Hippocampal Inhibitory Synapses Deficits Induced by α5-containing GABAA Receptors Mediates Chronic Neuropathic Pain-related Cognitive Impairment

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Research Article

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Abstract

Chronic neuropathic pain often leads to cognitive impairment, but the exact mechanism remains unclear. Gamma-aminobutyric acid A receptors ($GABA_ARs$) are the major inhibitory receptors in the brain, of which the α5-containing $GABA_ARs$ ($GABA_ARs$-α5) are implicated in a range of neuropsychiatric disorders with cognitive deficits. However, whether $GABA_ARs$-α5 are involved in chronic neuropathic pain-related cognitive impairment remains unknown. In this study, the rats with chronic neuropathic pain induced by right sciatic nerve ligation injury (SNI) exhibited cognitive impairment with declined spontaneous alternation in Y-maze test and discrimination index in novel object recognition test. The $GABA_ARs$-α5 expressing on parvalbumin and somatostatin interneurons increased remarkably in hippocampus, which resulting in decreased mean frequency of spontaneous inhibitory postsynaptic currents in hippocampal pyramidal neurons. Significantly, antagonizing the $GABA_ARs$-α5 by L655,708 rescued weakened inhibitory synaptic transmission and cognitive impairment induced by chronic neuropathic pain. Taken together, these data suggest that the $GABA_ARs$-α5 play a crucial role in chronic neuropathic pain-induced cognitive impairment by weakening inhibitory synaptic transmission, which may provide insights into the pharmacologic treatment of chronic neuropathic pain-related cognitive impairment.

1. Introduction

Chronic neuropathic pain is a serious public health problem worldwide [1]. It has been reported that patients with chronic pain usually exhibit symptoms of various neuropsychological disorders, such as chronic fatigue, depression, anxiety, sleep disturbances and cognitive impairment [2, 3]. Among these symptoms, cognitive impairment which contains impaired attention, learning, memory, planning and problem-solving abilities has been of great concern. Importantly, patients with chronic neuropathic pain tend to present with cognitive decline that has a huge impact on their daily functioning and living quality. Thus, it is imperative to uncover the cellular and molecular mechanisms underlying chronic neuropathic pain-related cognitive impairment. However, the exact mechanism remains elusive.

Inhibitory interneurons are critical for regulating complex network functions in the brain, and dysfunction of them would damage cognitive function. Parvalbumin (PV) and somatostatin (SST) interneurons are two main subtypes of interneurons, accounting for about 40–50% and 30% of total interneurons respectively [4, 5]. The suppressed interneurons would bring about weakened synaptic transmission which play a vital role in shape hippocampal output and memory [4, 6]. Indeed, in the early stages of neuropsychiatric disorders, such as schizophrenia, Alzheimer disease, post-traumatic stress disorder, deficits in inhibitory neurotransmission might drastically account for significant cognitive impairment [7–9]. However, little is known concerning the alterations of inhibitory synaptic neurotransmission in cognitive impairment caused by chronic neuropathic pain.

Gamma-aminobutyric acid receptors ($GABA_ARs$), a main inhibitory neurotransmitter ligand-gated ion channel in the brain, are composed of five different protein subunits: α1–6, β1–3, γ1–3, ρ1–3 and one

each for δ, ε, θ or π subunits [10]. Among them, the α5-containing $GABA_A$Rs ($GABA_A$Rs-α5) are concentrated in hippocampus and show a high sensitivity to GABA with slow desensitization rate. Of note, $GABA_A$Rs-α5 are closely associated with learning and memory [10–12]. The increased activity of $GABA_A$Rs-α5 was observed in different animal models such as traumatic brain injury, depression and inhalation anesthesia-related memory impairment [13–15]. The overactivity of $GABA_A$Rs-α5 has been reported to result in severe memory impairment, while systemic inhibition the function of $GABA_A$Rs-α5 using L655,708 significantly improved short-term recognition and memory in rodents [13]. However, the studies about $GABA_A$Rs-α5 in chronic neuropathic-related cognitive impairment were few. In this study, we examined whether $GABA_A$Rs-α5 were involved in memory impairment after unilateral sciatic nerve injury (SNI) in rats. Moreover, whether $GABA_A$Rs-α5 inverse agonist, L655,708, could improve chronic neuropathic pain-related cognitive Impairment. Finally, we explored the mechanisms of how $GABA_A$Rs-α5 participated in these processes.

2. Materials And Methods

2.1 Animals

Adult male Sprague-Dawley (SD) rats (8–10 weeks) were obtained from Nanjing Qinglongshan Animal Breeding Farm, China. The SD rats were reared in an air-conditioned, 12-hour light/black cycle, pathogen free facility at 25°C ± 1°C, and have free access to food and water. All experiments were approved by the Zoological Committee of Nanjing Medical University (Ethical code IACUC:2101035), and performed to comply strictly with the guidelines accepted by the International Association for the Study of Pain.

