blue) in DG, CA1 and CA3 from saline or EFV-treated mice. Scale bar indicates 75 μm. Quantification of relative mean fluorescent intensity of MAP2 in CA1 (B), CA3 (C) and DG (D) of hippocampus compared with control group. (brain slices from 3 mice in each group). Unpaired *t*-test was performed. Error bars indicate SEM. (E and F) Western blot and quantification of PSD95, Synaptophysin, Synapsin-1, Synaptotagmin-1 in hippocampus. (Control, n = 3; 40 mg/kg, n = 3; 80 mg/kg, n = 3). Unpaired *t*-test was performed. Error bars indicate SEM. Asterisks indicate significant differences (*, $P < 0.05$; **, $P < 0.01$). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

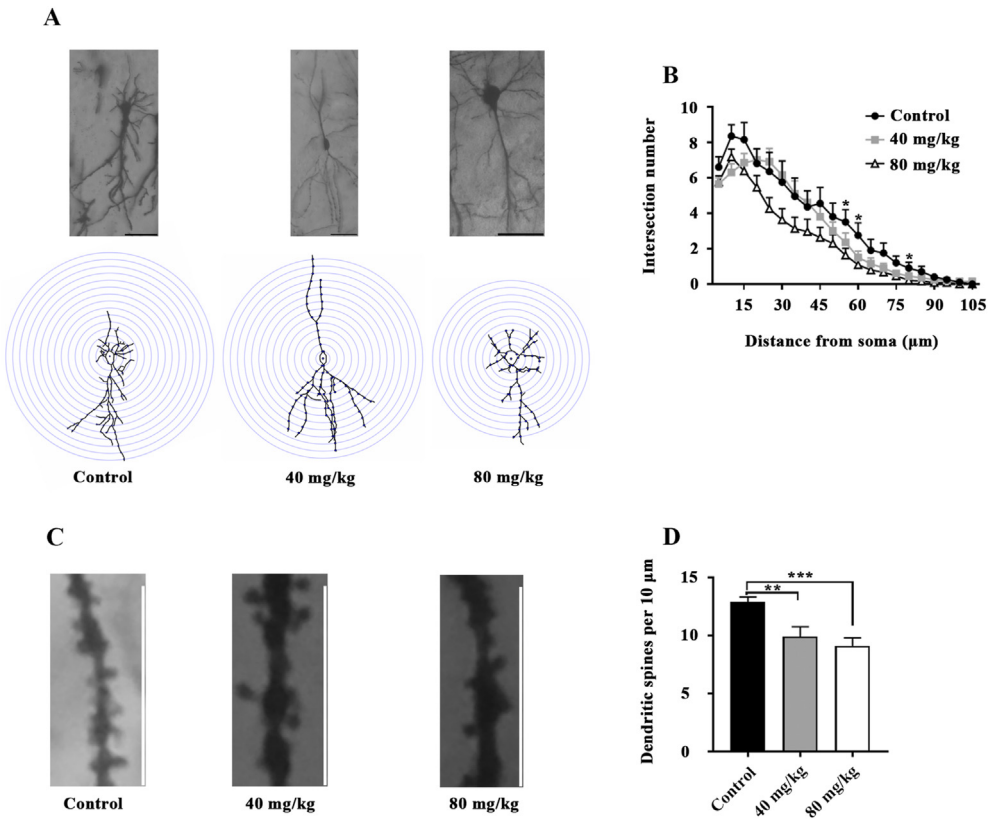

Fig. 3. Effects of long-term administration of efavirenz (40 and 80 mg/kg/day) on dendritic morphology.
(A) Representative morphology of neurons in CA1 of hippocampus. Scale bar indicates 20 μm. (B) Quantification of intersection number of dendritic crossings in CA1 of hippocampus per 5 μm from soma compared with control group. (Control, n = 20; 40 mg/kg, n = 20; 80 mg/kg, n = 24). Unpaired *t*-test was performed. Error bars indicate SEM. Asterisks indicate significant differences (*, $P < 0.05$). (C) Representative morphology of dendritic spines in CA1 of hippocampus. Scale bar indicates 10 μm. (D) Quantification of number of dendritic spines per 10 μm in CA1 of hippocampus compared with control group. (Control, n = 11; 40 mg/kg, n = 11; 80 mg/kg, n = 11). Unpaired *t*-test was performed. Error bars indicate SEM. Asterisks indicate significant differences (**, $P < 0.01$; ***, $P < 0.001$).

