Influence of Cerebral Glucose Metabolism by Chronic Pain–Mediated Cognitive Impairment in Adolescent Rats.

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Abstract

Chronic pain during adolescence can lead to mental health disorders in adulthood, but the underlying mechanism is still unclear. Furthermore, the homeostasis of cerebral glucose metabolism and neurotransmitter metabolic kinetics are closely associated with cognitive development and pain progression. The present study investigated changes in cognitive function and glucose metabolism in adult rats, which had experienced chronic pain during their adolescence. Here, spared nerve injury (SNI) surgery was conducted in 4-week-old male rats. Mechanical nociceptive reflex thresholds were analyzed, and SNI chronic pain (SNI-CP) animals were screened. Based on animal behavioral tests (open field, three-chambered social, novel object recognition, and the Y maze), the SNI-CP animals showed learning and memory impairment and anxiety-like behaviors, compared to SNI no chronic pain (SNI-NCP) animals. The cerebral glucose metabolism in the prefrontal cortex and hippocampus of adult SNI-CP animals was decreased with positron emission tomography/computed tomography. $GABA_2$ and Glu_4 levels in the metabolic kinetics study were significantly decreased in the hippocampus, frontal cortex and temporal cortex, and the expression of GLUT3 and GLUT4 was also significantly downregulated in the prefrontal cortex and hippocampus of adult rats in the SNI-CP group. These findings suggest that the rats which suffered chronic pain during adolescence have lower cerebral glucose metabolism in the cortex and hippocampus, which could be related to cognitive function during the development of the central nervous system.

Keywords: Neuropathic pain; Hippocampus; Cortex; Cognitive impairment; Glucose metabolism; Metabolic kinetics

Introduction

Chronic pain (such as neuropathic pain, postsurgical pain, visceral pain) is recognized as a multidimensional and subjective experience, which includes sensory, affective, and cognitive components, and can have a serious negative impact on quality of life [1-3]. Psychiatric comorbidities including anxiety [4], depression [5], poor sleep [6] and cognitive impairment [7] are always associated with chronic pain. Functional cognitive disorder has also been reported as one of the most common complications of chronic pain [7], which supports the notion that chronic pain and cognitive impairment share some common pathogenetic mechanisms [8,9]. For example, previous studies have demonstrated that chronic pain preferentially engages the regions involved in cognition and emotion modulation related to pain, such as the medial prefrontal cortex (mPFC), anterior cingulate cortex (ACC), amygdala and hippocampus, leading to comorbid cognitive and emotional deficits [10-13]. The development of chronic pain involves long-term multiple changes, including peripheral, spinal cord and brain neural pain networks [14]. The integration of pain information transmitted from peripheral to central perception leads to complex neuroanatomical, neurochemical and emotional changes [15,16] Multiple brain regions that are involved in processing pain are also implicated in processing affective, motivational, and emotional events [17,18].

Chronic pain during childhood and adolescence has been shown to contribute to a heightened risk of anxiety disorders, impaired emotional decision-making, and working memory loss in adulthood [19], which is associated with blocked structural development in brain regions [20], abnormal cerebral metabolism [21,22], hormonal changes [23], central sensitization [24], *etc*. Among these changes, the variations of glucose metabolism and neurotransmitters in affective and somatosensory regions induced by SNI may play a crucial role in the onset and development of cognitive and negative affective

components, such as anxiety and depression [25,26]. Research has shown that glucose metabolism in patients with chronic pain was reduced in the dorsal prefrontal cortex and primary motor cortex, possibly due to pain duration [21]. There is also increasing evidence suggesting that glutamatergic and GABAergic neurons contribute to the majority of oxidative glucose metabolism in the brain [27,28]. Another study showed that the SNI model induced, overall, decreased activity in mPFC pyramidal neurons with a subsequent reduction in glutamate levels correlated with pain-related depression-like behavior and cognitive impairments [29,30]. To our knowledge, there is no study that has investigated the long-term consequences of chronic pain on brain metabolism and metabolic kinetics on individuals suffering from chronic pain since adolescence.