2.2 Experiment

To determine whether peripheral nerve injury led to cognitive deficits in behaviors, sciatic nerve injury (SNI) or Sham surgery (n = 8/group) was performed. The neuropathic pain was induced by SNI in keeping with the procedure described by Decosterd and Woolf.[16] In Sham group, after peritoneal anesthesia with 1% pentobarbital sodium (0.5ml/100g) in rats, the right common peroneal nerve and tibial nerve were exposed, ligated tightly with 5 – 0 wires and transected at the distal end to remove 2–4 mm length of each nerve. Any contact or stretching of the sural nerve was avoided. Then, the muscle and skin layer were sutured in turn. In Sham group, the sciatic nerve and its terminal branches were exposed without ligation. The mechanical paw withdrawal threshold (MPWT) was examined before surgery and on days 3, 7, 14, and 21 after surgery. The open field (OF) was carried on days 7, 14, and 21 after surgery The novel object recognition (NOR) test was performed on days 8, 15, and 22 after surgery, whereas the Y-maze on days 9, 16, and 23 after surgery.

To evaluate the effect of the $GABA_A$Rs-α5 inverse agonist L655,708 on nociceptive behaviors and SNI-induced cognitive deficits in behaviors, 2 groups of rats (n = 8/group) were used. L655,708 (Sigma-Aldrich, Oakville, ON, Canada), dissolved in 10% dimethylsulfoxide solution, was used to antagonise $GABA_A$Rs-α5 receptors. The rats in SNI L655 group were intraperitoneally injected with L655,708

(1mg/kg) 30 minutes before the behavioral tests according to previous report.[17] The rats in SNI VEH group were intraperitoneally injected with 10% dimethylsulfoxide. Rats were subjected to the following tests: MPWT, NOR, Y maze tests, on 21 to 23 days after surgery. MPWT were performed on 21, 22, 23 days. In NOR test, inhabiting period was performed on 21 days after surgery and then training and testing periods on 22 days. Y-maze was performed on 23 days. After all of the behavioral tests, the brains were collected (Fig. 1).

2.3 Nociceptive behavioral test

The mechanical paw withdrawal threshold (MPWT) was measured according to “up-down” method. Briefly, the rats were placed in a transparent plexiglas box, and allowed to acclimate for 15 minutes. When the rats were quiet, Von-Frey filaments (0.6, 1.0, 1.4, 2.0, 4.0, 6.0, 8.0, 15g) were used to vertically stimulate the median planar paw of rats successively (the lateral planar skin of rats was stimulated after SNI), and the reaction of paw withdrawal was observed. The maximum force applied was 15 g to avoid any injury.

2.4 Open field test

Motion analysis in open field test is the most standardized measure for motor function. The rats were placed in the center of square chamber (100×100cm) with walls 40 cm in height and allowed to explore freely for 10 minutes. Each rat was gently placed in the center of the area and followed by a video tracking system (XR-XZ301; Shanghai Softmaze Information Technology Co., Ltd., Shanghai, China) automatically recording their activities. The total distance traveled was recorded.

2.5 Novel object recognition test

The novel object recognition test was conducted in the square chamber (100×100 cm). The rats were placed in a chamber without any object to explore and adapt the environment freely for 10 minutes. Twenty-four hours later, the rats were placed in a training chamber with two objects with same shape and texture and allowed to explore the objects freely for 10 minutes. The time of exploring each object was recorded. After an interval of 4 hours, a test was carried out in the same chamber in which a familiar object was replaced by a novel object with the same texture but different shape. The rats were allowed to explore the objects freely for 10 minutes, and the time spent on each object was recorded again. The recognition index is defined as the ratio of the time spent exploring novel objects to the total exploration time.

2.6 Y-maze test

The Y-maze device (XR-XY1032; Shanghai Softmaze Information Technology Co., Ltd., Shanghai, China) consists of three equidistant arms(120°;50 cm×10 cm×25 cm). The rat was placed at the end of one arm and allowed to explore freely for 8 minutes. It was considered to have visited the arm when the rat’s body and tail were completely inside the arm. Total number of arm entries and spontaneous alternation were recorded during this period. Spontaneous alternation was defined as a continuous selection into all three

arms (ABC, BCA, BAC et al.), which was calculated with the following formula: spontaneous alternation/ (sum of arm runs − 2) *100%.

2.7 Western blot

After behavioral tests, all of the rats were anesthetized by intraperitoneal injection pentobarbital sodium 50 mg/kg, and then their hippocampus were quickly collected and stored at -80℃. Hippocampal tissues were well mixed with RIPA lysis buffer containing protease inhibitors and the mixture were centrifuged at 12,000 r/min for 30 minutes to retain the supernatant. BCA protein kit was used to detect protein concentration. The proteins were separated by 12.5% SDS- PAGE gels and transferred to PVDF membranes. The membrane was sealed with 5% BSA for 1 h at room temperature (RT) and incubated with the following primary antibodies: rabbit anti-GAPDH (10494-1-AP; 1:10000; Proteintech); mouse anti-c-fos (sc-166940; 1:1000; Santa Cruz); rabbit anti $GABA_A$Rs-α5 (NBP2-75497; 1:1000; Novus). Then, the membrane was blotted with secondary antibody rabbit anti- IgG (GB23303; 1:3000; Servicebio) or mouse anti-IgG (GB23302;1:3000□Servicebio) for 2 h at RT. The protein bands were detected by chemiluminescence method and Image J Software (National Institute of Health, USA) was used to measure the band intensity.

