Mechanical acupuncture stimulation of HT7 attenuates alcohol intake in alcohol self-administration rats via the medial prefrontal cortex-habenula-ventral tegmental area pathway

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Abstract

Shenmen (HT7) acupuncture point stimulation is effective in alleviating alcoholism. Our study confirmed the effect of HT7 stimulation on reducing alcohol consumption via the up- and downregulation of habenula (Hb) microglial activity. To investigate the role of the Hb in mediating acupuncture effects, we evaluated the role of Hb, medial prefrontal cortex (mPFC) and ventral tegmental area (VTA) after acupuncture stimulation in the rat brain after alcohol intake. The number of active lever presses in alcohol (10% v/v) intake rats was significantly reduced following HT7 stimulation. HT7 stimulation not only attenuated the increased microglial activity and Sigma1 receptor level in the Hb and BDNF in the mPFC but also decreased TH in the VTA. Phospho-TrkB inhibition via ANA-12 microinjection into the mPFC reduced Hb microglial activity, decreased VTA TH expression and reduced alcohol intake. Blocking the Sigma1 receptor via BD1047 microinjection and inhibiting microglia via minocycline microinjection into the Hb reduced VTA TH expression and reduced alcohol intake. Controlling mPFC and Hb activity with acupuncture consequently regulated VTA TH neurons, reducing alcohol consumption. These results indicate that acupuncture treatment can alleviate alcohol consumption via the mPFC-Hb-VTA signaling pathway and could be a candidate for alcoholism therapy.

1. Introduction

Alcohol consumption has been linked to various social and economic problems throughout society. Unlike other illicit substances of abuse, alcohol is both legal and widely accepted, making its use exceptionally prevalent within our society. Excessive alcohol consumption causes various neuropathies, including depression anxiety and insomnia. According to the World Health Organization (WHO, 2018), 2.3 billion people were current drinkers in 2016. Alcoholism is also a trend that increases year after year. To date, there are only 4 pharmacological treatments approved in Europe for alcohol use disorders (AUDs): acamprosate, disulfiram, naltrexone, and nalmefene[1]. Although these pharmacological treatments reduce cravings for heavy drinking and improve abstinence rates, the problem of side effects cannot be avoided.

Acupuncture, a well-known form of alternative therapy, has been used for treating various disorders, including pain, certain drug addiction, and withdrawal syndromes. The name of acupuncture point HT7 (Shenmen) means the “gate of spirit”, and this acupuncture point is linked to traditional treatment for disorders such as amnesia, stupor, mania, insomnia, irritability and addiction[2,3]. For this reason, many clinical and preclinical studies have shown the effect mechanism of HT7 acupuncture point stimulation. For example, HT7 stimulation inhibits GABA neuron activity in the ventral tegmental area (VTA) and reduces alcohol self-administration[4–6]. In addition, HT7 acupuncture point stimulation improves alcohol-induced anxiety and alcohol intake via the modulation of corticotropin-releasing factor and neuropeptide Y in the amygdala[7]. Recently, HT7 stimulation was shown to attenuate alcohol dependence through the activation of endorphinergic input to the nucleus accumbens (NAc) from the arcuate nucleus[8]. There is much evidence that HT7 stimulation modulates neuronal and glial activity. For example, HT7 stimulation

was shown to inhibit microglial activation and modulate astrocyte proliferation in the pain model[9]. However, research on the effect of acupuncture stimulation on the regulation of the activity of glia cell in alcoholism studies are insufficient.

Growth factors regulate responses to drugs of abuse, including alcohol. Brain-derived neurotrophic factor (BDNF) is a representative growth factor. BDNF is expressed in various brain regions, including the hippocampus, NAc, amygdala, VTA and prefrontal cortex. Aberrant BDNF signaling in the medial prefrontal cortex (mPFC) contribute to the long-lasting structural changes seen in alcoholism[10,11]; the inhibition of BDNF expression in the mPFC increased alcohol self-administration, and an increase in BDNF expression decreased alcohol self-administration. From this, it can be seen that BDNF expression in the mPFC plays an important role in the impulsive consumption of alcohol. BDNF increases the release of glutamate. For this reason, an increase in BDNF in the mPFC promotes glutamate secretion, which affects various brain regions connected to glutamatergic neurons in the mPFC. Interestingly, the habenula (Hb) is one of the sites that receives glutamate projection neurons from the mPFC. There are many strategies to increase BDNF in the brain, and acupoint stimulation is one method. According to a recent study, acupuncture stimulation has been reported to increase BDNF in various brain regions, including the mPFC[12,13].

The Hb is divided into two distinct regions: the medial Hb and the lateral Hb (LHb). In recent years, research on the role of the LHb in various mental disorders has been actively conducted. According to recent studies, the LHb is thought to have a role in the progression of depression and drug abuse[14–16]. The Hb receives afferents from forebrain structures and sends projections to brainstem regions such as the raphe nuclei and VTA[17]. The LHb receives relay signal from the basal ganglia and limbic forebrain structures to mainly all midbrain monoaminergic centers, containing the serotonergic raphe and dopaminergic midbrain nuclei[18,19], regulatory mood and emotions and playing a critical part in the response to reward. Recent studies have shown that the main non-neuronal cell type of the Hb is microglia[20]. For this reason, the range of cells within the Hb has been expanded from neurons to glial cells, and studies on the function of the Hb are actively being conducted[21]. Recently, evidence has suggested that acupuncture stimulation can regulate the activity of neurons in the Hb region. Electroacupuncture was shown to alleviate hyperalgesia during ethanol (ETOH) withdrawal through a mechanism involving mu opioid receptors in the Hb[22]. The effect of HT7 stimulation involved in inhibiting cocaine-induced hyperlocomotion was shown to be induced by the c-fos activity of the dorsal columnal course and LHb[23]. Many studies have shown that acupuncture is effective in the treatment of mood and emotional disorders and drug abuse. However, despite the presence of many microglia in the Hb, studies on their role are insufficient.

This study investigated whether acupuncture had a significant effect on reducing alcohol consumption in a rat model of alcohol self-administration. We confirmed the activity of microglia in the Hb and clarified the mechanisms underlying the upregulation and downregulation of Hb activity by acupuncture stimulation.