3.3. Long-term EFV exposure activates NF-κB pathway resulting in the expression of pro-inflammatory cytokines

Since the increased pro-inflammatory cytokines can lead to synaptic dysfunction [18], and the level of phosphorylated p65 can represents the alteration of NF-κB pathway [30], several NF-κB-associated proteins were analyzed (Fig. 4A and C). Significant increases were observed in expression level of pro-IL-1β and a similar tendency of TNF-α and phosphorylation of p65 of 80 mg/kg group compared with control group (Fig. 4B and D). Additionally, there were no difference in prefrontal cortex and cortex (Supplementary Fig. 2).

4. Discussion

As a result of using cART, lifespan of patients has been prolonged [11], along with prolonged time of drug administration, making them facing the increasing risks of cognitive disorders.

The number of newly reported HIV-infected individuals in young adults are rising, the following 10 years are their most important phases during lifespan, as their both physique and mentality getting matured, starting learning and working in college and workplace [16,17,31,32]. In order to determine the effect of long-term EFV exposure on cognitive performances at young adult stage and underlying molecular mechanisms, 2-month old C57BL/6 mice were employed. Three behavioral tests were used: NOR test, Barnes maze test and MWM. The NOR test is a commonly used test for investigating short-term memory [22], and relied on natural proclivity for exploring of rodents [33]. The Barnes maze test and MWM is widely used for investigating spatial learning and memory [23,24]. Our results demonstrated that 5-month-EFV-treated groups showed significantly short-term memory impairment in NOR test, but only 80 mg/kg group showed substantial impairment of spatial learning and memory in Barnes maze and MWM, without affecting spontaneous activity.

It was reported that 10 mg/kg-EFV treatment, lasting for 36 days, induced anxiety-like behavior and impairment of aversive memory in mice [34]. 10 mg/kg-EFV treatment, lasting for 34 days, induced depression-like behavior and up-regulating the expression of pro-inflammatory cytokines in rats [24]. All evidences proved that the severity of EFV induced behavioral side effect in rodent model is time and dose dependent.

Since the hippocampus plays a critical role in spatial learning memory, cognition [25], and shot-term memory, spatial learning and memory are sensitive to hippocampal impairment [35–37]. Former study showed that EFV had neurotoxicity by detecting MAP2 expression on primary neurons [38], we performed IF assay with MAP2 antibody to determine the effect of EFV on dendritic integrity. Results showed decreased tendencies in CA1 and DG of hippocampus in EFV-treated groups, suggesting that the EFV treatment decreased MAP2 density. We further performed WB for synapse-associated proteins, which are closely associated with learning and memory [39]. Results showed decreased Synapsin-1 expression level in 80 mg/kg group and decreased PSD95 expression level in 40 mg/kg group, but in 80 mg/kg group showed no difference as compared with control group. The potential reason