Here, we defined the period of adolescence in rats based on the criteria outlined in the reference [31], which suggests that adolescence begins roughly around PND28. Consequently, four-week-old Sprague-Dawley rats were used as experimental subjects to establish the spared nerve injury (SNI) model. A comprehensive set of animal behavioral studies were implemented at the time of eight weeks after SNI. Then, the cerebral glucose metabolism and glucose transporter protein expressions were initially estimated, and the metabolic dynamics in glutamatergic/GABAergic neurons in the hippocampus and mPFC were investigated using nuclear magnetic resonance (NMR) spectroscopy. The results showed that rats which experienced chronic pain during adolescence had lower glucose metabolism and neurotransmitter disorders in the hippocampus and mPFC in adulthood, which could be the mechanism for chronic pain-mediated cognitive impairment during adolescence, and thus, provide basic neuronal mechanisms for developing novel drugs to treat chronic pain associated with cognitive impairment.

Materials and Methods

Animal preparation

The experimental protocol was approved by the Animal Ethics Committee of Zhongnan Hospital of Wuhan University (Ethics approval number: ZN2021097), and all experiments were performed in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals. The animal experiments were performed in the Animal Experiment Center of Zhongnan Hospital of Wuhan University and complied strictly with the guidelines accepted by the International Association for the Study of Pain. Male Sprague-Dawley rats were purchased from Beijing Vital River Laboratory Animal Technology (4 weeks old, weighing 80-100 g). The rats were group-housed under controlled temperature (23 ± 3 °C) and a 12-h light-dark cycle and had free access to food and water.

Animal surgery

Spared nerve injury (SNI) surgery was performed according to the experimental scheme of a previous work [32]. Briefly, the rats were anesthetized with isoflurane, the fur was shaved on the lateral surface of the left thigh, and the area was disinfected with iodophor. A longitudinal incision was made through the skin caudal to the femur to expose the sciatic nerve and its three terminal branches: sural, common peroneal and tibial nerves. The common peroneal and tibial nerves were delicately dissected and separated from the surrounding tissue. Distal to the trifurcation of the sciatic nerve, sutures (4-0 sutures) were applied to ligate the two branches (the tibial and common peroneal nerves). Sectioned distal to the ligation, 2 to 4 mm of the distal nerve stump was removed. During the entire experiment, the operators were very careful not to touch the sural branch, to ensure it was left completely intact. The mechanical paw withdrawal threshold (MPWT) was used to detect hyperalgesia in rats 28 days after the establishment of the model. The rats were divided into two groups: the SNI chronic pain (SNI-CP) group and the SNI nonchronic pain (SNI-NCP) group.

Mechanical paw withdrawal threshold

The mechanical paw withdrawal threshold (MPWT) was assessed one day before the surgery and on Days 1, 3, 7, 14, 28, 42 and 56 postsurgery using an electronic von Frey aesthesiometer (Fig. 1). The withdrawal threshold of the left hind paw was measured in response to a mechanical stimulus. The rats were placed in suspended Perspex frames in a quiet environment at room temperature. The bottom of the frame was covered with wire mesh that had evenly distributed round holes with a diameter of approximately 0.5 cm, which gave access to the plantar surface of the hind paw. Animals were left to habituate to the environment for 30 min. A plastic tip attached to the aesthesiometer was applied to the lateral region of the left plantar surface, which is mostly innervated by the sural nerve through the floor below. Brisk withdrawal and paw flinching were considered positive responses. The withdrawal threshold was determined as the average force (*g*) required to withdraw the stimulated paw in three trials, with 30 s intervals between the trials.

Behavioral tests

All animals gradually underwent different behavioral studies from postsurgery Day 56 to Day 63 (Fig. 1). Between tests, the boxes were wiped with 75% ethanol to eliminate olfactory cues.