2. Results

2.1. Effect of mechanical acupuncture treatment on alcohol self-administration

We first tested whether mechanical acupuncture stimulation of HT7 and the nonacupuncture point had a suppressive effect on alcohol intake when using the self-administration operant chamber in alcohol self-administration rats (Fig. 1a). The administration of mechanical acupuncture at HT7significantly decreased the number of alcohol-induced lever presses in the alcohol-treated group ($F (3, 26) = 7.256$, $p = 0.005$, $n = 7–8$ per group) (Fig. 1b).

2.2. Stimulation at HT7 reduced the increased microglial activity and Sigma receptor level of the Hb in the alcohol self-administration rats

Next, we measured microglial activity and sigma receptor activity in the rat Hb (Fig. 2a). We investigated whether acupuncture stimulation could change microglial and sigma receptor activity markers. Alcohol self-administration significantly increased the Arginase-1 level in the Hb. The stimulation of HT7 by mechanical acupuncture significantly decreased Arginase-1 levels in the Hb in the alcohol self-administration group ($F (3, 10) = 16.26$, $p = 0.0004$, $n = 3–4$ per group) (Fig. 2b). The Iba-1 level also increased in the alcohol self-administration group. However, the stimulation of HT7, but not the nonacupuncture point, significantly decreased the Iba-1 level in the Hb in the alcohol self-administration group ($F (3, 10) = 6.380$, $p = 0.0109$, $n = 3–4$ per group) (Fig. 2c). The sigma receptor-1 level increased in the Hb in the alcohol self-administration group. However, the stimulation of HT7, but not the nonacupuncture point, significantly decreased the sigma1 receptor level in the Hb in the alcohol self-administration group ($F (3, 9) = 6.93$, $p = 0.0103$, $n = 3–4$ per group) (Fig. 2d).

2.3. Stimulation at HT7 increased the BDNF level in the mPFC and decreased TH in the VTA in the alcohol self-administration rats

In the next experiment, the expression levels of BDNF in the mPFC and TH in the VTA were measured when HT7 was stimulated in alcohol self-administration rats (Fig. 3a). Alcohol self-administration significantly decreased the BDNF level in the mPFC. The stimulation of HT7, but not the nonacupuncture point, by mechanical acupuncture significantly increased BDNF levels in the mPFC in the alcohol self-administration group ($F (3, 10) = 37.64$, $p = 0.0001$, $n = 4$ per group) (Fig. 3b and d). In addition, the expression of TH in the VTA was measured when HT7 was stimulated in alcohol self-administration rats. Alcohol self-administration significantly increased TH expression in the VTA. The stimulation of HT7, but not the nonacupuncture point, by mechanical acupuncture significantly decreased dopamine neuron expression in the VTA in the alcohol self-administration group ($F (3, 10) = 29.11$, $p = 0.0001$, $n = 3–4$ per group) (Fig. 3c and d).

2.4. Inhibition of pTrkB in the mPFC reduced the expression of microglia in the Hb, decreased TH expression in the VTA and reduced alcohol intake

We used ANA-12, an antagonist of TrkB, to determine how the BDNF increase in the mPFC affected sigma receptor and microglial activity in the Hb. As shown in Fig. 4, the administration of a low dose (50 nmol/μl) of ANA-12 did not significantly decrease arginase-1 in the Hb (Fig. 4a). However, the administration of 100 and 200 nmol/μl ANA-12 significantly decreased Arginase-1 in the Hb ($F (3, 11) = 11.64$, $p = 0.0010$, $n = 3–4$ per group) (Fig. 4b), and the administration of 200 nmol/μl ANA-12 dose significantly decreased TH levels in the VTA ($F (3, 11) = 9.768$, $p = 0.0020$, $n = 3–4$ per group) (Fig. 4c). Next, we tested whether the microinjection of 100 nmol/μl ANA-12 had a suppressive effect on alcohol intake in alcohol-addicted rats in the self-administration operant chamber. The microinjection of 100 nmol/μl ANA-12 into the mPFC significantly decreased the number of alcohol activation lever presses compared with the injection of vehicle ($t = 2.586$, $p = 0.0271$, $n = 6$ per group) (Fig. 4d).

2.5. Blocking the sigma receptor and inhibiting microglia in the Hb reduced the expression of TH in the VTA and reduced alcohol intake

We used a microglial inhibitor, minocycline (MINO), and a sigma receptor antagonist, BD1047 (BD), to determine how the activation of the sigma receptor and microglia in the Hb affected TH in the VTA region (Fig. 5a). The administration of a low dose (50 nmol/μl) of BD did not significantly decrease TH in the VTA. However, the administration of 100 and 200 nmol/μl BD significantly decreased TH in the VTA ($F (3, 10) = 16.44$, $p = 0.0003$, $n = 3–4$ per group) (Fig. 5b). Similarly, the administration of all doses (50, 100 and 200 nmol/μl) of MINO significantly decreased TH in the VTA ($F (3, 10) = 32.05$, $p = 0.0001$, $n = 3–4$ per group) (Fig. 5c). Next, we tested whether the microinjection of 50 nmol/μl MINO and 100 nmol/ml BD had a suppressive effect on alcohol intake in alcohol-addicted rats in the self-administration operant chamber. The microinjection of 50 nmol/μl MINO and 100 nmol/μl BD into the Hb significantly decreased the number of alcohol activation lever presses compared with the microinjection of vehicle ($F (2, 16) = 3.089$, $p = 0.0734$, $n = 7$ per group) (Fig. 5d).

3. Discussion

The present study showed that the number of activation lever presses in alcohol self-administration rats was reduced by mechanical acupuncture at HT7 (also known as “Shenmen”). Alcohol self-administration increased the activity of two types of microglia and increased the expression of sigma1 receptors in the Hb. Repeated HT7 stimulation reduced the activity of microglia increased by alcohol administration and attenuated the expression of the sigma1 receptor. Alcohol self-administration decreased the expression of BDNF in the mPFC and increased the expression of TH in the VTA. HT7 stimulation increased BDNF expression and decreased TH levels. Moreover, the administration of a BDNF receptor antagonist to the mPFC inhibited microglial activity in the Hb and TH activity in the VTA in a concentration-dependent manner. Furthermore, the administration of a microglial inhibitor and sigma1 receptor antagonist to the Hb inhibited VTA TH activity in a concentration-dependent manner. The microinjection of antagonists and inhibitors into the brain regions decreased the number of activation lever responses in alcohol self-administration rats. Based on these data, it is possible that acupoint stimuli operate through the mPFC-Hb-VTA pathway.

