Biochemical and Biophysical Research Communications 652 (2023) 46–54

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Therapeutic effect of implanted and non-invasive vagus nerve stimulation on heroin-induced anxiety

Yingbiao Yue [a], Lei Zou [b], Hong Li [c], Yu Xia [d], Zhouyang Ren [c], Fazhen Yang [c], Deshenyue Kong [a], Guofen Re [a], Huayou Luo [e], Zunyue Zhang [f, *], Kunhua Wang [f, **], Mei Zhu [g, ***]

[a] National Health Commission Key Laboratory of Drug Addiction Medicine, Kunming Medical University, Kunming, 650032, China
[b] Department of Hepatobiliary Surgery, The First People's Hospital of Yunnan Province, Kunming, 650032, Yunnan, China
[c] Narcotics Control Bureau of the Ministry of Public Security of Yunnan Province, Kunming, 650032, China
[d] Peking University Health Science Center, Beijing, 100191, China
[e] Department of Gastrointestinal and Hernia Surgery, First Affiliated Hospital of Kunming Medical University, Kunming, 650032, China
[f] Yunnan University, Kunming, 650032, China
[g] First Affiliated Hospital of Kunming Medical University, Kunming, China

ARTICLE INFO

Article history:
Received 25 January 2023
Received in revised form
1 February 2023
Accepted 15 February 2023
Available online 16 February 2023

Keywords:
Anxiety
Heroin
Vagus nerve stimulation
Transcutaneous auricular vagus nerve stimulation
Neuroinflammation
Microglia

ABSTRACT

Substance addiction causes anxiety, which in turn reinforces the maintaining of substance use, resulting in a vicious circle. And this circle is one of the reasons why addiction is so hard to cure. However, there is no treatment involved in addiction-induced anxiety at present. We tested whether VNS (vagus nerve stimulation) can improve heroin-induced anxiety, and made a comparison between nVNS (transcervical vagus nerve stimulation) and taVNS (transauricular vagus nerve stimulation) on therapeutic effect. Mice were subjected to nVNS or taVNS before heroin administration. By observing c-Fos expression in the NTS (nucleus of the solitary tract), we assessed vagal fiber activation. Using the OFT (open field test) and the EPM (elevated cross maze test), we evaluated the anxiety-like behaviors of the mice. Using immunofluorescence, we observed the proliferation and activation of microglia in the hippocampus. And ELISA was used to measure the levels of proinflammatory factors in the hippocampus. Both nVNS and taVNS significantly increased the expression of c-Fos in the nucleus of solitary tract, suggesting the feasibility of nVNS and taVNS. The anxiety level of heroin-treated mice was significantly increased, microglia in the hippocampus was significantly proliferated and activated, and the proinflammatory factors (IL-1β, IL-6, TNF-α) in the hippocampus were significantly up-regulated. Crucially, both nVNS and taVNS reversed the above changes caused by heroin addiction.

Significance: It was confirmed that the therapeutic effect of VNS on heroin-induced anxiety may be an effective treatment method to break the "addiction-anxiety" cycle and provides some insights for subsequent treatment of addiction.

1. Introduction

Heroin, as a traditional addictive drug, brings great losses to individuals and society. In order to improve this situation, the main cure method at present applies methadone or buprenorphine substitution therapy, which achieves positive effect. However, this treatment still has many limitations, such as the side effects brought by drugs and the lack of medical conditions in many places [1,2].

Heroin inhalation causes anxiety [3,4], and this anxiety also shows a special connection with heroin addiction. According to previous researches, substance addiction leads to anxiety [5,6], which is also an important contributor to substance abuse, dependence and relapse [7–9]. For instance, anxiety induced by alcohol addiction will in turn lead to the maintenance and recurrence of pathological alcohol use, resulting in a vicious circle [10].

* Corresponding author.
** Corresponding author.
*** Corresponding author.
E-mail addresses: zhangzunyue@ynu.edu.cn (Z. Zhang), khwang@ynu.edu.cn (K. Wang), zhumei@ydyy.cn (M. Zhu).

https://doi.org/10.1016/j.bbrc.2023.02.041
0006-291X/

Among heroin addicts, higher levels of anxiety and depression are significantly associated with higher levels of psychosis, neuroticism, crime, and addiction [11]. In the meantime, opioid-addicts have significant decision-making disorders, which are significantly affected by anxiety levels [12]. Anxiety and addiction are mutually reinforcing. High levels of anxiety promote the use of addictive drugs. The use of addictive drugs also causes anxiety. This cycle may be one of the reasons why it is difficult to quit addictive drugs. Therefore, the alleviation of heroin-induced anxiety may have a positive effect on the treatment of heroin addiction, but there is no effective treatment for heroin-induced anxiety at present.

The vagus nerve is a widely distributed neural network which acts as a bridge between the brain and peripheral organs. Among all vagus nerves, vagal afferent nerves form the majority whose work is transmitting impulses to the nucleus of the solitary tract (NTS) when receiving signals. Then NTS, the vagus nerve center, projects signal directly or indirectly to various regions of the brain, including hypothalamus, cortex, amygdala, hippocampus and so on [13–15]. According to previous studies, vagus nerve stimulation plays an active role in treating many diseases, such as depression, epilepsy, Parkinson's disease (PD), autistic spectrum disorder (ASD) and traumatic brain injury (TBI) [15–18]. In addition, vagus nerve stimulation can reduce anxiety-like behaviors to some extent [19–25]. But the mechanism of vagus nerve stimulation has not been fully clarified, and whether vagus nerve stimulation can improve anxiety caused by addictive drugs also has not been explored.

The hippocampus not only plays a great role in learning, memory formation and memory consolidation, but also involves in regulating mood, fear, anxiety and stress [26]. Microglia play a key role in the development of central nervous system, immune detection and maintaining the normal function of neurons [27]. Hippocampal microglia activation can significantly lead to anxiety-like behaviors in rats [28–30], which can be inhibited by minocycline (a kind of microglial inhibitor) [31]. At the same time, the inflammatory factors also increase in the hippocampus of anxious mice and rats [32,33]. And, lentiviral knockdown of IL-1 β in the hippocampus of mice can significantly reduce lipopolysaccharide (LPS) -induced anxiety-like behaviors [34]. The above evidences suggest that hippocampal microglia play an important role in anxiety. So, we hypothesize that vagus nerve stimulation can reduce microglial neuritis in the hippocampus and improve anxiety-like behaviors caused by addiction.

The most common way to stimulate the vagus nerve is to implant stimulation electrodes in the cervical vagus nerve, but this invasive way has many side effects. In recent years, transauricular vagal nerve stimulation (taVNS) has attracted more and more attention because of its non-invasive method and simplicity. In addition, some studies show that taVNS has similar effects as invasive vagus nerve stimulation. Considering the physical quality and surgical tolerance of heroin abusers, both of transcervical vagus nerve stimulation and transauricular vagus nerve stimulation were applied to evaluate the therapeutic effect of vagus nerve stimulation on anxiety caused by addiction.