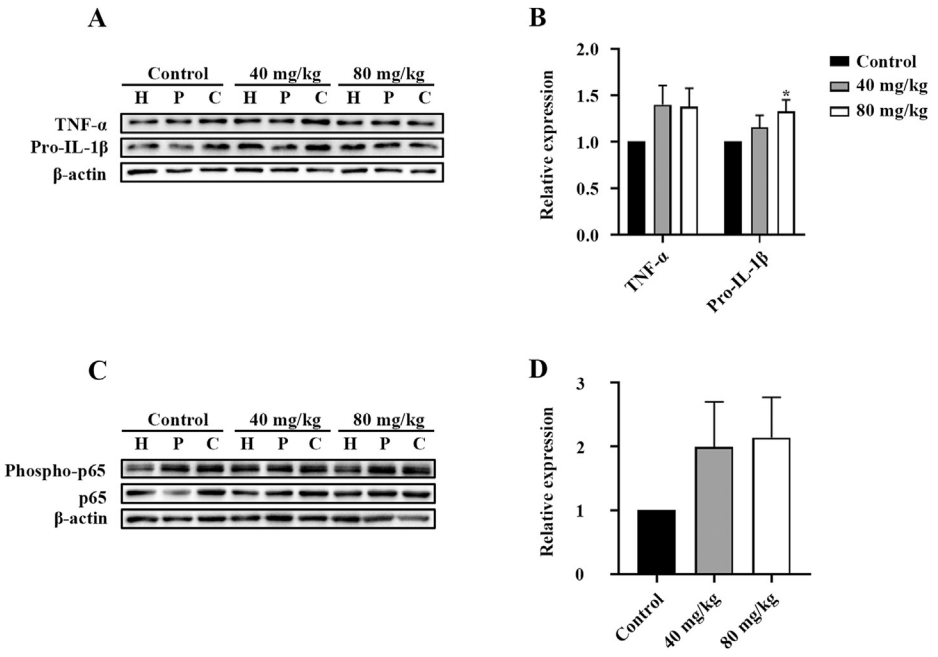

Fig. 4. Effects of long-term administration of efavirenz (40 and 80 mg/kg/day) on expression of pro-inflammatory cytokines and activation of NF-κB pathway in hippocampus. (A) Representative western-blot antibodies against TNF-α, Pro-IL-1β and β-actin in hippocampus. (B) Quantification of relative expressions of pro-inflammatory cytokines compared with control group in hippocampus. (Control, n = 6; 40 mg/kg, n = 6; 80 mg/kg, n = 6). (C) Representative western-blot antibodies against p65, phospho-p65 and β-actin in hippocampus. (D) Quantification of expression levels of phosphorylated p65/p65 compared with control group in hippocampus. (Control, n = 3; 40 mg/kg, n = 3; 80 mg/kg, n = 3). Unpaired *t*-test was performed. Error bars indicate SEM. Asterisks indicate significant differences (*, $P < 0.05$).

could be that the mechanisms of EFV toxicity vary between different concentrations.

Dendritic spines structure plasticity plays a critical role in memory acquisition, storage and processing [40,41]. Result of Golgi-Cox staining indicated that dendritic complexity and synaptic plasticity were decreased in 80 mg/kg group. Overall, these results strongly suggest that EFV may induced synaptic dysfunction that is responsible for the cognitive impairments.

It is well established that the release of pro-inflammatory cytokines can lead to synaptic dysfunction [18], and EFV can cause the activation of NF-κB pathway in HCAECs cell [42]. Pro-IL-1β and TNF-α are two main downstream pro-inflammatory cytokines of NF-κB [19,20]. We showed that pro-IL-1β and TNF-α were overexpressed and NF-κB pathway was activated in hippocampus of 80 mg/kg group. The results suggest that the activation of NF-κB induced by EFV might cause synaptic dysfunction and cognitive impairments.

There are different kinds of adverse neuropsychiatric effects of EFV reported before. Most of reports showed sleep disorders and dizziness, headaches, confusion, stupor and attention deficit [43]. Once comes to long-term exposure, the side effects turn into anxiety and depression, along with high serum EFV concentration [44,45]. And there are improvements of depression and anxiety after discontinuation [46]. All evidences strongly suggest that severity of the CNS side effects may be related with EFV exposure time in patients.

Taken all together, our study revealed C57BL/6 mice, which received EFV for 5 months displayed cognitive deficits. This could be due to the activation of NF-κB pathway thus overexpressed and released pro-inflammatory cytokines, ended up with synaptic dysfunction. Further studies are still necessary to confirm whether other cells may be involved in this neuroinflammation such as microglia and astroglia. Thus, it is highly important to use lower dose of EFV or even switch to other anti-HIV drugs to improve cognitive performances and live quality of patients.

Funding statement

This study was supported by the Grants from the National Science Foundation of China (Nr.31470270), Jianghan University (Nr.3015/06210035) to B. Sun and from Hubei University of Technology (Nr. 337203) to K. Hu.

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.

Acknowledgement

We would like to thank Xiaokang Gong, Yang Zhao, Weiling Li and Qian Peng for technical support.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbrc.2021.11.015.

References

[1] R. Esnouf, J. Ren, C. Ross, Y. Jones, D. Stammers, D. Stuart, Mechanism of inhibition of HIV-1 reverse transcriptase by non-nucleoside inhibitors, Nat. Struct. Biol. 2 (4) (1995) 303–308.
[2] R.A. Spence, W.M. Kati, K.S. Anderson, K.A. Johnson, Mechanism of inhibition of HIV-1 reverse transcriptase by nonnucleoside inhibitors, Science 267 (5200) (1995) 988–993.
[3] B.M. Best, M. Goicoechea, Efavirenz—still first-line king? Expet Opin. Drug Metabol. Toxicol. 4 (7) (2008) 965–972.
[4] G. Lapadula, D.P. Bernasconi, F. Bai, E. Focà, A. Di Biagio, S. Bonora, et al., Switching from efavirenz to rilpivirine improves sleep quality and self-perceived cognition but has no impact on neurocognitive performances, AIDS 34 (1) (2020) 53–61.
[5] W.H. Organization, Consolidated Guidelines on the Use of Antiretroviral Drugs