Our results showed that 10% (v/v) alcohol administration significantly increased the number of activation lever presses in alcohol self-administration rats compared to the administration of saline. This result is consistent with previous reports that the administration of mechanical acupuncture at HT7 significantly decreased 10% (v/v) alcohol consumption compared with the control treatment[24].

In the present study, alcohol self-administration significantly increased microglial markers (Arginase-1 and Iba-1 levels) in the Hb. The stimulation of HT7 by mechanical acupuncture significantly decreased Arginase-1 and Iba-1 levels in the Hb compared to the stimulation of the nonacupuncture point. Consistent with these findings, alcoholism increases microglia activity in the human brain[25]. Additionally, intermittent alcohol exposure in adult rats also triggers transitory cell activation and the expression of inflammatory cytokines[26]. Microglial proliferation is commonly observed after cell death[27], and a model of binge drinking produces neuronal cell death[28,29]. There are previous studies investigating the inhibition of microglial hyperactivity by acupuncture in a traumatic brain injury model and vascular dementia model[30,31], but studies in an alcoholism model are insufficient. This study is the first attempt to control microglial hyperactivity in the Hb of rats due to alcoholism with acupuncture treatment. According to our results, when microglia activity increased, Sigma-1 receptor expression also increased in the Hb. Sigma-1 receptors are highly expressed in neurons as well as microglia[32]. This receptor has been shown to play a key role in motivation, learning and memory. The Sigma-1 receptor is considered among the most promising targets in addiction. For this reason, several studies have shown that Sigma11 receptors are involved in the motivational effect of psychostimulants such as cocaine ,methamphetamine and alcohol[33–35]. Our results suggest that alcohol self-administration increases microglia activity and Simga-1 receptors, and HT7 may decrease their activity.

The mPFC is the main brain region to send glutamate neuron projections to the Hb[36]. Various neurotransmitters and growth factors activate glutamate neurons. BDNF is a representative growth factor contributing to the activation of glutamate neurons. Increased BDNF plays an important neuroprotective role in AUDs in the mPFC. Many studies investigating the mechanism of acupuncture have shown that BDNF activity in the mPFC mediates various alcohol-related behaviors[37,38]. The increase in BDNF levels in the mPFC and the depressive behavior of rats in a maternal separation in a rat model of depression model were alleviated by HT7 acupoint stimulation[6]. Consistent with these findings, our results showed that the reduced BDNF expression in the mPFC due to alcohol intake was alleviated by HT7 stimulation.

The mesolimbic dopaminergic system is thought to play an important role in mediating the positive reinforcing effects of alcohol and other drugs of abuse[39,40]. This circuit comprises dopaminergic neurons in the VTA and their projections to the NAc and numerous other brain regions. The LHb sends inhibitory reward signals to VTA-DA neurons and inhibit DA release in the NAc, and neurons in the LHb can be excited by peripheral stimulation[41]. Interestingly, alcohol self-administration significantly increased TH activity and HT7 stimulation reduces the activity of TH increased by alcohol administration in the VTA. This result can explain the increase in the number of active levers by alcohol administration as the activation of VTA DA neurons.

Next experiment, we demonstrated the up- and downregulation mechanisms contributing to the regulation of microglial and sigma1 receptor activity in the Hb. First, an antagonist of the BDNF receptor TrkB (ANA-12) was used to determine whether the increase in BDNF in the mPFC affected the Hb and VTA. According to our results, the inhibition of BDNF activity in the mPFC reduced the activity of microglia in the Hb and decreased the activity of TH in the VTA. Decreased activity of TH in the VTA resulted in decreased alcohol intake. Our behavioral test results are consistent with the results of a study showing that the microinjection of BDNF into the mPFC reduced alcohol consumption in alcohol-dependent subjects[42]. On the basis of this result, it can be suggested that the BDNF activity of the mPFC can regulate alcohol consumption through the regulation of proteins in the Hb and VTA. Interestingly, this study is also the first to examine the Hb and VTA after the administration of a TrkB antagonist to the mPFC in an alcohol self-administration model.

Next, an inhibitor of microglia (MINO) and an antagonist of the sigma1 receptor (BD1047) were used to determine whether the decrease in Hb activity affected TH expression in the VTA. According to our results, microinjections of MINO into the Hb significantly reduced TH in the VTA. Our results also showed that the microinjection of 50 nmol/μl MINO into the Hb significantly reduced alcohol intake. In addition, microinjections of BD into the Hb significantly reduced TH in the VTA. Our results also showed that the microinjection of 100 nmol/μl BD into the Hb significantly reduced alcohol intake. According to an alcohol consumption study related to MINO administration, treatment with 50 mg/kg MINO significantly reduced alcohol intake in male and female C57Bl/6J mice using a free choice voluntary drinking model[43]. In addition, MINO (50 mg/kg/day, 4 days) reduced alcohol intake in a binge drinking model[44]. A selective antagonist, BD, blocked alcohol-induced locomotor stimulation, conditioned place preference, and conditioned taste aversion[45]. The sigma1 receptor antagonist BD-1063 reduced alcohol consumption in alcohol-dependent rats have been shown to have higher levels of the sigma1 receptor in the NAc[46]. Our results are also consistent with those of these previous studies. Taken together, our results show that alcohol self-administration increased the activity of microglia and the sigma1 receptor in the Hb, and HT7 stimulation alleviated this activity in the Hb. The upstream regulator of this Hb activity was an increase in the BDNF activity of the mPFC. Controlling the activity of the mPFC and Hb with acupuncture consequently regulated TH neurons in the VTA, reducing alcohol intake. These results indicate that acupuncture treatment alleviates alcohol consumption via the mPFC-Hb-VTA signaling pathway and could be a candidate for alcoholism therapy.

4. Methods

4.1. Animal preparation

Adult male Wistar rats (250–300 g) were purchased from Orient Bio (Seongnam, Korea). The rats were provided with sufficient food and water, and the light-dark cycle was maintained at a 12-hour cycle throughout the study period. This study was conducted after being reviewed and approved by the Animal Care Committee of the Korea Institute of Oriental Medicine (KIOM, Daejeon, Korea).