2.8 Immunofluorescence

Animals were instilled with 0.1M phosphate buffer saline (PBS) and 4% paraformaldehyde (PFA) under deep anesthesia after the last behavioral test. Then, the brains were fixed in 4% PFA for 12 h and dehydrated in 30% sucrose at 4℃. The freezing microtome was applied to slice the brains in coronal sections of 20 μm thickness. Sections were permeabilized in PBS mixed with 0.1% Triton X-100 (PBT) and blocked with 10% fetal calf serum for 2 h at RT. Then, sections were incubated in primary antibody: rabbit anti-$GABA_A$Rs-α5 (NBP2-75497; 1:200; Novus), mouse anti- $GABA_A$R-α5 (NBP2-59700;1:200; Novus), mouse anti-SST (sc-74556; 1:50; Santa Cruz), rabbit anti-PV (ab11427; 1:500; Abcam), rabbit anti-c-fos (ab222699; 1:100; Abcam), mouse anti-CaMKII-α (MA1-048; 1:100; ThermoFisher) overnight at 4℃. After being washed in PBT, sections were incubated in secondary antibody: Alexa Fluro 594 goat anti-mouse (SA00013-3; 1:500; Proteintech), Alexa Fluro 594 goat anti-Rabbit (SA00013-4; 1:500; Proteintech), CoraLite488-conjugated Affinipure Goat Anti-Mouse (SA00013-1; 1:500; Proteintech), CoraLite488-conjugated Affinipure Goat Anti-Rabbit (SA00013-2; 1:500; Proteintech). The nuclei were stained by DAPI (Sigma). The ImageJ was used to analyse images viewed at 10×magnification with fluorescence microscope (MF31, Mshot, Guangzhou, China).

2.9 Electrophysiology

According to previously described electrophysiology,[18, 19] brain slices were placed in the recording chamber and completely submerged in ACSF (bubbled with 95% O_2/5% CO_2). The pyramidal neurons in hippocampus CA1 were recorded. The infrared optics, using an upright microscope equipped with a × 40 water-immersion lens (Olympus BX51W1, Japan), and an infrared-sensitive CCD camera were employed to record the whole-cell. The pipette solution contained the following: 8 NaCl, 125 potassium D-gluconate, 0.2 EGTA, 0.3 NaGTP ,10 HEPES, 2 Mg-ATP. Patch pipettes were pulled on a horizontal pipette puller (P-97,

Sutter Instrument). The pyramidal neurons with stable series resistance were used for analysis. Data were recorded by an Axon patch 700B amplifer (Molecular Devices), low-pass filtered at 2 kHz and digitally sampled at 10 kHz online and Clampft software (Molecular Devices) was used. Spiking patterns were recorded in the current-clamp configuration by injecting a series of current pulses (400 ms duration, -50 to 350 pA intensity with an increment of 50 pA) to characterize the intrinsic membrane properties of neurons. After the rupture of the neuronal membrane, the resting membrane potential was recorded immediately and the action potential threshold was defined as the first 400 ms rectangular current injection that elicited a spike. The spontaneous inhibitory postsynaptic currents (sIPSCs) and spontaneous excitatory postsynaptic currents (sEPSCs) were recorded for at least 5 min after baseline stabilization. The sIPSCs and sEPSCs were analyzed by Mini Analysis Program (Version 6.0.3, Synaptosoft). To characterize the intrinsic membrane properties of pyramidal neurons, spiking patterns were recorded in the current-clamp confguration by injecting a series of current pulses (400 ms duration, 100 to 350 pA intensity with an increment of 50 pA).

2.10 Statistical analyses

All data were presented as mean ± SEM. To compare the differences between two groups, student *t* tests were used. Two-way analysis of variance followed by Dunnett's post hoc test was applied to calculate the values of MPWT, OF, NOR and Y-maze tests. The data were analyzed by using GraphPad prism 8.3. *P* value less than 0.05 was considered statistically significant.

3. Results

3.1 Chronic sciatic nerve injury led to mechanical hyperalgesia and cognitive impairment

The MPWT, OF, NOR and Y-maze tests were conducted after surgery (Fig. 2A). Compared with Sham group, the SNI group showed significant reductions in MPWT on 3, 7, 14, and 21 days after surgery (Fig. 2B). There was no difference in locomotor function between the Sham and SNI groups in OF test (Fig. 2C). The NOR and Y-maze tests were used to assess cognitive function. In NOR test, compared with Sham group, the discrimination index and the exploration time of novel object were significantly decreased on 21 days but not 7 and 14 days after SNI (Figs. 2D-G). The representative traveling traces exploring familiar object A1 and novel object B were shown in Figs. 2D-F. In Y-maze test, the spontaneous alternation was remarkably declined on 21 days but not 7 and 14 days after SNI compared with Sham group and no differences in total number of arm entries were observed between 2 groups (Figs. 2H and I). Collectively, our data show that the cognitive function was impaired in neuropathic pain rats.