2. Material and methods

2.1. Chemicals

Heroin applied in this study was provided by Public Security Department of Yunnan province, China. Programmed electrical stimulator was bought from Chengdu Instrument Factory, located in Sichuan province, China. Anti-c-Fos (ab222699) and secondary antibody (Alexa Fluor® 488 , ab150077; Alexa Fluor® 647 , ab150075) were bought from abcam (Cambridge, UK). Anti-Iba-1 (#17198) and CD68(Alexa Fluor® 488 Conjugate, #51644) were from Boston, US. Mounting Medium, antifading (with DAPI) was from Solarbio (Beijing, China). IL-1β, TNF-α and IL-6 ELISA kit were from Jianglai biological company (Shanghai, China).

2.2. Animals

8 weeks-old male C57BL/6 mice (21 g–23 g) were purchased from Sipeifu (Beijing) Biotechnology Co., Ltd., and the mice were housed under microbarrier system. The mice were kept at environment with appropriate temperature and humidity and a 12 h reverse light/dark cycle (lights off at 7:00 a.m.) with access to food and water ad libitum. This study was approved by the Animal Ethics Committee of Kunming Medical University (Grant No.: KMMU2021684). And all animal experiments were conducted according to National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications, revised 1978).

2.3. Animal grouping

Mice were randomly divided into 6 groups: saline group, heroin group, heroin + nVNS group, heroin + nVNS + sham group, heroin + taVNS group and heroin + taVNS + sham group.

In saline group, mice received intraperitoneal injection of 0.2 ml saline for 7 days. In heroin group, heroin (10 mg/kg), dissolved in 0.2 ml normal saline, was injected intraperitoneally once a day for 7 days. In heroin + nVNS group, heroin was injected intraperitoneally for 7 days (the injection mode and dose were the same as those in the heroin group). At the same time, 1 h before the heroin administration, nVNS was administered (square wave, 0.05 mA, 50 ms pulse width, 15 Hz, 10 mins). In heroin + nVNS + sham group, all treatments were the same as those in heroin + nVNS group, but there was no electricity during stimulation. In heroin + taVNS group, mice received intraperitoneal injection of heroin for 7 days (same as heroin group), and underwent taVNS 1 h before heroin injection (stimulation parameters were the same as those of heroin + nWNS group). In heroin + taVNS + sham group, all the administrations were the same as those in heroin + taVNS group, but there was no electricity during stimulation.

2.4. nVNS model

One day before surgery, mice had no access to food, but had free access to water. During the operation, mice anesthetized with isoflurane were fixed on the constant-temperature operating table and kept at constant temperature during the operation. Then detailed procedures were followed: disinfecting and cutting the skin of neck after removing the hair on neck; exposing vagus nerve on the left; carefully wrapping electrodes around the vagus nerve; disinfecting the skin of head after removing the hair on it; exposing the bone by cutting the scalp; drilling two 0.5 mm-holes into the donum with a bone drill; carefully screwing sterile screws into the holes; wrapping wires around the vagus nerve running subcutaneously; fixing wires to the screws on the donum; using dental cement to fix insulated screws and wires; disinfecting the wound and suture. The operation procedures were kept as sterile as possible and all instruments were sterilized before operation. After operation, mice were housed in an environment with constant temperature and given a special nutrient solution, and mice were stimulated after 2-weeks recovery. Before the stimulation, mice were fixed, and the wires on their heads were connected to a 0.2 mm wire linking to the programmed electrical stimulator. When being stimulated (square wave, 0.05 mA, 50 ms pulse width, 15 Hz, 10min), mice were placed in an open field of 50 cm × 50 cm to move freely (Fig. 1).

2.5. taVNS model

A special clamp electrode was used to fix the mouse's left auricular-concha region and the other end of it was connected with the programmed electrical stimulator through a wire. When being stimulated (square wave, 0.05 mA, 50 ms pulse width, 15 Hz, 10min), mice were placed in an open field of 50 cm × 50 cm to move freely. (Fig. 1).

2.6. Behavioral tests

Open field test (OFT), as a method to evaluate spontaneous behaviors, exploratory behaviors and tensions of experimental animals in a new environment, has been extensively applied to evaluate anxiety. The experiment device of OFT in our study was an open box of 50 × 50 × 50 cm with white floor and walls. In the test, mice were placed in the central area of the floor with their backs to the experimenter, and they were allowed to move freely in this device for 5 min with a camera on the top of the device recording their movements. The time spent at the central area, the total distance of movement and the number of feces were counted to evaluate the levels of anxiety in mice.

Elevated plus maze (EPM) is an experimental method to evaluate anxiety responses of rodents. EPM device applied in this study was composed of open arms and closed arms, showing a cross shape. Open arms were 50 cm long and 5 cm wide, closed arms were 75 cm long and 5 cm wide, the surrounding baffle was 15 cm high, and the whole device was 75 cm high from the ground. Mice were placed in a central area facing the closed arms with their backs to the experimenter, and the time spent in each area and entries to each area were recorded.

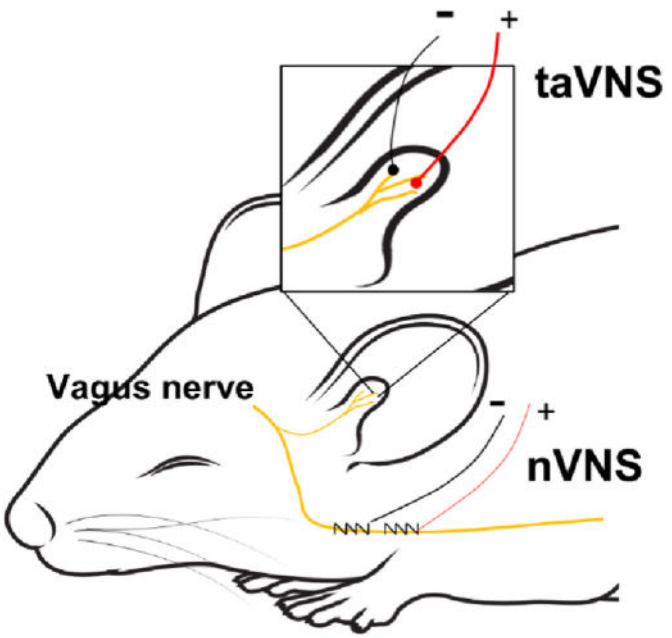

Fig. 1. Schematic diagram of nVNS and taVNS in mice. nVNS was administered by implanting electrodes into the vagus nerve of left-side neck, and the electrode was connected to a programmed electrical stimulator (square wave, 0.05 mA, 50 ms pulse width, 15 Hz, 10 mins). TaVNS was administered by fixing the electrode on left concha auriculae of mice. After fixing the electrodes, the electrodes were connected to a programmed electrical stimulator with the same stimulation parameters as VNS.