4.2. Alcohol self-administration

Alcohol self-administration was achieved in an operant chamber (measuring 31.8 cm × 25.4 cm × 26.7 cm, MED Associates Inc., Georgia, VT, USA) equipped with two response levers and a house light. When the experimental animal pressed the activation lever once, a 10% alcohol solution was dropped into the 0.1ml chamber central dish, and the animal ate it. And the lights were turned on during self-administration. In contrast, when the animals pressed the inactivation lever, the response was noted but there was no significant consequence. The animals experienced behavioral training five days a week for 20 min a day. Rats were trained to become addicted to alcohol using a modified sucrose fading method. In brief, rats gradually became accustomed to pressing the lever while training to learn the activation lever. In the initial self-administration training, 20% sucrose solution was used to adapt the lever pressing process. After the rats learned to press the lever to drink the 20% sucrose solution, the sucrose solution was lowered to 10% in the next training session. After a stable baseline response was established, the concentration of alcohol and sucrose was adjusted with 1 week with 2% alcohol in 10% sucrose, 1 week with 5% alcohol in 10% sucrose, and 1 week with 10% alcohol in 5% sucrose. Acupuncture was started when the rats began to show consistent responses to 10% alcohol. The criteria for the alcohol baseline were determined by the mean value of three consecutive alcohol intakes, using the animals that showed a stable response to 10% alcohol, with a variation of less than 20%.

4.3. Acupuncture treatment

Manual acupuncture is a method in which a needle is inserted into acupuncture points and the needle is twisted. A mechanical acupuncture instrument (MAI) was used to quantitatively provide the effectiveness of manual acupuncture. Daegu University of Oriental Medicine (Daegu, Korea) made this equipment and was provided for this study. The needles (0.18 × 8 mm, Dong Bang Medical, Gyeonggi-do, Korea) were connected to the MAI, and the MAI provided stimulation to the acupoint site with an intensity of 1.3 m/s2 and a frequency of 85 Hz. The acupuncture point HT7 (located on the ulnar aspect of the wrist) and a nonacupuncture point located on the upper part of the left buttock were used in this study. All rats were acclimatized to the experimental procedure including acupuncture without needle insertion for one week before the start of the experiment. The MAI was applied bilaterally at the acupuncture points and the left buttock for 30 s and maintained for up to 1 min after needle insertion.

4.4. Microinjection and treatment

General anesthesia was administered by intraperitoneal injection of Rumpun (0.1 ml, Bayer, Korea) and Zoletil (0.4 ml, Zoletil 50, Virbac Lab, Carros, France). During deep anesthesia, the rats' heads were placed in a stereotaxic apparatus (Stoelting; Wood Dale, IL, USA). The cranium was exposed, and a drill (35000 rpm; Saeshin Dental Lab, South Korea) was used to drill a hole to implant a cannula into the mPFC (AP = + 3.4 mm; ML = − 8 mm; DV = − 5 mm) and Hb (AP = − 3.9 mm; ML = ± 1 mm; DV = − 4.7 mm) according to the stereotactic coordinates of the rat brain atlas (1998 edition). After surgery, lidocaine (Laboratorios PISA, Mexico City, Mexico) was applied topically to the area around the cannula implant for pain relief. After a week of recovery, the operated rats were reintroduced into the alcohol self-administration chamber.

Vehicle was injected through a stainless steel cannula connected to a 5 μl Hamilton syringe. The environment was created for the mice to move freely. And 2 μl of vehicle was microinjected over 5 min using an automatic infusion pump (KD Scientific, Holliston, MA, USA) (speed: 0.4 μl/min). To prevent the administered vehicle from returning due to capillary action, it was left for 5 minutes after microinjection.

4.5. Western blot

After 20 minutes of providing the final acupoint stimulation, the rats were anesthetized with N2O/O2 gas, and the whole brain was extracted. After the brain extracted from the rat was cut using a matrix, only our target brain regions were selectively collected. Brain samples were lysed in RIPA buffer, subjected to 3 cycles of 20 s sonication, and incubated for 1 h on ice. Brain samples were precipitated by centrifugation (60 min at 13000 rpm) and only the supernatant was isolated, and the protein content of the supernatant was anal yzed using the Bradford method. The Samples were electrophoretically separated on a Bis-Tris gel (4–10%) and then transferred to a nitrocellulose membrane. The membrane was treated with a blocking reagent (TOYOBO Life Science, Osaka, Japan) to block non-specific antibody binding. Membranes were incubated with primary antibodies (anti-BDNF, anti-Argimase-1, anti-Iba-1, anti-BiP, anti-Sigma Receptor1, anti-TH and anti-β-actin) at a dilution of 1:1000 in blocking solution in PBS overnight at 4°C. After overnight at 4°C, membranes were washed 3 times and incubated with secondary antibody for 1 hour at room temperature The secondary antibody was horseradish peroxidase (HRP)-labeled goat anti-rabbit or goat anti-mouse (1:1000, KPL, Gaithersburg, MD, USA). An enhanced chemiluminescent reagent (Thermo Fisher) was used to visualize the protein bands on the membrane. The amplified fluorescence signal of the membrane was imaged using a Fusion SL4 imaging system (Vilber Lourmat, Eberhardzell, Germany), and the results were obtained using ImageJ software (http://rsb.info.nih.gov/ij/) has been quantified.

4.6. Statistical analysis

tatistical analysis was performed using Prism 6 (GraphPad Software, San Diego, CA, USA). All data analyzed were expressed as mean ± standard error of the mean (SEM). For the alcohol intake analysis, data were compared using a one-way analysis of variance (ANOVA) or unpaired t-test. Post hoc analysis was performed using Tukey’s multiple comparison test to determine differences in values among experimental groups. Statistical significance was considered when $p < 0.05$.

Declarations

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Author Contributions

Participated in the research design: SYS and YR. Conducted the experi-ments: SYS and SKB. Contributed new reagents or analytical tools: SYK and SKB. Performed the data analysis: SJC and KHC. Wrote or contributed to the writing of the manuscript: SYS and YR. All authors read and approved the final manuscript.

Competing Interests

The authors declare no competing interests.