Open field test

The open field test was performed in a gray polyvinylene box (100 × 100 × 40 cm) as previously described [33]. Rats were brought into the testing room 2 h prior to the start of testing to acclimate to the environment. The box was divided into 25 equal squares virtually, with a central area (60 × 60 cm) and a peripheral area (20 cm on each side). Each rat was gently placed in the center of the field and observed for 5 min. The activities of the rats were recorded by a camera fixed above the box. The total distance moved, and the time spent in the central zone were quantified.

Three-chambered social test

The three-chambered social test was conducted in a chamber (90 × 40 ×30 cm) made from acrylic resin to assess social interaction and social novelty [34]. Rats were allowed to acclimate to the environment in the center chamber for 5 min and were then confined to the central compartment. For social interaction testing, an empty wire cup and a wire cup with an unfamiliar sex-matched rat (S1) were placed on the left or right side of the chamber, and the walls were removed to allow the testing rat to explore for 10 min. For social novelty testing, another strange rat (S2) was introduced to the previous empty cup, and the behaviors of the rat being tested were recorded for 10 min. The time spent in each chamber was recorded. For social interaction testing, the social preference index (SPI) was calculated as follows: time sniffing S1 divided by time sniffing S1 plus time sniffing empty cup (EC). For the social novelty testing, the social preference index (SPI) was calculated as follows: time sniffing S2 divided by time sniffing S1 plus time sniffing S2.

Novel object recognition (NOR) test

The NOR test was performed according to our previous experiment [33]. Before the experiment, every rat was randomly placed in the apparatus (100 cm×100 cm×40 cm) for 5 min to adapt to the new environment, and then the animals were allowed to freely explore two similar objects for 5 min. Twenty-four hours later, one object was replaced with a novel object with a different shape and color, but the other properties were the same. Every rat was allowed to explore for 5 min. A computer system recorded the movement of the rats. The recognition index (RI) was calculated as follows: time spent exploring a new object divided by time spent exploring a new object plus time spent exploring a familiar object.

Y maze test

The Y maze test consisted of two tests to assess spatial memory [35]. In the first test, one arm was randomly selected as the novel arm and was closed. The rat was placed at the starting arm and allowed to freely explore the other two arms for 10 min. After 1 hour, a second test was conducted. All the arms were opened, and the rats were put back in the same starting arm and allowed to move freely among the three arms for 5 min. The behaviors of rats were recorded using a video camera. The time spent in each arm, the total entries into the novel arm and the total distance moved were analyzed. The percentage of the residence time in the new arm divided by the residence time in the new arm and in the familiar arm was taken as the preference index of the new arm.

^{18}F-FDG PET/CT scan

^{18}F-FDG PET/CT scans were performed 56 days after the surgery. The rats were fasted for 12 h before scanning. Then, approximately 500 ± 25 μCi ^{18}F-FDG was injected through the tail vein with 2% isoflurane. After a 45-min uptake period of ^{18}F-FDG, rats were placed on the scanning bed and subjected to PET-CT scanning (Raycan Technology Co., Ltd., Suzhou, China). The PET-CT images were obtained using the following parameters: PET scan mode, static; scan time, 10 min; CT scan mode, normal; scan time, 3.5 min; PET reconstruction algorithm, OSEM3D/PSF; iterations, 2; subsets, 12; post filter, slightly; CT reconstruction algorithm, FDK; image size, 256; TFOV scale, 1. The PET images were reconstructed using the three-dimensional (3D) OSEM method with a voxel size of 0.5×0.5×0.5 mm^3. The AMIDE software package (Free Software Foundation, Inc., Boston, Massachusetts, USA) was used to perform the region-of-interest (ROI) analysis. The average glucose uptake of the ROI is presented as the mean standardized uptake value (SUV = mean pixel value with decay-corrected ROI activity (μCi/kg)/(injected dose [μCi]/weight [kg])).