3.2 The hippocampal inhibitory and excitatory synaptic transmission were disrupted in SNI rats

The whole-cell patch-clamp was performed to record the sIPSCs and sEPSCs of pyramidal neurons in hippocampal CA1 region slices between the Sham and SNI rats (Fig. 3A). The increased mean frequency of sEPSCs in SNI group was observed, and there was no statistical significance in mean amplitude between 2 groups (Figs. 3B-D). The mean frequency of sIPSCs were significantly reduced in the SNI neurons and there was no statistical difference in the amplitude (Figs. 3E-G). These results indicated that chronic neuropathic pain disrupted the balance of inhibitory and excitatory synaptic transmission in hippocampus.

3.3 The expression of $GABA_A$Rs-α5 on PV and SST interneurons were increased in hippocampus

Since the decrease of inhibitory synaptic transmission was observed in SNI rats, which had been poorly explored in SNI model, we further investigated the mechanism of it. Firstly, we used western blot analysis to detect the protein levels of PV and SST, and no significant differences were observed between 2 groups in hippocampus (Figs. 4A-D). Immunohistochemical staining showed that the amount of γ-aminobutyric acid (GABA) receptor subunits, $GABA_A$Rs-α5, were increased in hippocampus CA1 and CA3 region (Figs. 4E and F). Consistent with these findings, western blot result showed the level of $GABA_A$Rs-α5 in SNI group was remarkably higher than that of Sham group (Figs. 4G and H). In addition, the co-immunostaining showed that $GABA_A$Rs-α5 expressing on PV and SST interneurons increased in hippocampal CA1 region (Figs. 4I-K).

3.4 The glutamate decarboxylase which was specialized to synthesize GABA was downregulated

Given that $GABA_A$Rs-α5 loaded on PV and SST interneurons were upregulated which could suppress the function of them, we next explored the change of GABAergic system. The two main enzymes of the GABAergic system, glutamate decarboxylase 67 (GAD67) and 65 (GAD65), were detected. The protein expressions of GAD67 were obviously downregulated in SNI group (Figs. 5A and B). However, the level of GAD65 had no statistical difference between Sham and SNI group (Figs. 5C and D). Our results suggested that the GABAergic system was partly suppressed in chronic neuropathic pain.

3.5 Antagonizing $GABA_A$Rs-α5 by L655,708 improved the cognitive impairment in SNI rats

We further examined the effects of the $GABA_A$Rs-α5 inverse agonist L655,708 on nociceptive hypersensitivity and cognitive deficits (Fig. 6A). Following the administration of L655,708 or vehicle, MPWT had no statistical difference (Fig. 6B). In NOR test, the exploration time spent on two identical object was similar in the training session. However, in testing session, L655,708 significantly improved the decreased exploration time of novel object and the reduction in discrimination index in SNI rats

(Figs. 6C and D). Beside the Fig. 4C were the representative traveling traces exploring familiar object A1 and novel object B. In Y-maze, L655,708 remarkably blocked the decreased spontaneous alternation of SNI rats and total number of arm entries had not differences among the 2 groups (Figs. 6E and F). These results suggested that $GABA_A$Rs-α5 were involved in the cognitive dysfunction.

3.6 Antagonizing $GABA_A$Rs-α5 by L655,708 reversed the weakened GABAergic system and inhibitory synapses

Western blot analysis showed that L655,708 application did not change the protein expression of $GABA_A$Rs-α5 (Figs. 7A and B), while up-regulated the level of GAD67 (Figs. 7C and D). Next, we detected whether L655,708 administration had effect on reduction frequency of sIPSCs in SNI rats. L655,708 increased the frequency of sIPSCs compared with the vehicle-treated neurons. The amplitude of sIPSCs had no significant difference between the 2 groups. These results suggested that $GABA_A$Rs-α5 were involved in the dysfunction of GABAergic system.

4. Discussion

In the present study, we confirmed the previous finding that chronic neuropathic pain induced by SNI challenge impaired cognitive function. Importantly, we showed that chronic neuropathic pain induced excessive $GABA_A$Rs-α5 expressing on PV and SST interneurons, resulting in weakened inhibitory synaptic transmission and sequentially contributing to cognitive impairment in rodents (Fig. 8).