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Figures

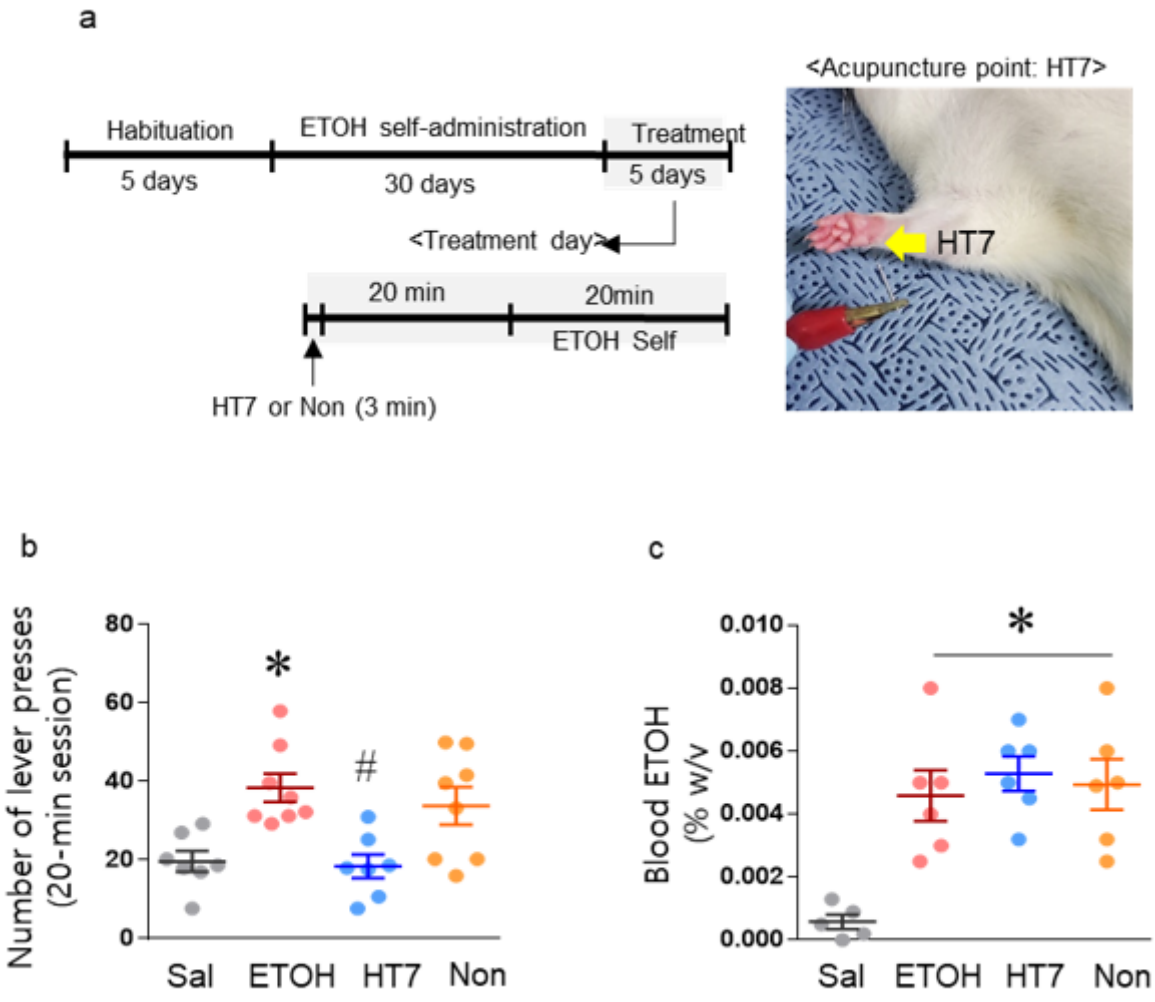

Figure 1

Effect of mechanical acupuncture treatment on alcohol self-administration. The experimental schedule for acupuncture stimulation was performed at acupuncture point Shenmen (HT7) (**a**). The change in the number of lever presses and HT7 stimulation in alcohol self-administration rats (n = 8 for each group) (**b**). The data were analyzed using repeated-measures one-way ANOVA followed by Tukey's test. *p < 0.05 vs. the Saline (Sal) group; # p < 0.05 vs. the ETOH group. Values are expressed as the mean ± SEM.