2.7. Immunofluorescence

Mice were anesthetized with 1% sodium pentobarbital and transcardially perfused with 4% paraformaldehyde before the whole brain was dissected. Then intact brains were fixed, dehydrated and embedded in OCT compound. The whole brain was placed on a frozen slicer to cut into slices. The solitary nucleus sections were labeled with c-Fos antibody (ab222699) and Alexa Fluor® 488 (ab150077). The sections of the hippocampus were labeled with Alexa Fluor® 647 (ab150075), Iba-1 antibody (#17198), and CD68 (Alexa Fluor® 488 Conjugate, #51644), and photographed using confocal microscopy.

2.8. Inflammatory cytokines ELISA assay

The levels of inflammatory cytokines including IL-1β、TNF-α、IL-6 were examined by ELISA. To get the sample, brains were removed from mice killed with cervical dislocation, and hippocampuses were stripped from the brains. Then the supernatant was diluted after grinding and centrifugation of tissue, followed by steps according to manufacturers' instructions about ELISA kits. Finally, UV absorbance was assessed by a microplate reader (OD at 450 nm) , and the levels of the samples were calculated. Each sample was run in duplicate, and the variation was less than 10%.

2.9. Statistical analysis

Statistical analysis software was used to compare multiple groups by one-way ANOVA, and $p < 0.05$ was considered statistically significant. Data were represented as mean ± standard deviation.

3. Results

3.1. Both nVNS and taVNS significantly increase C-Fos expression in NTS

Mice were placed in an open field and subjected to either nVNS or taVNS, with the nVNS group stimulating the cervical vagus and the taVNS group stimulating the auricular vagus (square wave, 0.05 mA, 50 ms pulse width, 15 Hz, 10min), and mice remained awake and could move freely during the stimulation process (Fig. 1). After the stimulation, the brains were removed immediately from mice killed with cervical dislocation and c-Fos expression in the nucleus of solitary tract (NTS) was observed by immunofluorescence.

It was found that nVNS significantly increased c-Fos expression in NTS ($p < 0.0001$), and so did taVNS ($p < 0.005$). This result proved the feasibility of these two stimulation methods. Crucially, the number of c-Fos$^+$cells activated by nVNS was significantly higher than that by taVNS ($p < 0.05$) (Fig. 2).

3.2. Both nVNS and taVNS significantly reduce anxiety in heroin-treated mice

Mice recovered for two weeks after surgery, with normal wound healing and normal weight gaining. After heroin administration, the speed of weight gain was significantly slowed down in mice, and neither nVNS nor taVNS could promote the weight gain of mice (Fig. 3B). OFT and EPM, as experimental paradigms of evaluating anxiety levels in mice, were used in this study. It was found through OFT that there was no statistical difference in total distance among

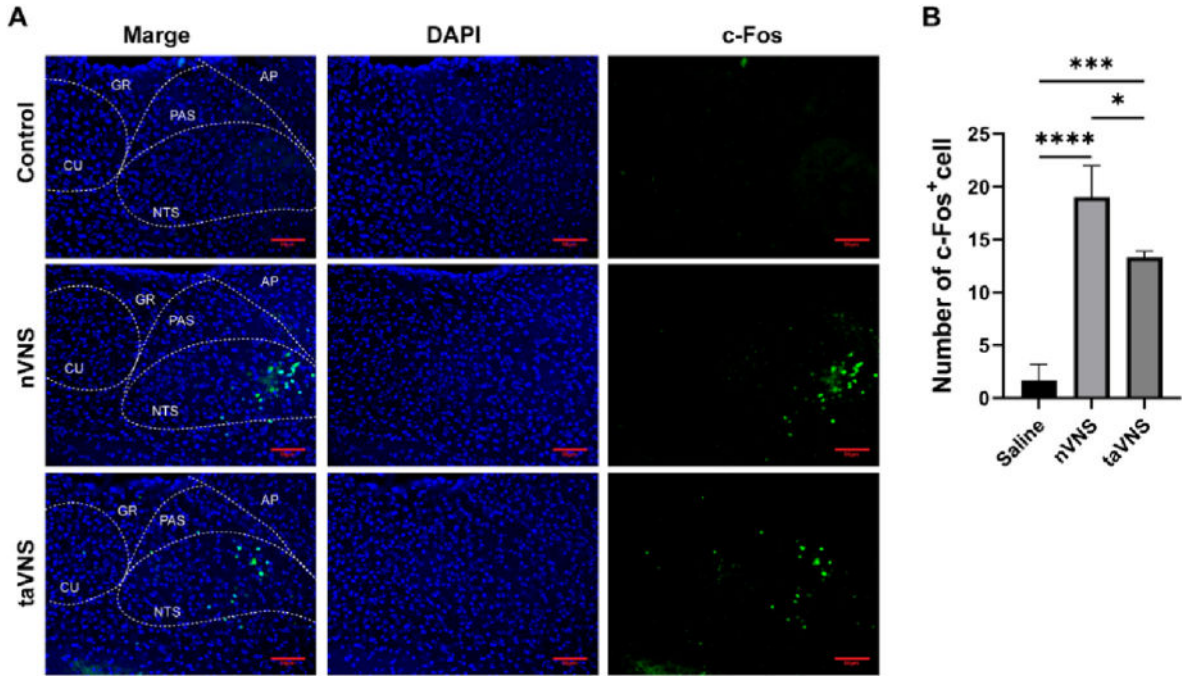

Fig. 2. Both nVNS and taVNS administrations significantly increased c-Fos expression in NTS. And compared with taVNS, nVNS had a more significant neuronal activation effect. Scale bar = 50 μm.

all groups ($p > 0.05$) (Fig. 3 E). In addition, heroin significantly reduced the time spent in the central region in mice ($p < 0.05$), and the number of feces in mice increased significantly in the course of experiment ($p < 0.0001$). Time spent in the central region was significantly increased by nVNS treatment, which was done before heroin administration ($p < 0.0001$) and the number of feces in mice was reduced ($p < 0.0001$). Similarly, Similarly, taVNS also increased time spent in the central region ($p < 0.005$) and decreased the number of feces ($p < 0.0001$) (Fig. 3C–F). Notably, mice underwent nVNS stayed in the central region longer than those administered by taVNS, although the difference was not statistically significant ($p = 0.054$) (Fig. 3 E). EPM, another classic test, was also applied to assess anxiety in this study. It was found that heroin significantly reduced both the time spent in open arms and open arms entries ($p < 0.005$), and mice exhibited anxiety-like behaviors. However, nVNS administration significantly increased the time spent of anxious mice in open arms ($p < 0.0005$), and taVNS had a similar effect ($p < 0.005$) (Fig. 3G–I). In summary, heroin caused significant anxiety in mice, but nVNS and taVNS before heroin administration significantly improved anxiety-behaviors in mice.