for Treating and Preventing HIV Infection: Summary of Key Features and Recommendations, June 2013, World Health Organization, 2013.
[6] L.-T.V.S.W. Group, Long-term virological suppression on first-line efavirenz+ tenofovir+ emtricitabine/lamivudine for HIV-1, AIDS 33 (4) (2019) 745–751.
[7] E.H. Decloedt, P.Z. Sinxadi, G.U. van Zyl, L. Wiesner, S. Khoo, J.A. Joska, et al., Pharmacogenetics and pharmacokinetics of CNS penetration of efavirenz and its metabolites, J. Antimicrob. Chemother. 74 (3) (2019) 699–709.
[8] E.H. Decloedt, G. Maartens, Neuronal toxicity of efavirenz: a systematic review, Expet Opin. Drug Saf. 12 (6) (2013) 841–846.
[9] C.A. Kenedi, H.W. Goforth, A systematic review of the psychiatric side-effects of efavirenz, AIDS Behav. 15 (8) (2011) 1803–1818.
[10] Q. Ma, F. Vaida, J. Wong, C.A. Sanders, Y-t Kao, D. Croteau, et al., Long-term efavirenz use is associated with worse neurocognitive functioning in HIV-infected patients, J. Neurovirol. 22 (2) (2016) 170–178.
[11] N. Ciccarelli, M. Fabbiani, S. Di Giambenedetto, I. Fanti, E. Baldonero, L. Bracciale, et al., Efavirenz associated with cognitive disorders in otherwise asymptomatic HIV-infected patients, Neurology 76 (16) (2011) 1403–1409.
[12] D.B. Clifford, S. Evans, Y. Yang, E.P. Acosta, H. Ribaudo, R.M. Gulick, et al., Long-term impact of efavirenz on neuropsychological performance and symptoms in HIV-infected individuals (ACTG 5097s), HIV Clin. Trials 10 (6) (2009) 343–355.
[13] D.B. Clifford, S. Evans, Y. Yang, E.P. Acosta, K. Goodkin, K. Tashima, et al., Impact of efavirenz on neuropsychological performance and symptoms in HIV-infected individuals, Ann. Intern. Med. 143 (10) (2005) 714–721.
[14] C. Pinnetti, P. Balestra, R. Libertone (Eds.), Use of Efavirenz Is Not Associated to an Increased Risk of Neurocognitive Impairment in HIV-Infected Patients [Abstract THAB0101], 20th International AIDS Conference July, 2014.
[15] Efavirenz use is not associated with increased risk of neuropsychological impairment, in: S. Rourke, J. Gill, C. Power (Eds.), Annual Conference on Retroviruses and Opportunistic Infections, 2015. Seattle, WA.
[16] UNICEF. HIV and AIDS in adolescents, Turning the Tide against AIDS Will Require More Concentrated Focus on Adolescents and Young People, 2019 [Cited 2020 Jun 10].
[17] U. Unaids, UNAIDS Data 2019, UNAIDS data 2019, 2019.
[18] F. Leng, P. Edison, Neuroinflammation and microglial activation in Alzheimer disease: where do we go from here? Nat. Rev. Neurol. 17 (3) (2021) 157–172.
[19] M. Zusso, V. Lunardi, D. Franceschini, A. Pagetta, R. Lo, S. Stifani, et al., Ciprofloxacin and levofloxacin attenuate microglia inflammatory response via TLR4/NF-kB pathway, J. Neuroinflammation 16 (1) (2019) 1–12.
[20] C.E. Bryant, D.R. Spring, M. Gangloff, N.J. Gay, The molecular basis of the host response to lipopolysaccharide, Nat. Rev. Microbiol. 8 (1) (2010) 8–14.
[21] J.G. Fox, S. Barthold, M. Davisson, C.E. Newcomer, F.W. Quimby, A. Smith, The Mouse in Biomedical Research: Normative Biology, Husbandry, and Models, Elsevier, 2006.
[22] L.M. Lueptow, Novel object recognition test for the investigation of learning and memory in mice, JoVE (126) (2017), e55718.
[23] M.W. Pitts, Barnes maze procedure for spatial learning and memory in mice, Bio-protocol 8 (5) (2018).
[24] S.M. O'Mahony, A.-M. Myint, H. Steinbusch, B.E. Leonard, Efavirenz induces depressive-like behaviour, increased stress response and changes in the immune response in rats, Neuroimmunomodulation 12 (5) (2005) 293–298.
[25] J. Lisman, G. Buzsáki, H. Eichenbaum, L. Nadel, C. Ranganath, A.D. Redish, Viewpoints: how the hippocampus contributes to memory, navigation and cognition, Nat. Neurosci. 20 (11) (2017) 1434–1447.