^{1}H-NMR

Brain sample preparation: According to our previous study [36], before the experiment, all animals were fasted overnight and only had free access to water (16-18 hours) in order to reduce endogenous glucose levels. On the following experimental day, the rats were anesthetized with 2.0% isoflurane, and a PE50 tube (Intech, PA, USA) was catheterized into the tail vein for the infusion of [1-^{13}C] glucose (Qingdao Tenglong Weibo Technology Co., LTD, Qingdao, P.R. China). After the rat had recovered for approximately 15 min and began moving freely, one end of the infusion tube was connected to a swivel (Instech, PA, USA), and the other side of the swivel was connected to the pump (Fusion 100, Chemyx, TX, USA) using PE50 tubing. Finally, [1-^{13}C] glucose was infused through the lateral tail vein in 2 min at a fixed infusion rate (the dosage was based on the previous infusion protocol [37]) under awake and free moving states. The rats were allowed to move freely in the cages for approximately 20 min. Then, all animals were deeply anesthetized with isoflurane and euthanized using head-focused microwave irradiation (Tangshan Nanosource Microwave Thermal Instrument Manufacturing Co. Ltd., China). The brains were manually dissected into 12 different regions: the left frontal cortex (FC_L), right frontal cortex (FC_R), left temporal cortex (TC_L), right temporal cortex (TC_R), left parietal cortex (PC_L), right parietal cortex (PC_R), left occipital cortex (OC_L), right occipital cortex (OC_R), hippocampus (HIP), thalamus (THA), midbrain (MID) and cerebellum (CE). The tissue was weighed and immediately frozen at -80 °C for further processing.

Metabolite extraction: Briefly, the brain tissue samples were mixed with 400 μL of HCl/methanol (0.1 M) and homogenized with a Tissuelyser (QIAGEN, Germany) for 90 s at a frequency of 20 Hz. Then, 800 μL of ethanol (60%, vol/vol) was added, and the mixture was homogenized again. The homogenate was centrifuged at 14,000 *g* for 10 min, and the supernatant was collected. The above process was repeated twice with 1200 μL of 60% ethanol for adequate extraction. Then, the supernatant

was collected and lyophilized (Thermo Scientific, Germany) after removal of organic solvents (methanol and ethanol) in a vacuum environment at 45 °C. The dry product was dissolved with a D_2O buffer solution (600 μL of D_2O with 0.2 M Na_2HPO_4/NaH_2PO_4, pH=7.2) for [^{1}H-^{13}C]-NMR analysis, and the chemical TMSP (3-(trimethylsilyl) propionic-2,2,3,3-d4 acid sodium salt, 5 mM) was selected as the internal standard chemical in the buffer.

NMR spectrum acquisition: All NMR spectra were obtained at 298 K with a BrukerAvance III 500 MHz NMR spectrometer (BrukerBioSpin, Germany). With POCE (proton-observed carbon editing, ^{1}H-[^{13}C]-NMR) pulse sequence, the ^{13}C enrichment of metabolites was presented as the subtraction between one spin-echo measurement without inversion pulse applied at the ^{13}C frequency (the total metabolite concentrations, ^{12}C+^{13}C) and the other with the inversion pulse (the difference of metabolites, ^{12}C-^{13}C). The collection parameters used were described in our previous study: number of scans, 64; repetition time, 20 s; sweep width, 20 ppm; acquisition data, 64 K; and echo time, 8 ms.

NMR data processing: The phase and baseline correction of the NMR spectra were manually performed in Topspin (Version 2.1, Bruker BioSpin, Germany). Then, all the spectra were automatically processed and analyzed with the homemade software NMRSpec based on MATALAB (freely available by request: jie.wang@wipm.ac.cn). After the peak alignment in NMRSpec, the ^{13}C enrichment in different kinds of metabolites was automatically integrated and calculated.

Western blotting

The rats in different groups were anesthetized with isoflurane, and the frontal cortex and hippocampus were collected at 4 °C and stored at -80 °C. Tissue proteins were extracted using RIPA total protein lysate with proteinase inhibitor. The protein concentrations were measured by the BCA Protein Assay Kit. Then, the mixed loading buffer samples were denatured in 95 °C water for 10 minutes.