Chronic neuropathic pain was often caused by lesions or diseases of the somatosensory system, such as major surgeries or traumas, diabetic neuropathy, stroke and herpes zoster [20]. It is estimated that chronic neuropathic pain afflicts 7–10% of the population, which has tightly associated with cognitive impairment [21]. Recently, preclinical studies also proved memory deficits in the animal model of chronic neuropathic pain by using NOR and Y-maze tests [22]. In our study, the SNI model was used to induce chronic neuropathic pain. According to previous finding that right sciatic nerve injury showed more significant cognitive impairment than the left in rodents, we adopted the former method to establish SNI model. The results demonstrated that SNI rats with reduced long-lasting nociceptive threshold presented the deficits of recognition and working memory as evaluated by the NOR and Y-maze tests. Due to SNI surgery did not alter total moving distance of rats, these deficits were unlikely attributed to the injury in locomotor function caused by the surgery. As illustrated in the neurocognitive behavioral tests, rats appeared memory deficits in NOR and Y-maze tests on 21 rather than 7 and 14 days after surgery, which was most partly consistent with previous studies [22, 23]. Specifically, unlike our results, a previous study showed that SNI produced the working memory impairments on 14 days after surgery in Y-maze test [22]. The inconsistency in animal species (mice vs rats) or basal nociceptive response levels might account for these differences.

$GABA_A$Rs-α5 are most concentrated in hippocampus, accounting for about 25% of all hippocampal $GABA_A$Rs [24]. $GABA_A$Rs-α5 are highly sensitive to GABA and show a slow desensitization rate, mainly mediating low concentration GABA-mediated tonic inhibition outside the synapses and inhibitory postsynaptic current in inhibitory postsynaptic membrane [25]. This specific distribution suggests a potential role of $GABA_A$Rs-α5 in regulating the activity of neurons and hippocampal dependent cognition. In preclinical models, increased expression or activity of $GABA_A$Rs-α5 contributed to cognitive impairment associated with many neuropsychiatric diseases such as traumatic brain injury, perioperative neurocognitive disorders and stroke, and inhibiting these receptors can mitigate the deficits [13, 26, 27]. Whereas, up to now, there has been little research focusing on whether $GABA_A$Rs-α5 were involved in cognitive impairment under chronic neuropathic pain conditions. Our study showed that the expression of $GABA_A$Rs-α5 in hippocampus were up-regulated under the existence of chronic neuropathic pain, and antagonizing $GABA_A$Rs-α5 by L655,708 improved cognitive function, which consistent with previous reports [15, 28]. This finding suggested that $GABA_A$Rs-α5 may play a crucial part in chronic neuropathic pain-related cognitive impairment. Additionally, previous studies have demonstrated that chronic pain increased $GABA_A$Rs-α5 expression in the spinal cord and dorsal root ganglia, this receptor could be a valid pharmacological target to treat chronic pain states [29, 30]. Whether the up-regulated $GABA_A$Rs-α5 on spinal level are associated with chronic neuropathic-mediated hippocampus memory impairment is unknown and remains to be investigated in future studies. The reverse agonist L655,708 used in this study was carefully selected to preferentially inhibit $GABA_A$Rs-α5, which shows a preference for $GABA_A$Rs-α5 receptors and occupy 60–70% of $GABA_A$Rs-α5 at a dose of 1mg/kg [31]. Moreover, L655,708 did not cause significant off-target behavior effects, such as dyskinesia and sedation [31], which avoided its impact on the accuracy of behavioral results. Additionally, the chronic neuropathic pain caused by SNI surgery was not relieved by L655,708 injection, suggesting that L655,708 alleviated cognitive impairment by directly antagonizing increased $GABA_A$Rs-α5 rather than relieving pain.

So far, changes in the inhibitory synaptic transmission in hippocampus linked with allodynia and neuropsychiatric aspects of chronic neuropathic pain have been poorly explored. Deficits in inhibitory synaptic transmission may account for underlying mechanism for impaired cognition [32], GABAergic interneurons are of great importance in regulating synaptic plasticity and synchronizing activity in the CA1 region of the hippocampus, both of which are essential in proper cognitive function [33, 34], PV and SST interneurons, two main subsets of inhibitory interneurons, have been reported to have a great impact in coding of neuronal information and the regulation of learning and memory [35, 36]. Interneuron-specific plasticity at PV and SST inhibitory synapses onto CA1 pyramidal neurons shapes hippocampal output [6]. On the one hand, previous studies had found that photogenetic activation of PV interneurons in SNI rats led to mechanical hypersensitivity which were migrated by suppression of PV interneurons [37]. In chronic inflammatory pain models, the decreased function of PV interneurons was observed [38]. In addition, it has been reported that SST interneurons were less active in SNI model [39]. On the other hand, a down-regulation or a disruption of PV interneurons in hippocampus would contribute to sepsis-induced cognitive impairment [40]. Suppressing the function of SST neurons reduces synchronized

cellular and neural activity, and resulted in cognitive dysfunction [41]. Taken together, PV and SST interneurons are not only involved in chronic pain but also in cognitive regulation. Therefore, it is reasonable to deduce that interneurons might have a great effect on the development of chronic neuropathic pain-related cognitive impairment.