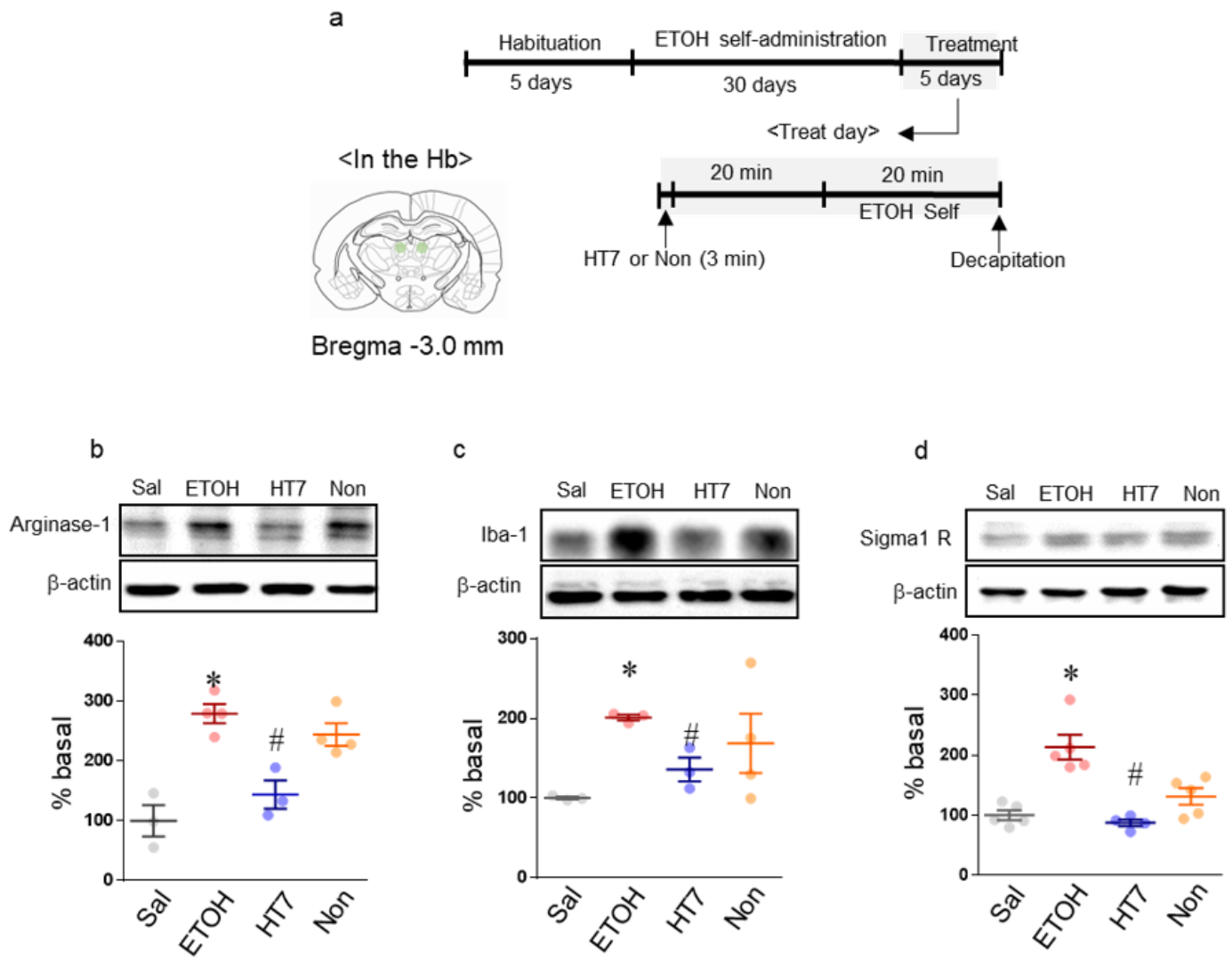

Figure 2

The effects of acupuncture stimulation on microglial activity and sigma receptor levels in the LHb. A schematic illustration showing the region of the Hb and the experimental schedule (**a**). The effects of mechanical acupuncture on microglial marker (arginase-1 and Iba-1) expression in the Hb in alcohol-addicted rats (n = 3-4 for each group) (**b** and **c**). The effects of HT7 stimulation on Sigma1-receptor activity and BiP expression in the Hb in alcohol-addicted rats (n = 4-5 for each group) (**d**). The original western blot images are available in Supplementary Figure S1, respectively. The data were analyzed using repeated-measures ANOVA followed by Tukey's test. *p < 0.05 vs. the saline (Sal) group; # p < 0.05 vs. the ETOH group. Values are expressed as the mean ± SEM. Non = nonacupuncture point.

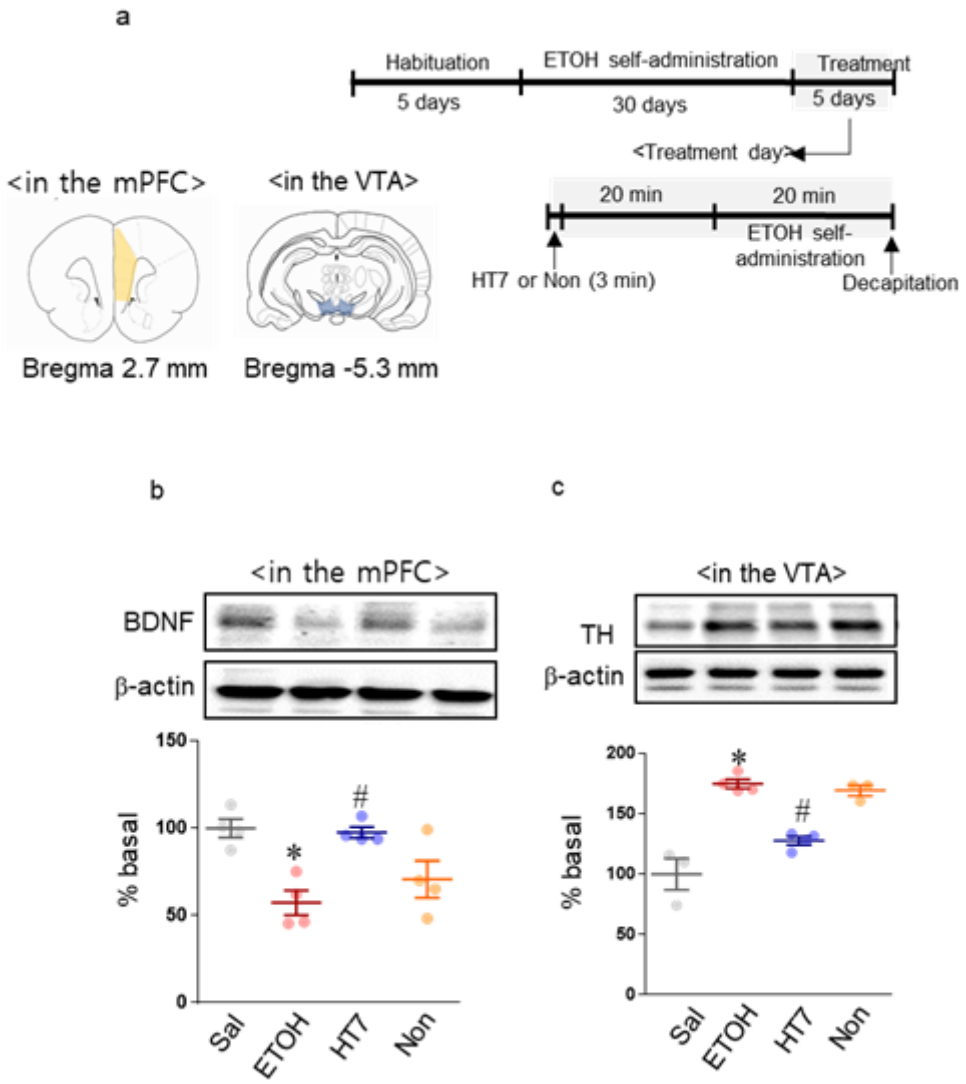

Figure 3

Effects of acupuncture stimulation on BDNF expression in the mPFC and TH expression levels in the VTA. Schematic illustrations showing the regions of the mPFC and VTA and the experimental schedule (**a**). The effects of mechanical acupuncture on BDNF expression in the mPFC in alcohol self-administration rats (n = 3-4 for each group) (**b** and **d**). The effects of mechanical acupuncture on TH expression in the VTA in alcohol addicted-like rats (n = 3-4 for each group). (**c** and **d**). The original western blot images are available in Supplementary Figure S2, respectively. The data were analyzed using repeated-measures ANOVA followed by Tukey's test. *p < 0.05 vs. the saline (Sal) group; # p < 0.05 vs. the ETOH group. Values are expressed as the mean ± SEM. Non = nonacupuncture point.