3.3. Both nVNS and taVNS significantly reduce the proliferation and activation of microglia in hippocampus of heroin-addicted mice

According to previous studies, hippocampus played an important role in regulating mood and cognitive function, and microglia activation in hippocampus was one of the important mechanisms leading to anxiety. In our study, heroin significantly induced the proliferation of hippocampal microglia (Iba-1+ cells) and increased the number of activated cells (CD68+cells). And both nVNS and taVNS significantly reduced the proliferation and activation of microglia (Fig. 4A). Compared with taVNS, nVNS had a more significant effect on microglia proliferation ($p < 0.05$), but there was no significant difference in the effect on microglia activation.

3.4. Both nVNS and taVNS reduce the levels of pro-inflammatory cytokines in hippocampus of heroin-treated mice

In our study, ELISA was used to detect the levels of pro-inflammatory cytokines in hippocampus of heroin-treated mice. The results showed that heroin significantly increased the levels of IL-1B, TNF-an and IL-6. After nVNS and taVNS administration, the levels of IL-1B, TNF-α and IL-6 in hippocampus of heroin-treated mice decreased significantly (Fig. 5A–C). Significantly, compared with taVNS group, the levels of IL-1B and TNF-α decreased more in nVNS ($p < 0.05$).

4. Discussion

VNS played a therapeutic role in many in many disease, such as treatment-resistant depression [17,35], epilepsy [36,37] and anxiety [19,21,25]. However, the role of VNS in the treatment of addiction-induced anxiety had not been reported. In our study, it was found that VNS had a therapeutic effect on heroin-induced anxiety, and this effect might be achieved by ameliorating neuroinflammation in hippocampus. In addition, by comparing the effects of two different stimulation methods, it was found that there was a similar effect between nVNS and taVNS. But some differences still existed between them.

Addiction was characterized by uncontrollable compulsive use of drugs, which could also lead to anxiety. The epidemiology of addiction and anxiety had been well established [38]. The use of heroin leaded to higher levels of anxiety [11] and increased anxiety sensitivity [39]. Addiction-induced anxiety had long been neglected, but in recent researches, it was increasingly believed that comorbidities, including anxiety, were important factors for addiction [5,7]. Anxiety levels could predict the age of first substance abuse in men [40], and anxiety sensitivity had also been shown to predict mental or criminal problems caused by addiction [41]. More importantly, when addiction caused anxiety [42], anxiety in turn promoted the maintenance and relapse to addiction

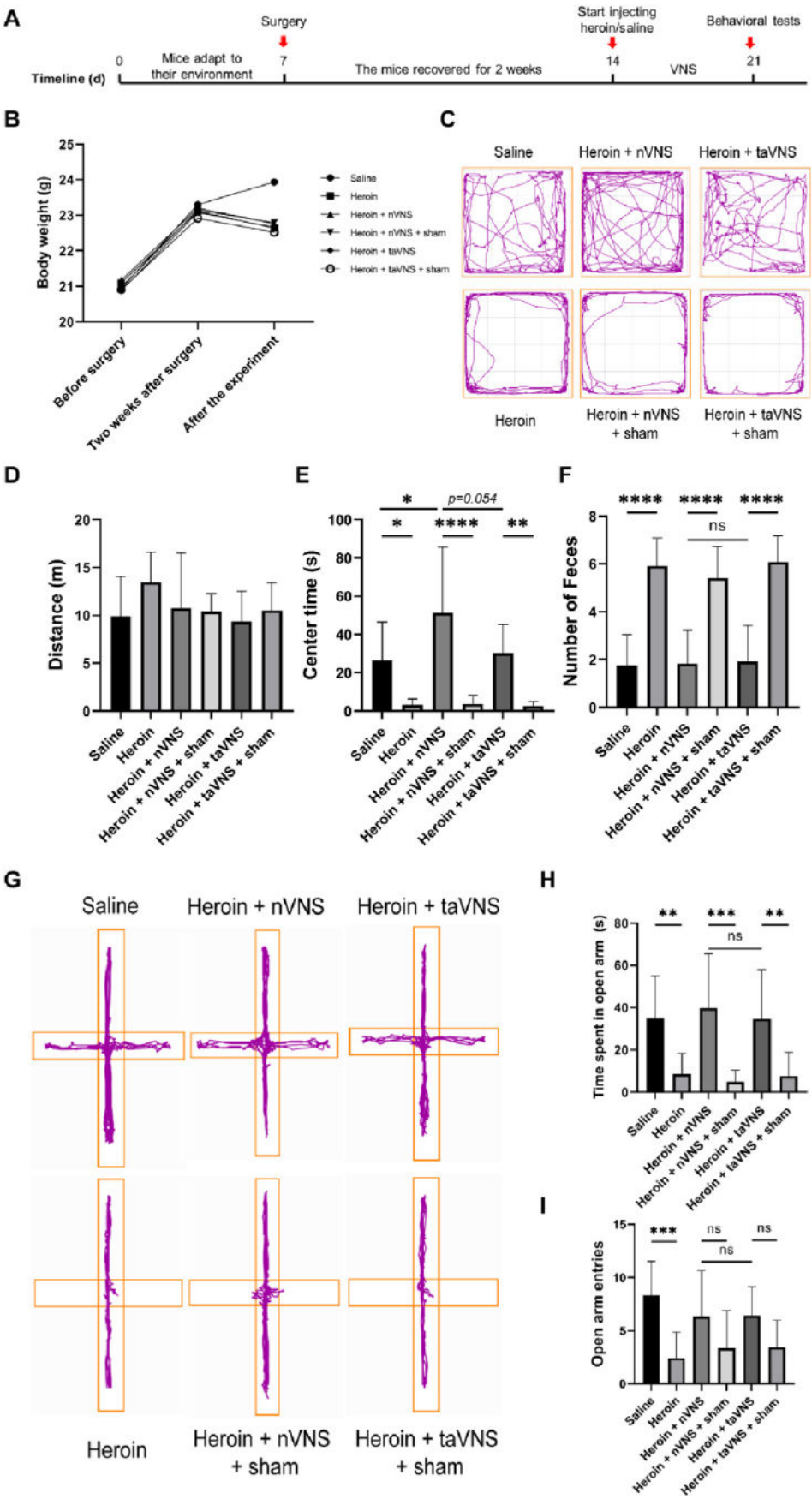

Fig. 3. Heroin induced significant anxiety in mice. But both nVNS and taVNS significantly improved anxiety in mice. (A) Timeline for the experiment. (B) Body weights of mice during the experiment. After two-weeks recovery from the operation, there was no significant differences in body weight of all groups, but the weight growth of mice significantly slowed down after heroin administration, which wasn't reversed by nVNS and taVNS (C) Representative figures of tracking in the OFT. (D) Total distance traveled in OFT. There was no significant difference in the total distance among all groups. (E) Time spent in center. (F) Number of feces in OFT. (G) Representative figures of tracking in the EPM. (H) Time spent in open arms. (I) Open arm entries.