PV interneurons provide intensive rhythmic inhibition to pyramidal neurons by forming inhibitory synapses, embracing the soma and axon initial segment of pyramidal neurons [42], and SST interneurons regulate local synaptic and dendritic conductances by forming dendritic inhibition to pyramidal neurons [43]. $GABA_A$Rs-α5 on nonpyramidal cells are the essential effectors controlling plasticity in learning and memory [44]. Our results showed that $GABA_A$Rs-α5 loaded in PV and SST interneurons was increased in hippocampus of SNI rats, indicating that $GABA_A$Rs-α5 coordinated SST and PV interneurons in hippocampus to disinhibit pyramidal neurons and damage cognition. These data demonstrated that $GABA_A$Rs-α5 were essential in cognitive processes by modulating a component of synaptic transmission in hippocampal CA1 region. The accumulating evidence demonstrated that reduced inhibitory synaptic transmission induced by GABAergic interneurons dysfunction would bring about impaired information processing in the hippocampus [45, 46]. Recently, Magnin et al.[47] found that $GABA_A$Rs-α5 were highly expressed in SST interneurons in stratum oriens/alveus layer and targeted by vasoactive intestinal peptide (VIP) and calretinin to form inhibitory synaptic connections and L655,708 could improve spatial memory in CA1-VIP-off mice. We observed that the sIPSCs mean frequency of pyramidal neurons significantly decreased in SNI rats which likely suggested a selective suppression of GABAergic inhibitory interneurons, resulting in failing to form powerful presynaptic inhibition projecting to pyramidal neurons. Consistent with previous study that the rate of sIPSCs decay was highly sensitive to modulators of the $GABA_A$Rs [48], L655,708 administration in this study effectively rescued the decrease frequency of sIPSCs. What's more, glutamate decarboxylase is a key enzyme in γ-aminobutyric acid (GABA) synthesis, in which GAD67 plays a major role. The down-regulated level of GAD67 suggested the deficits in GABAergic activity in this study, and may contribute to damaged cognitive processes. According to previous study, the attenuated inhibitory synaptic transmission may disrupting hippocampus-mediated memory function of everyday-type memory performance, as assessed on the watermaze delayed-matching-to-place task [49]. The selective inverse agonist at $GABA_A$Rs-α5 enhanced watermaze delayed-matching-to-place task performance and the induction of long-term potentiation at hippocampal synapses. Thus, the weakened GABAergic synaptic transmission caused by upregulated $GABA_A$Rs-α5 in hippocampus was likely the underlying mechanism for cognitive impairment after peripheral nerve injury.

In summary, our behavioral and electrophysiological evidence indicated $GABA_A$Rs-α5 play a vital role in chronic neuropathic pain-related cognitive impairment, likely through suppressing inhibitory synaptic transmission. It could be a prospective molecular target for the treatment strategy of cognitive impairment under chronic neuropathic pain conditions in clinical practice.

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Declarations

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Author Contribution

Xuechun Cai, Jie Sun and Wei Zhu contributed to study design; Xuechun Cai, Jie Sun and Wei Zhu contributed to manuscript editing; Lili Qiu, Chaoran Wang, Hang Yang, Zhenhui Zhou, Meng Mao, and Yunqing Zhu contributed to experimental studies; Xuechun Cai, Lili Qiu, Chaoran Wang, Yazhou Wen and Wenlan Cai contributed to data analysis. All authors read and approved the final manuscript.

Declaration of Competing Interest

The authors report no declarations of interest.

Ethics declarations

The experimental procedures were approved by the Animal Care and Use Committee of Nanjing Medical University (Nanjing, China: Approval No.: IACUC- 2101035).

Consent to Participate

Not applicable.

Consent for Publication

All authors consent to the publication of this manuscript.

Data Availability

The datasets used during the present study are available from the corresponding author upon reasonable request.

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None.

Conflict of interest statement

The authors declare they have no conflicts of interest.