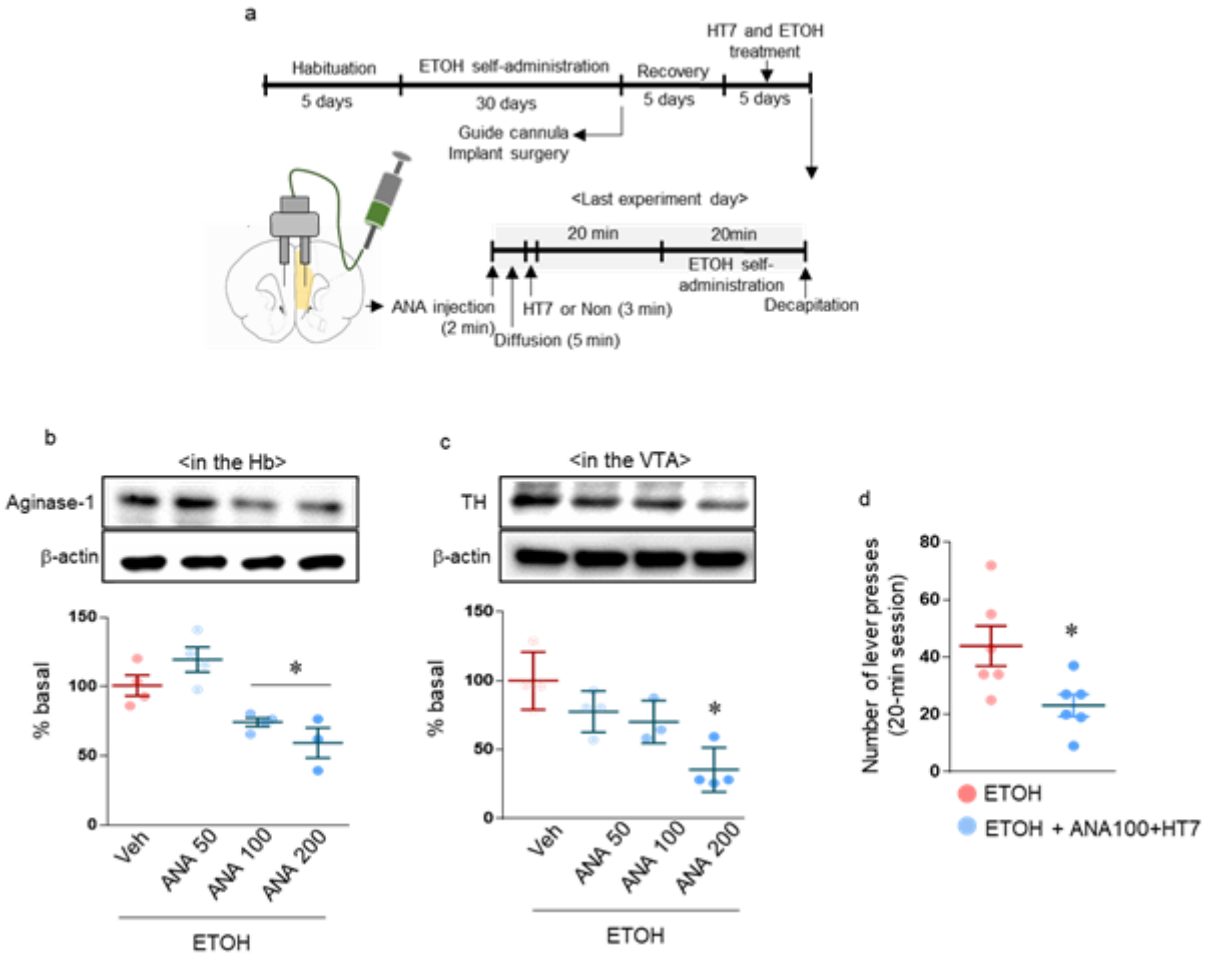

Figure 4

Effects of BDNF receptor antagonist microinjection on decreased levels of Arginase-1 and TH. A schematic illustration showing the configuration of the microinjection needle placement in the mPFC experimental schedule (**a**). The restoration of Arginase-1 levels after the microinjection of the BDNF receptor antagonist ANA-12 into the mPFC (n = 3-4 for each group) (**b**). The restoration of TH levels after the microinjection of the BDNF receptor antagonist ANA-12 into the mPFC (n = 3-4 for each group) (**c**). The change in the number of lever presses after the microinjection of ANA-12 into the Hb in alcohol self-administration rats (n = 6 for each group) (**d**).). The original western blot images are available in Supplementary Figure S3, respectively. The data were analyzed using repeated-measures ANOVA followed by Tukey's test. *p < 0.05 vs. the vehicle (Veh) group. Values are expressed as the mean ± SEM.