Equal amounts of protein samples were separated by electrophoresis on 8-15% SDS–PAGE gels and transferred onto PVDF membranes (Aspen). After incubation with western blotting-specific blocking solution (5% skimmed milk powder (AS1107, Aspen, China) diluted in TBST), the PVDF membranes were incubated with anti-GLUT4 (1:1000, AGT-024, Alomone), anti-(extracellular) GLUT3 (1:1000, AGT-023, Alomone) and anti-β-actin (1:10,000, Aspen) antibodies at 4 °C overnight. Then, the blots were washed, incubated with HRP-conjugated secondary antibody for 1.5 h and detected by a chemiluminescent imaging system (Tanon, China).

Immunofluorescence

The rats were anesthetized with isoflurane, transcardially perfused with PBS and subsequently incubated with 4% paraformaldehyde. The fixed brain was then rapidly removed, postfixed in paraformaldehyde at 4 °C for 48 h, embedded, and sectioned for immunofluorescence. The tissue sections were washed and incubated with IF-specific blocking solution (10% goat serum (AR0009, Booster, China) diluted in PBS) for 30 min. The sections were incubated with anti-GLUT3 (1: 100, AGT-023, Alomone) and anti-GLUT4 (1: 200, AGT-024, Alomone) overnight at 4 °C. After three washes with PBS, the sections were incubated with Cy3 goat anti-rabbit secondary antibody (1:50, AS-1109, Aspen) at 37 °C for 2 h in the dark. The sections were imaged using an Aperio VERSA 8 microscope (Leica, Germany), and the fluorescence intensity of the images was analyzed using imaging software.

Statistical analysis

Statistical analysis was performed using SPSS 21.0 statistical software (IBM, New York, USA). Student's t-test was used to compare the differences between the 2 groups. Two-way analysis of variance was applied to calculate the values of the mechanical paw withdrawal threshold (MPWT). $P < 0.05$ was considered statistically significant. All results are expressed as the mean ± SEM.

Results

Chronic pain and nonchronic pain following SNI

Mechanical hyperalgesia was assessed one day before the surgery and on Days 1, 3, 7, 14, 28, 42 and 56 postsurgery using the electronic von Frey aesthesiometer. The mechanical paw withdrawal threshold (MPWT) was used to separate the rats with chronic pain from those with nonchronic pain at 28 days after establishment of the model. All animals were divided into two groups: the SNI chronic pain (SNI-CP) group and the SNI nonchronic pain (SNI-NCP) group. The results suggest that mechanical hypersensitivity did not develop continuously in approximately one-quarter of the rats, and MPWT gradually recovered to baseline within one week. The rats in the SNI-CP group developed marked hypersensitivity to stimulation with von Frey filaments from the first day after surgery that lasted until two months after SNI surgery (Fig. 2a).

Effect of chronic pain in adolescence on animal behaviors

The open field test was used to evaluate emotion-related behaviors. The results indicate that SNI-CP rats spent less time in the central zone than SNI-NCP rats did (Fig. 2b). The three-chamber social test was used to assess the sociability and social novelty of the animals. For the sociability test, compared with that of SNI-NCP rats, the SNI-CP group exhibited a significantly lower social preference index (SPI). For the social novelty test, rats in the SNI-CP group did not show a preference for the Stranger 2 chamber (S2), and the social preference index (SPI) in the SNI-CP group was lower than that in the SNI-NCP group (Fig. 2c). The novel object recognition test (NOR) and Y maze test were used to estimate the recognition memory and spatial memory of the rats. In the novel object recognition test, the recognition index (RI), which is calculated as the time spent exploring a new object divided by the time spent

exploring a new object plus the time spent exploring a familiar object, was used as a measure of NOR. Compared to that of the SNI-NCP group, the SNI-CP group exhibited a significantly lower RI (Fig. 2d). In the Y maze test, the SNI-CP group spent less time in the novel arm than the SNI-NCP rats did (Fig. 2e). These data suggest that the chronic pain in adolescence induced anxiety-like behaviors, social deficits and recognition and spatial memory deficits in adulthood.