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Figures

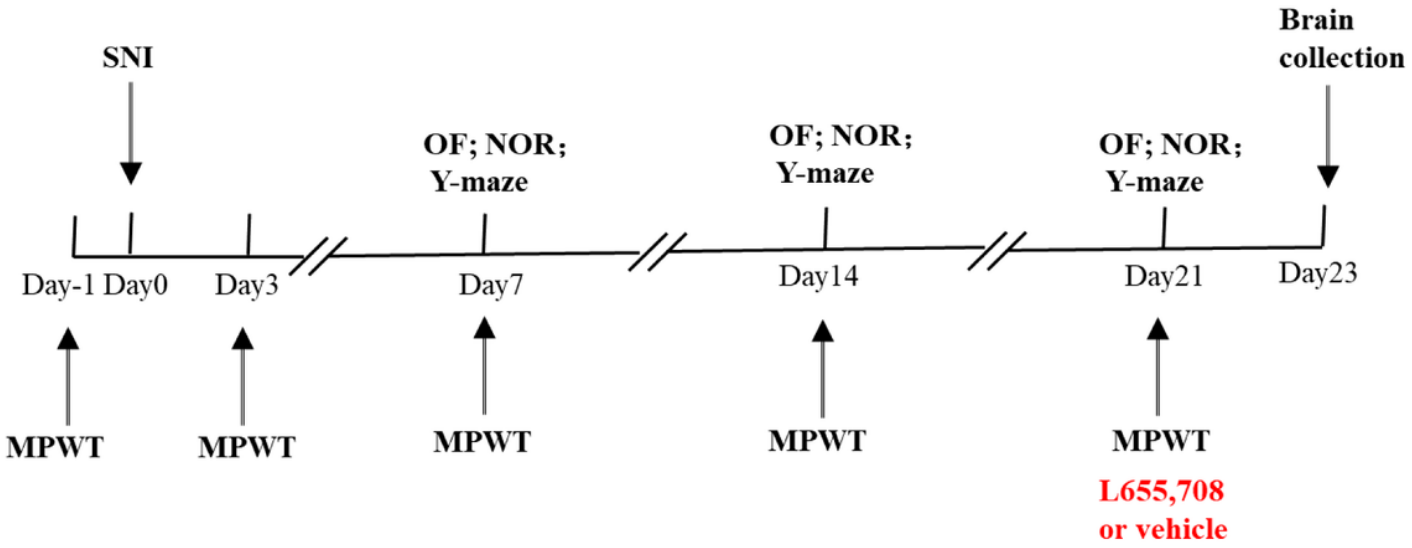

Figure 1

Flowchart diagram showed the timeline of experimental procedures in this study. SNI, sciatic nerve injury; MPWT, mechanical paw withdrawal threshold; NOR, novel object recognition; OF, open-field.

Figure 2

Chronic sciatic injury induced nociceptive mechanical hypersensitivity and cognitive deficits.

(A) Schematic of modeling and behavioral tests. (B) Mechanical allodynia was observed in SNI rats (2-way repeated measures ANOVA: Interaction, F (4,56) =20.42, *P*<0.0001; Time, F (4,56) =35, *P*<0.0001; Surgery, F (1,14) =171.3, *P*<0.0001). (C) Open field test was used to examine locomotor activity (two-way repeated measures ANOVA: Interaction, F (2,28) =0.2207, *P*=0.8033; Time, F (2,28) =0.1287, *P*=0.8798; Surgery, F (1,14) =0.4026, *P*=0.5360). (D) In NOR test, the exploration time of objects was recorded in the training and testing periods after 7 days of surgery (n=8). (E) In NOR test, the investigation time of objects was recorded in the training and testing periods after 14 days of surgery (n=8). (F) In NOR test, the investigation time of objects was recorded in the training and testing periods after 21 days of surgery (n=8). Beside the figure D-F were representative traveling traces of exploring familiar object A1 and novel object B. (G) In NOR test, the discrimination indexs were analyzed (n=8). (H) In Y-maze test, the spontaneous alternation was analyzed (2-way repeated measures ANOVA: Interaction, F (2, 28) = 6.298, *P*=0.0055; Time, F (2, 28) = 0.6232, *P*=0.5435; Surgery, F (1, 14) = 0.0758, *P*=0.7871). (I) In Y-maze test,

number of arm entries had no significant difference (2-way repeated measures ANOVA: Interaction, F (2, 28) = 0.2427, *P*=0.7861; Time, F (2, 28) = 0.6554, *P*=0.5270; Surgery, F (1, 14) = 3.164, *P*=0.0970). A1 and A2, same objects; B, novel object; ANOVA, analysis of variance. MPWT, mechanical paw withdrawal threshold. Data are presented as mean±SEM. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

Figure 3

The decreased inhibitory synapses and increased excitatory synapses were observed in hippocampus.

(A) Schematic of electrophysiology in Sham and SNI rats.　B　Spontaneous excitatory postsynaptic currents paths from hippocampal CA1 pyramidal neurons in the Sham and SNI rats.　C and D　Compared with the Sham group, the frequency of sEPSCs exclusively increased in SNI group (*P*=0.0065), but there was no difference in amplitude (*P*=0.2883). (E) Spontaneous inhibitory postsynaptic currents paths from hippocampal CA1 pyramidal neurons in the Sham and SNI rats.　F and G　Compared with the Sham group, the frequency of sIPSCs decreased in SNI group (*P*=0.0100), but there was no difference in amplitude (*P*=0.3078). n=15 cells. Data are presented as mean±SEM. sIPSCs, Spontaneous inhibitory postsynaptic currents. Student's t test. Data are presented as mean±SEM. *$p < 0.05$, **$p < 0.01$, ns　$p > 0.05$.

Figure 4

Chronic neuropathic pain resulted in increased $GABA_A$Rs-α5 expressing on PV and SST interneurons in hippocampus.