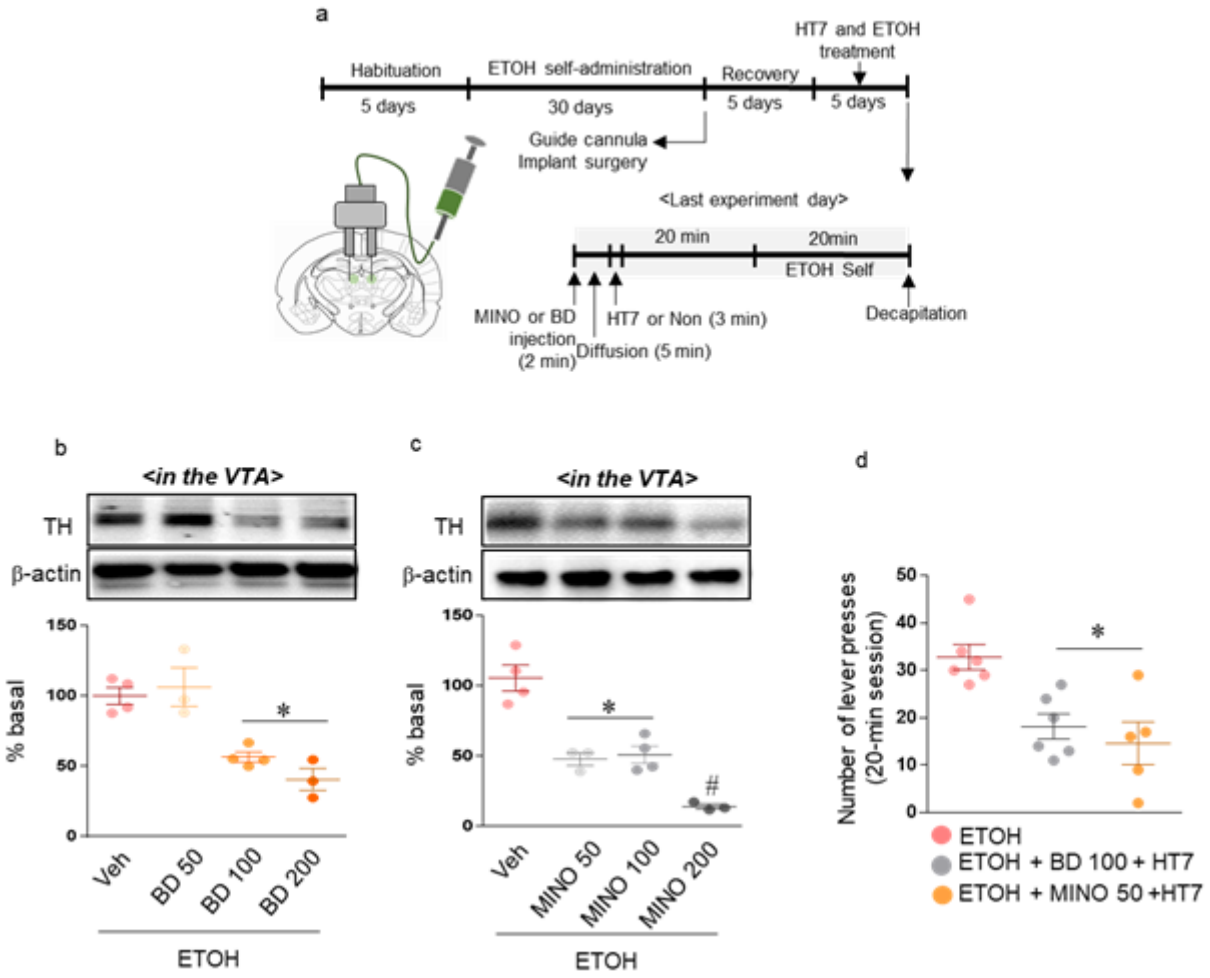

Figure 5

Effects of a microglial inhibitor and Sigma receptor antagonist on decreased TH levels. A schematic illustration showing the configuration of the microinjection needle placement in the Hb and the experimental schedule (**a**). The restoration of TH levels in the VTA after the microinjection of the microglial inhibitor MINO into the Hb (n = 3-4 for each group) (**b**). The restoration of TH levels in the VTA after the microinjection of the Sigma1 receptor antagonist BD into the Hb (n = 3-4 for each group) (**c**). The change in the number of lever presses after the microinjection of MINO and BD into the Hb of alcohol self-administration rats (n = 7 for each group (**d**). The original western blot images are available in Supplementary Figure S4, respectively. The data were analyzed using repeated-measures ANOVA followed by Tukey's test. *p < 0.05 vs. the vehicle (Veh) group; # p < 0.05 vs. the MINO 50 and 100 nmol groups. Values are expressed as the mean ± SEM.

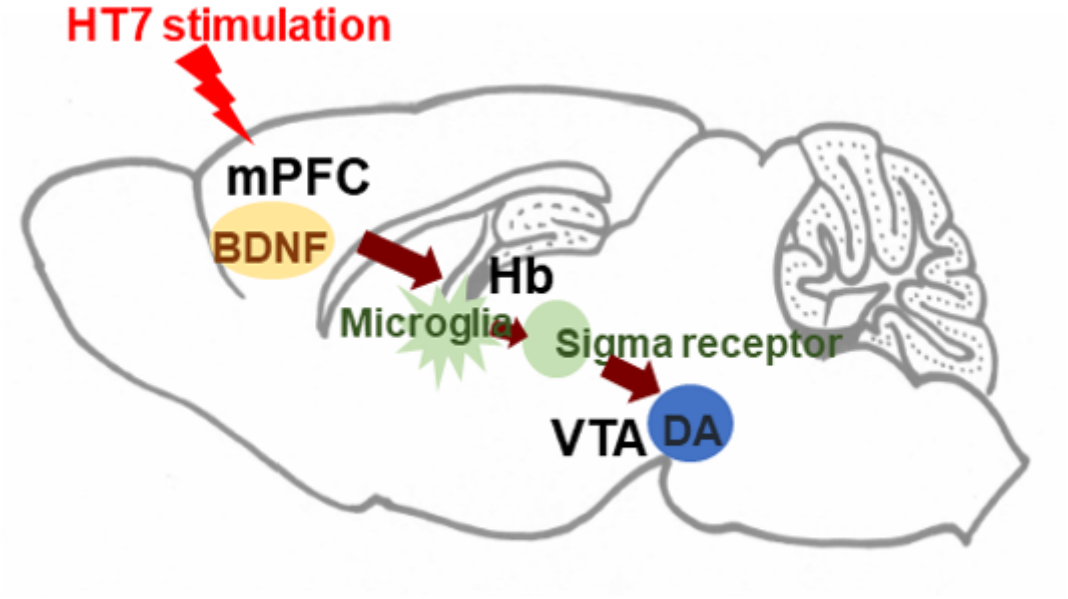

Figure 6

Schematic diagram illustrating the mechanism of the effects of acupuncture stimulation. The mPFC-Hb-VTA-based mechanism to reduce alcohol intake by acupoint stimulation.

Supplementary Files

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