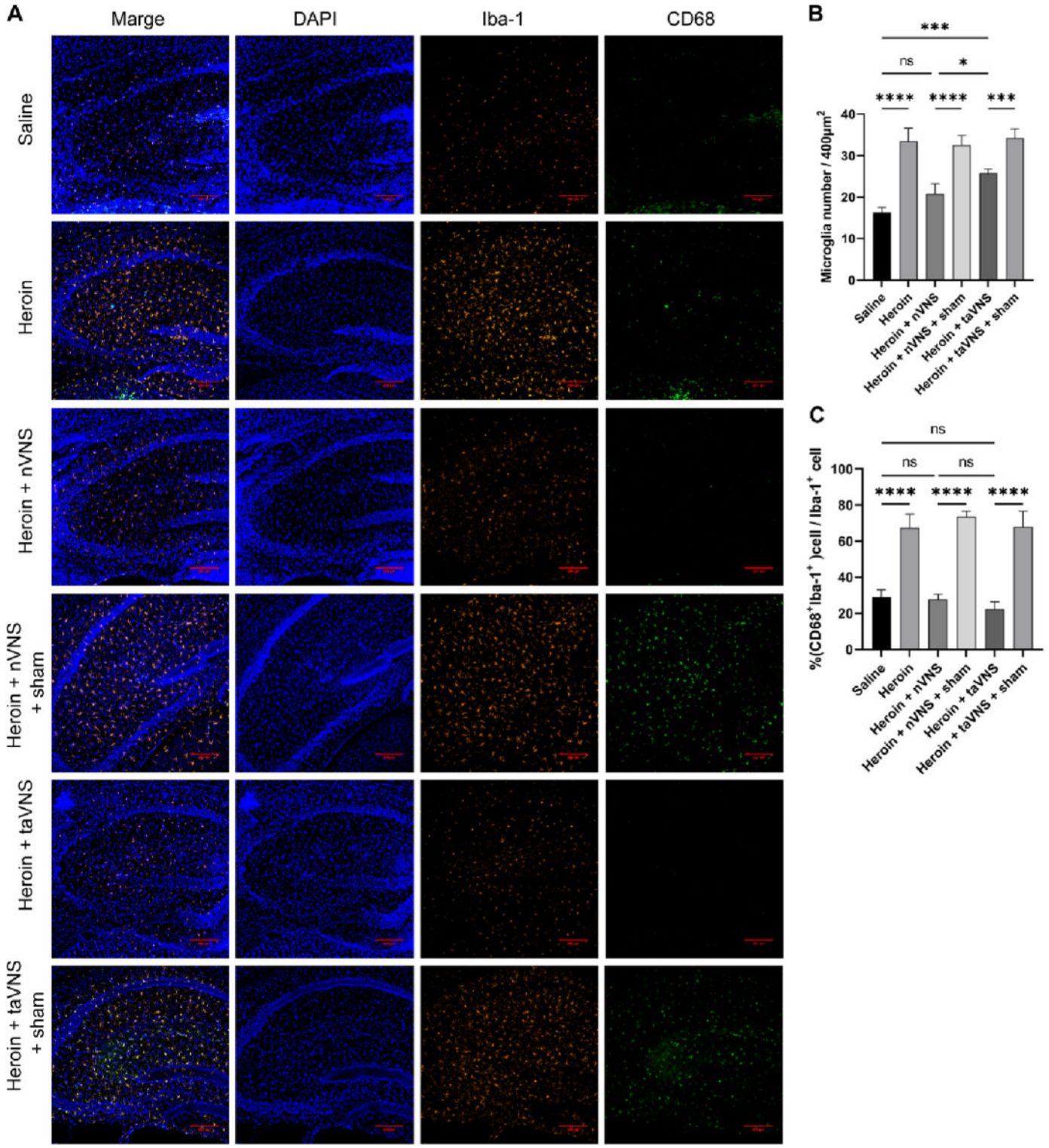

Fig. 4. Proliferation and activation degree of microglia in hippocampus of mice. (A) Results of immunofluorescence in the hippocampus of mice. Blue: DAPI, orange: Iba-1, green: CD68. (B) Number of Iba-1$^+$ cells in the same size area. (C) Ratio of CD68$^+$Iba-1$^+$ cells/Iba-1$^+$ cells in the same size area. Scale bar = 200 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

[43]. For example, alcohol addiction leaded to anxiety, and anxiety in turn promoted the maintenance and relapse to pathological alcohol use, thus forming a vicious circle [10]. Addiction and anxiety shared genetic underpinnings [44] and there might be overlap of their neural mechanisms [45], which might be the basis of the interaction between addiction and anxiety. However, there was no specific treatment for addiction-induced anxiety at present.

Vagus nerve was a bridge between brain and its peripheral organs. Its afferent fibers projected signals through nucleus tractus solitarius to important brain areas such as cortex, hypothalamus