(A-D) Western blot results showed there was no significant difference in PV (*P*=0.7284) and SST (*P*=0.5171) protein expression in SNI group relative to the Sham group (n=4). (E and F) Immunofluorescence results demonstrated SNI surgery significantly increased $GABA_A$Rs-α5 in the hippocampus CA1 and CA3 (n=4). (G and H) The level of $GABA_A$Rs-α5 expression was higher in SNI rats (n=4, *P*=0.003). (I-K) Immunofluorescence co-labeled results demonstrated the $GABA_A$Rs-α5 (green) expressed on PV (red, *P*=0.0156) and SST (red, *P*=0.0014) interneurons were increased (n=4). scale bar = 100 μm. O/A, stratum oriens / alveus. PYR, pyramidal layer. RAD, stratum radiatum. Student's t test. Data are presented as mean±SEM. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

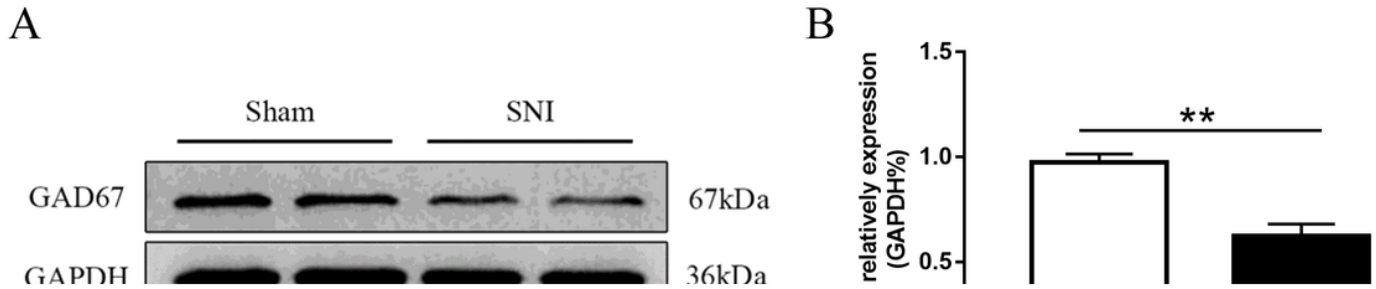

Figure 5

The GABAergic system was suppressed. (A and B) Western blot result showed the down-regulated level of GAD67 in the hippocampus (*P*=0.0024). (C and D) No statistical difference of GAD65 level was observed between 2 groups (*P*=0.8050). n=4. Student's t test. Data are presented as mean±SEM. ***p* < 0.01, ns *p* > 0.05.

Figure 6

$GABA_A$Rs-α5 reverse agonist L655,708 rescued the cognitive dysfunction without affecting basal nociceptive responses. (A) Schematic of intraperitoneal injection L655,708 or vehicle. (B) L655,708 or vehicle did not affect hyperalgesia in SNI rats (2-way repeated measures ANOVA: Interaction, F (6,84)=1.668, *P*=0.1389; Time, F (6,84)=309.7, *P*<0.0001; Surgery, F (1,14)=0.297, *P*=0.5943); (C, D) In NOR test, the exploration time (2-way repeated measures ANOVA: Interaction, F (3,42)=2.175, *P*=0.2052; Time, F (3,42)=0.5234, *P*=0.6686; Surgery, F (1,14)=4.03, *P*=0.0644) and discrimination index P=0.0129 of novel object in SNI L655 group were significantly increased compared to the that of SNI VEH group (n=8). Beside the figure C were representative traveling traces of exploring familiar object A1 and novel object B. (E) In Y-maze test, the spontaneous alternations were significantly increased compared to that of SNI VEH group (n=8) (P=0.0262). (F) In Y-maze test, number of arm entries had no significant difference

(n=8) (P=0.5644). A1, familiar object; B, novel object; ANOVA, analysis of variance. MPWT, mechanical paw withdrawal threshold. (B): Two-way repeated-measures ANOVA, compared with the SNI VEH group. (C): Two-way repeated-measures ANOVA, compared with the SNI VEH group. (D-F): Student's t test. Data are presented as mean±SEM. $*p < 0.05$, $**p < 0.01$.

Figure 7

L655,708 administration improved weakened GABAergic system and inhibitory synaptic transmission in hippocampus.

(A and B) There was no significant difference in the level of $GABA_A$Rs-α5 expression (n=3, *P*=0.4164). (C and D) L655,708 administration up-regulated the level of GAD67 in SNI L655 group compared with SNI VEH group (n=3, *P*=0.0392). (E) Schematic of electrophysiology in L655,708 or vehicle injected rats. (F) Spontaneous inhibitory postsynaptic currents paths from hippocampal CA1 pyramidal neurons in SNI VEH and SNI L655 groups. (G and H) L655,708 exclusively increased the mean frequency of sIPSCs (*P*=0.0186), but there was no difference in mean amplitude (*P*=0.9423) (n=15 cells). sIPSCs, spontaneous inhibitory postsynaptic currents. Student's t test. Data are presented as mean±SEM. $*p < 0.05$, ns $p > 0.05$.

Figure 8

The schematic diagram of the pathogenesis of chronic neuropathic pain-induced cognitive impairment.

Chronic neuropathic pain induced excessive expression of $GABA_A$R-α5 in PV and SST interneurons, which resulted in weakened synapses and cognitive impairment.