[26] J.W. Dalley, R.N. Cardinal, T.W. Robbins, Prefrontal executive and cognitive functions in rodents: neural and neurochemical substrates, Neurosci. Biobehav. Rev. 28 (7) (2004) 771–784.
[27] V.J. Brown, E.M. Bowman, Rodent models of prefrontal cortical function, Trends Neurosci. 25 (7) (2002) 340–343.
[28] J.K. Seamans, C.C. Lapish, D. Durstewitz, Comparing the prefrontal cortex of rats and primates: insights from electrophysiology, Neurotox. Res. 14 (2) (2008) 249–262.
[29] M. Carlén, What constitutes the prefrontal cortex? Science 358 (6362) (2017) 478–482.
[30] S.L. Beesetti, M. Jayadev, G.V. Subhashini, L. Mansour, S. Alwasel, A.H. Harrath, Andrographolide as a therapeutic agent against breast and ovarian cancers, Open Life Sci. 14 (1) (2019) 462–469.
[31] B. Dick, B.J. Ferguson, Health for the world's adolescents: a second chance in the second decade, J. Adolesc. Health 56 (1) (2015) 3–6.
[32] A. Pettifor, M. Stoner, C. Pike, L.-G. Bekker, Adolescent lives matter: preventing HIV in adolescents, Curr. Opin. HIV AIDS 13 (3) (2018) 265.
[33] D.E. Berlyne, Novelty and curiosity as determinants of exploratory behaviour 1, Br. J. Psychol. General Sect. 41 (1-2) (1950) 68–80.
[34] P.R. Romao, J.C. Lemos, J. Moreira, G. de Chaves, M. Moretti, A.A. Castro, et al., Anti-HIV drugs nevirapine and efavirenz affect anxiety-related behavior and cognitive performance in mice, Neurotox. Res. 19 (1) (2011) 73–80.
[35] R.S. Astur, L.B. Taylor, A.N. Mamelak, L. Philpott, R.J. Sutherland, Humans with hippocampus damage display severe spatial memory impairments in a virtual Morris water task, Behav. Brain Res. 132 (1) (2002) 77–84.
[36] L. De Hoz, E.I. Moser, R.G. Morris, Spatial learning with unilateral and bilateral hippocampal networks, Eur. J. Neurosci. 22 (3) (2005) 745–754.
[37] H. Goddyn, S. Leo, T. Meert, R. D'Hooge, Differences in behavioural test battery performance between mice with hippocampal and cerebellar lesions, Behav. Brain Res. 173 (1) (2006) 138–147.
[38] V.T. Ciavatta, E.K. Bichler, I.A. Speigel, C.C. Elder, S.L. Teng, W.R. Tyor, et al., In vitro and ex vivo neurotoxic effects of efavirenz are greater than those of other common antiretrovirals, Neurochem. Res. 42 (11) (2017) 3220–3232.
[39] J.M. Henley, R. Seager, Y. Nakamura, K. Talandyte, J. Nair, K.A. Wilkinson, SUMOylation of synaptic and synapse-associated proteins: an update, J. Neurochem. 156 (2) (2021) 145–161.
[40] C.H. Duman, R.S. Duman, Spine synapse remodeling in the pathophysiology and treatment of depression, Neurosci. Lett. 601 (2015) 20–29.
[41] H. Qiao, M.-X. Li, C. Xu, H.-B. Chen, S.-C. An, X.-M. Ma, Dendritic spines in depression: what we learned from animal models, Neural Plast. 2016 (2016).
[42] M.S. Jamaluddin, P.H. Lin, Q. Yao, C. Chen, Non-nucleoside reverse transcriptase inhibitor efavirenz increases monolayer permeability of human coronary artery endothelial cells, Atherosclerosis 208 (1) (2010) 104–111.
[43] G.J. Treisman, A.I. Kaplin, Neurologic and psychiatric complications of antiretroviral agents, AIDS 16 (9) (2002) 1201–1215.
[44] F. Gutiérrez, A. Navarro, S. Padilla, R. Antón, M. Masiá, J. Borrás, et al., Prediction of neuropsychiatric adverse events associated with long-term efavirenz therapy, using plasma drug level monitoring, Clin. Infect. Dis. 41 (11) (2005) 1648–1653.
[45] T.A. Rihs, K. Begley, D. Smith, J. Sarangapany, A. Callaghan, M. Kelly, et al., Efavirenz and chronic neuropsychiatric symptoms: a cross-sectional case control study, HIV Med. 7 (8) (2006) 544–548.
[46] M. Mothapo K, A. Schellekens, R. van Crevel, M. Keuter, K. Grintjes-Huisman, P. Koopmans, et al., Improvement of depression and anxiety after discontinuation of long-term efavirenz treatment, CNS Neurol. Disord. - Drug Targets 14 (6) (2015) 811–818.