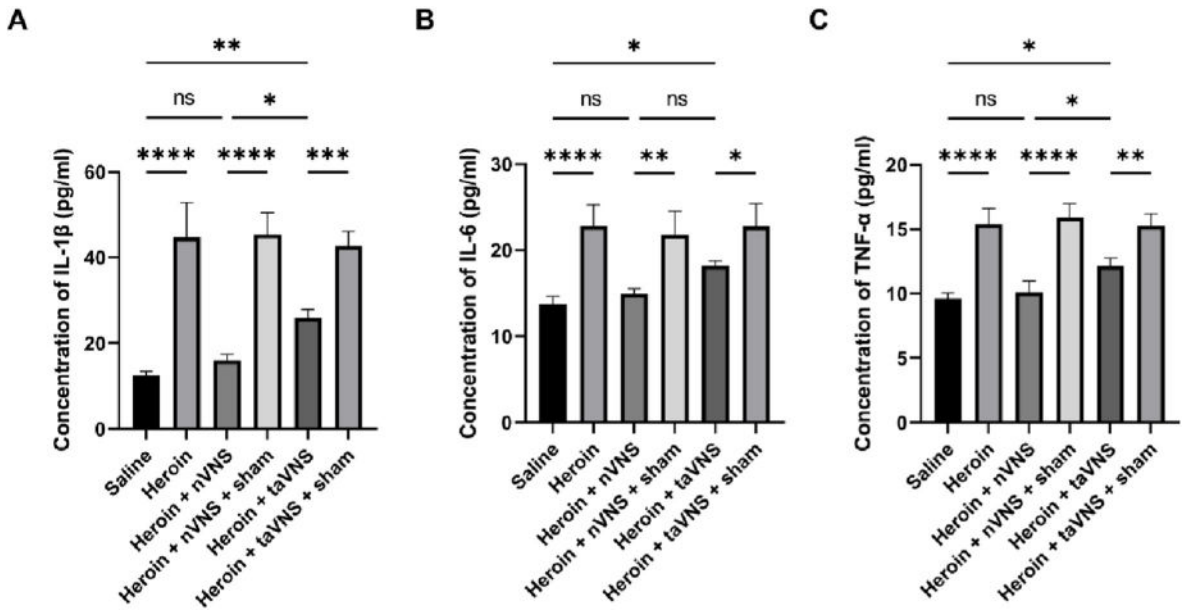

Fig. 5. Expression of pro-inflammatory cytokines IL-1B, IL-6, TNF- α in hippocampus of mice. (A) Concentration of IL-1β in hippocampus of mice. (B) Concentration of IL-6 in hippocampus of mice. (C) Concentration of TNF-α in hippocampus of mice.

and hippocampus, which was the basis of VNS treatment for mental illness. VNS showed therapeutic effects on many mental diseases, and anxiety was also included recently. And we found the therapeutic effect of VNS on drug-addicted anxiety. In our study, classical experimental paradigms were used to evaluate anxiety.

The results showed that heroin significantly caused anxiety in mice, and two different VNS methods significantly reduced the anxiety levels of heroin-treated mice, indicating the therapeutic effect of VNS on heroin-induced anxiety. Because of substance-caused anxiety played a great role in promoting the maintaining of addiction and the relapse to substance, the treatment of anxiety might improve addiction.

Neuroinflammation in hippocampus was one of the mechanisms for anxiety, and microglia activation in hippocampus could lead to anxiety-like behaviors in rats [28–30], but these behaviors could be inhibited by minocycline [31]. In our study, heroin resulted in significantly increased levels of hippocampal microglia and pro-inflammatory cytokine in mice. Both nVNS and taVNS significantly inhibited the proliferation and activation of hippocampal microglia cells and the expression of inflammatory factors induced by heroin. VNS significantly improved the microglia-mediated neuro-inflammation in heroin-addicted mice, which might be the mechanism of VNS in the treatment of heroin-induced anxiety.

In the past decades, invasive vagus nerve stimulation had been the main method of vagus nerve therapy. It worked by implanting a pacemaker-like power supply equipment below the collarbone and connecting the wires to the vagus nerve in the left carotid sheath. This scheme required surgical implantation of electrodes and power supply equipment, and the incidence of surgical complications of invasive vagus nerve stimulation was reported to be about 8.6%. The most common complications were postoperative hematoma, infection, vocal cord paralysis and hoarseness [46,47]. At the same time, surgery also brought higher economic costs, which was not a small burden for addicts with widespread financial difficulties. Physical fitness, as an important factor affecting adaptation and prognosis, was also needed to be considered, because addicts often had poor surgical condition such as malnutrition and drug abuse-related comorbidities. In recent years, more and more attention had been paid to the non-invasive technique, and it had been reported that the non-invasive technique had achieved similar therapeutic effects as the invasive technique in some diseases. However, the parameters and methods of stimulation and the therapeutic effect on other diseases were still controversial [48,49]. In our study, transcutaneous vagus nerve stimulation was selected, which was considered as a potential cure in a series of diseases [50–52].

Our results suggested that non-invasive transcutaneous auricular vagus nerve stimulation (taVNS) exhibited the similar efficacy as traditional invasive cervical vagus nerve stimulation (nVNS) in the treatment of addiction-induced anxiety in spite some difference. Compared with taVNS, nVNS activated more neurons in the NTS, and in animal behavior tests, mice receiving nVNS stayed longer in the central area than mice receiving taVNS, which was one of the important indicators to evaluate anxiety-like behaviors. In addition, nVNS showed stronger inhibitory effect on neuro-inflammation. But we couldn't simply attribute this difference to that nVNS was superior to taVNS. nVNS directly stimulated the main vagus nerve, while taVNS stimulated only one of its branches. In addition, as there was no mature stimulation program for taVNS at present, we chose the same stimulation parameters as nVNS, which might cause the loss of current conduction down through the skin. These might be the reasons for the difference between nVNS and taVNS. Despite these differences, taVNS had significant implications for substance use disorders due to its safety and convenience, and our study confirmed the therapeutic potential of taVNS in heroin-caused anxiety.

5. Conclusion

We confirmed the therapeutic role of VNS in heroin-induced anxiety, and found VNS inhibited neuroinflammation in hippocampus induced by heroin, which might be one of the important mechanisms in alleviating anxiety. Addiction leaded to anxiety, which in turn promoted the use of addictive drugs. This vicious circle was one of the reasons why addiction was difficult to treat. Therefore, the treatment of anxiety caused by addiction also had a

positive effect on addiction. At the same time, we also compared the therapeutic effects of nVNS and taVNS on anxiety caused by heroin. We found that there were similar effects between these two methods in spite of some differences. But compared with nVNS, taVNS caused less damage to patients and needed lower economic cost, which was more potential.

Data availability

The data used to support the findings of this study are available from the corresponding author upon request.

Funding

This study is funded and supported by Yunnan Provincial Government (NO.2019PT310003, NO. RLMY20200019), Yunnan Provincial Department of Health (2016NS040, 2017NS039, 2018NS122), Yunnan Provincial Department of Science and Technology (NO.202002AA100007, 202001AY070001-004) and Narcotics Control Bureau of the Ministry of Public Security of Yunnan Province.

Declaration of competing interest

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

References

[1] G. Gerra, I. Maremmani, B. Capovani, L. Somaini, S. Berterame, J. Tomas-Rossello, et al., Long-acting opioid-agonists in the treatment of heroin addiction: why should we call them "substitution, Subst. Use Misuse 44 (5) (2009) 663–671.
[2] P. Skolnick, The opioid epidemic: crisis and solutions, Annu. Rev. Pharmacol. Toxicol. 58 (2018) 143–159.
[3] A. Gutierrez, E.L. Harvey, K.M. Creehan, M.A. Taffe, The long-term effects of repeated heroin vapor inhalation during adolescence on measures of nociception and anxiety-like behavior in adult Wistar rats, Psychopharmacology 239 (12) (2022) 3939–3952.
[4] J.S. Wang, J.L. Liu, J. Zhang, J. Tan, T. Huang, C.X. Lu, et al., Descended social anxiety disorder and craving in women heroin dependence through exercise alerts plasma oxytocin levels, Front. Psychiatr. 12 (2021), 624993.
[5] F. Vorspan, W. Mehtelli, G. Dupuy, V. Bloch, J.P. Lépine, Anxiety and substance use disorders: co-occurrence and clinical issues, Curr. Psychiatr. Rep. 17 (2) (2015) 4.
[6] E.A. Prosek, A.L. Giordano, E.S. Woehler, E. Price, R. McCullough, Differences in emotion dysregulation and symptoms of depression and anxiety among illicit substance users and nonusers, Subst. Use Misuse 53 (11) (2018) 1915–1918.
[7] D.D. Brewer, R.F. Catalano, K. Haggerty, R.R. Gainey, C.B. Fleming, A meta-analysis of predictors of continued drug use during and after treatment for opiate addiction, Addiction 93 (1) (1998) 73–92.
[8] M.A. Dawes, S.M. Antelman, M.M. Vanyukov, P. Giancola, R.E. Tarter, E.J. Susman, et al., Developmental sources of variation in liability to adolescent substance use disorders, Drug Alcohol Depend. 61 (1) (2000) 3–14.
[9] R. Sinha, T. Fuse, L.R. Aubin, S.S. O'Malley, Psychological stress, drug-related cues and cocaine craving, Psychopharmacology 152 (2) (2000) 140–148.
[10] M.G. Kushner, K. Abrams, C. Borchardt, The relationship between anxiety disorders and alcohol use disorders: a review of major perspectives and findings, Clin. Psychol. Rev. 20 (2) (2000) 149–171.
[11] S. Tatalović Vorkapić, E. Dadić-Hero, K. Ružić, The relationship between personality traits and anxiety/depression levels in different drug abusers' groups, Annali dell'Istituto superiore di sanita 49 (4) (2013) 365–369.
[12] T. Lemenager, A. Richter, I. Reinhard, J. Gelbke, B. Beckmann, M. Heinrich, et al., Impaired decision making in opiate addiction correlates with anxiety and self-directedness but not substance use parameters, J. Addiction Med. 5 (3) (2011) 203–213.
[13] C.B. Nemeroff, H.S. Mayberg, S.E. Krahl, J. McNamara, A. Frazer, T.R. Henry, et al., VNS therapy in treatment-resistant depression: clinical evidence and putative neurobiological mechanisms, Neuropsychopharmacology : off. publ. Am. Coll. Neuropsychopharmacol. 31 (7) (2006) 1345–1355.
[14] O.F. O'Leary, E.S. Ogbonnaya, D. Felice, B.R. Levone, L. CC, P. Fitzgerald, et al., The vagus nerve modulates BDNF expression and neurogenesis in the hippocampus, Eur. Neuropsychopharmacol : J. Europ. Coll. Neuropsychopharmacol. 28 (2) (2018) 307–316.
[15] Y. Wang, G. Zhan, Z. Cai, B. Jiao, Y. Zhao, S. Li, et al., Vagus nerve stimulation in brain diseases: therapeutic applications and biological mechanisms, Neurosci. Biobehav. Rev. 127 (2021) 37–53.
[16] H.F.J. González, A. Yengo-Kahn, D.J. Englot, Vagus nerve stimulation for the treatment of epilepsy, Neurosurg. Clin. 30 (2) (2019) 219–230.
[17] F.R. Carreno, A. Frazer, Vagal nerve stimulation for treatment-resistant depression, Neurotherapeutics : J. Am. Soc. Exp. Neurother. 14 (3) (2017) 716–727.
[18] P. Mohr, M. Rodriguez, A. Slavíčková, J. Hanka, The application of vagus nerve stimulation and deep brain stimulation in depression, Neuropsychobiology 64 (3) (2011) 170–181.
[19] L.J. Noble, V.B. Meruva, S.A. Hays, R.L. Rennaker, M.P. Kilgard, C.K. McIntyre, Vagus nerve stimulation promotes generalization of conditioned fear extinction and reduces anxiety in rats, Brain Stimul. 12 (1) (2019) 9–18.
[20] T. Shivaswamy, R.R. Souza, C.T. Engineer, C.K. McIntyre, Vagus nerve stimulation as a treatment for fear and anxiety in individuals with autism spectrum disorder, J. Psychiatr. brain sci. 7 (4) (2022).
[21] L.J. Noble, A. Chuah, K.K. Callahan, R.R. Souza, C.K. McIntyre, Peripheral effects of vagus nerve stimulation on anxiety and extinction of conditioned fear in rats, Learn. Mem. 26 (7) (2019) 245–251.
[22] C.M. Driskill, J.E. Childs, B. Itmer, J.S. Rajput, S. Kroener, Acute vagus nerve stimulation facilitates short term memory and cognitive flexibility in rats, Brain Sci. 12 (9) (2022).
[23] R.R. Souza, N.M. Robertson, D.T. Pruitt, P.A. Gonzales, S.A. Hays, R.L. Rennaker, et al., Vagus nerve stimulation reverses the extinction impairments in a model of PTSD with prolonged and repeated trauma, Stress 22 (4) (2019) 509–520.
[24] E. Mathew, M.N. Tabet, N.M. Robertson, S.A. Hays, R.L. Rennaker, M.P. Kilgard, et al., Vagus nerve stimulation produces immediate dose-dependent anxiolytic effect in rats, J. Affect. Disord. 265 (2020) 552–557.
[25] M.S. George, H.E. Ward Jr., P.T. Ninan, M. Pollack, Z. Nahas, B. Anderson, et al., A pilot study of vagus nerve stimulation (VNS) for treatment-resistant anxiety disorders, Brain Stimul. 1 (2) (2008) 112–121.
[26] T. Bartsch, P. Wulff, The hippocampus in aging and disease: from plasticity to vulnerability, Neuroscience 309 (2015) 1–16.
[27] A. Sierra, S. Beccari, I. Diaz-Aparicio, J.M. Encinas, S. Comeau, M. Tremblay, Surveillance, phagocytosis, and inflammation: how never-resting microglia influence adult hippocampal neurogenesis, Neural Plast. 2014 (2014), 610343.
[28] A. Nagayach, R. Bhaskar, I. Patro, Microglia activation and inflammation in hippocampus attenuates memory and mood functions during experimentally induced diabetes in rat, J. Chem. Neuroanat. 125 (2022), 102160.
[29] T.A. Kunitsyna, O.I. Ivashkina, M.A. Roshchina, K.A. Toropova, K.V. Anokhin, Lentiviral transduction of neurons in adult brain: evaluation of inflammatory response and cognitive effects in mice, Bull. Exp. Biol. Med. 161 (2) (2016) 316–319.
[30] K. Riazi, M.A. Galic, A.C. Kentner, A.Y. Reid, K.A. Sharkey, Q.J. Pittman, Microglia-dependent alteration of glutamatergic synaptic transmission and plasticity in the hippocampus during peripheral inflammation, J. Neurosci. : Off. J. Soc. Neurosci. 35 (12) (2015) 4942–4952.
[31] Y.L. Wang, Q.Q. Han, W.Q. Gong, D.H. Pan, L.Z. Wang, W. Hu, et al., Microglial activation mediates chronic mild stress-induced depressive- and anxiety-like behavior in adult rats, J. Neuroinflammation 15 (1) (2018) 21.
[32] Y. Hao-Ran, L. Li-Na, G.E. Fei, Y. Jing, C. Qin-Lei, L.U. Wei-Ting, et al., [Effect of Xiaoyao San on OVX combined with CUS anxiety and depression model rats based on hippocampal microglia M1 polarization], Zhongguo Zhong yao za zhi = Zhongguo zhongyao zazhi = China journal of Chinese materia medica 45 (20) (2020) 4964–4970.
[33] T. Liu, Y. Ma, R. Zhang, H. Zhong, L. Wang, J. Zhao, et al., Resveratrol ameliorates estrogen deficiency-induced depression- and anxiety-like behaviors and hippocampal inflammation in mice, Psychopharmacology 236 (4) (2019) 1385–1399.
[34] M. Li, C. Li, H. Yu, X. Cai, X. Shen, X. Sun, et al., Lentivirus-mediated interleukin-1β (IL-1β) knock-down in the hippocampus alleviates lipopolysaccharide (LPS)-induced memory deficits and anxiety- and depression-like behaviors in mice, J. Neuroinflammation 14 (1) (2017) 190.
[35] J. Fang, P. Rong, Y. Hong, Y. Fan, J. Liu, H. Wang, et al., Transcutaneous vagus nerve stimulation modulates default mode network in major depressive disorder, Biol. Psychiatr. 79 (4) (2016) 266–273.
[36] P. Ryvlin, S. Rheims, L.J. Hirsch, A. Sokolov, L. Jehi, Neuromodulation in epilepsy: state-of-the-art approved therapies, Lancet Neurol. 20 (12) (2021) 1038–1047.
[37] A. Suller Marti, S.M. Mirsattari, K. MacDougall, D.A. Steven, A. Parrent, S. de Ribaupierre, et al., Vagus nerve stimulation in patients with therapy-resistant generalized epilepsy, Epilepsy Behav. : E&B 111 (2020), 107253.
[38] B.F. Grant, F.S. Stinson, D.A. Dawson, S.P. Chou, M.C. Dufour, W. Compton, et al., Prevalence and co-occurrence of substance use disorders and independent mood and anxiety disorders: results from the National Epidemiologic Survey on Alcohol and Related Conditions, Arch. Gen. Psychiatr. 61 (8) (2004) 807–816.
[39] C.W. Lejuez, A. Paulson, S.B. Daughters, M.A. Bornovalova, M.J. Zvolensky, The association between heroin use and anxiety sensitivity among inner-city individuals in residential drug use treatment, Behav. Res. Ther. 44 (5) (2006) 667–677.
[40] N.R. Marmorstein, H.R. White, R. Loeber, M. Stouthamer-Loeber, Anxiety as a predictor of age at first use of substances and progression to substance use problems among boys, J. Abnorm. Child Psychol. 38 (2) (2010) 211–224.

[41] G. Stathopoulou, A.K. Gold, D.L. Hoyt, M. Milligan, B.A. Hearon, M.W. Otto, Does anxiety sensitivity predict addiction severity in opioid use disorder? Addict. Behav. 112 (2021), 106644.
[42] F.M. Quitkin, A. Rifkin, J. Kaplan, D.F. Klein, Phobic anxiety syndrome complicated by drug dependence and addiction. A treatable form of drug abuse, Arch. Gen. Psychiatr. 27 (2) (1972) 159–162.
[43] D.T. George, D.J. Nutt, B.A. Dwyer, M. Linnoila, Alcoholism and panic disorder: is the comorbidity more than coincidence? Acta Psychiatr. Scand. 81 (2) (1990) 97–107.
[44] K. Hodgson, L. Almasy, E.E. Knowles, J.W. Kent, J.E. Curran, T.D. Dyer, et al., Genome-wide significant loci for addiction and anxiety, Eur. Psychiatr. : J. Assoc. European Psychiatrist. 36 (2016) 47–54.
[45] A. Lüthi, C. Lüscher, Pathological circuit function underlying addiction and anxiety disorders, Nat. Neurosci. 17 (12) (2014) 1635–1643.
[46] D. Révész, B. Rydenhag, E. Ben-Menachem, Complications and safety of vagus nerve stimulation: 25 years of experience at a single center, J. Neurosurg. Pediatr. 18 (1) (2016) 97–104.
[47] D.J. Englot, E.F. Chang, K.I. Auguste, Vagus nerve stimulation for epilepsy: a meta-analysis of efficacy and predictors of response, J. Neurosurg. 115 (6) (2011) 1248–1255.
[48] J.Y.Y. Yap, C. Keatch, E. Lambert, W. Woods, P.R. Stoddart, T. Kameneva, Critical review of transcutaneous vagus nerve stimulation: challenges for translation to clinical practice, Front. Neurosci. 14 (2020) 284.
[49] C.H. Liu, M.H. Yang, G.Z. Zhang, X.X. Wang, B. Li, M. Li, et al., Neural networks and the anti-inflammatory effect of transcutaneous auricular vagus nerve stimulation in depression, J. Neuroinflammation 17 (1) (2020) 54.
[50] A.D. Farmer, A. Strzelczyk, A. Finisguerra, A.V. Gourine, A. Gharabaghi, A. Hasan, et al., International consensus based review and recommendations for minimum reporting standards in research on transcutaneous vagus nerve stimulation (version 2020), Front. Hum. Neurosci. 14 (2020), 568051.
[51] J.A. Clancy, D.A. Mary, K.K. Witte, J.P. Greenwood, S.A. Deuchars, J. Deuchars, Non-invasive vagus nerve stimulation in healthy humans reduces sympathetic nerve activity, Brain Stimul. 7 (6) (2014) 871–877.
[52] M.F. Butt, A. Albusoda, A.D. Farmer, Q. Aziz, The anatomical basis for transcutaneous auricular vagus nerve stimulation, J. Anat. 236 (4) (2020) 588–611.