Effect of chronic pain in adolescence on cerebral glucose metabolism

To evaluate the changes in regional glucose metabolic activities between the two groups, we performed an ^{18}F-FDG PET/CT scan to detect glucose uptake at 56 days postsurgery (n=4), and the data were semi-quantitatively analyzed from the images after attenuation correction. Representative brain images of ^{18}F-FDG PET/CT of the two groups are shown in Fig. 3a (coronal section, sagittal section and horizontal section, from left to right). The results show that SUVs in the prefrontal cortex (PFC), motor cortex (MC), somatosensory cortex (SC), and hippocampus (HIP) of both the left and right sides were lower in SNI-CP rats than in SNI-NCP rats (Fig. 3b). We also compared the SUVs between the left and right brain regions from the SNI-NCP and SNI-CP groups, and the results demonstrate that there was no difference between the left and right brain regions in the SNI-NCP group and the SNI-CP group (Fig. 3c, $p>0.05$).

Effect of chronic pain in adolescence on cerebral metabolic kinetics

Here, a POCE NMR pulse sequence was employed to investigate the metabolic composition of the brain extracts. Examples of NMR spectra from the prefrontal cortex in two different groups are illustrated in Fig. 4a. The relative concentrations of the metabolites were obtained from the PQN normalized nonedited spectrum (upper two spectra), and the total concentrations of ^{13}C-labeled metabolites were calculated by subtracting the two series of spectra in POCE data, which are shown in the lower two

spectra. The NMR spectrum demonstrate that the enrichment rates of metabolites in SNI-CP were lower than those in SNI-NCP. The differences in metabolites between the two groups are shown by the heat map (Fig. 4b), and the statistical *t* values of metabolites for different brain regions between the two groups are illustrated by the color of every grid in the heat map, in which the grid with a white point is considered statistically significant ($p<0.05$). The heat map directly indicates the types of metabolites that are statistically significant between the two groups in the studied brain regions. These results show that it is necessary to further analyze the data in order to obtain more detailed implications.

With the infusion of [1-^{13}C]-glucose, glucose-related metabolites were gradually labeled through tricarboxylic acid (TCA) cycle flux in neurons and astrocytes. In the first cycle, Glu_4 was labeled in glutamatergic neurons, $GABA_2$ in GABAergic neurons and Gln_4 in astroglia. Compared with that of the SNI-NCP group, the results confirm that Glu_4 enrichment in the SNI-CP group was significantly decreased in the FC_L, FC_R, TC_L, TC_R, HIP, THA, MID and CE (Fig. 4c), while $GABA_2$ enrichment in the SNI-CP group was decreased in the FC_L, FC_R, TC_L, TC_R, HIP, THA and CE (Fig. 4d). Glx_3 (Glu + Gln) enrichment in the SNI-CP group was significantly lower in the FC_L, FC_R, TC_L, TC_R, HIP and THA than the enrichment in the SNI-NCP group (Fig. 4e). Gln_4 enrichment was significantly decreased in the FC_L, FC_R, TC_L, TC_R, HIP, THA and CE in the SNI-CP group relative to the SNI-NCP group (Fig. 4f). We also compared the differences in metabolites between the left and right frontal cortex (FC), temporal cortex (TC), parietal cortex (PC) and occipital cortex (OC) in the SNI-NCP and SNI-CP groups, and the results indicate that there was no significant difference in metabolites between the left and right cortex in SNI-NCP and SNI-CP rats (Fig. 5a, b, $p>0.05$), which is also similar to the former PET study.

Effect of chronic pain in adolescence on GLUT3 and GLUT4 protein expression in the hippocampus and frontal cortex.

304 Glucose metabolism in neurons is closely related to glucose transporters, especially for GLUT3 and
305 GLUT4; thus, the influence of these two transporters on chronic pain in adolescence was further
306 investigated to disclose the reason for the suppression of glucose utilization and metabolic kinetics in the
307 hippocampus and frontal cortex in adult rats. As shown in Fig. 6a-c, immunofluorescence staining
308 revealed a significant decrease in GLUT3 and GLUT4 levels in the hippocampus and prefrontal cortex
309 of the SNI-CP group compared with the SNI-NCP group. Western blot analysis showed that the SNI-CP
310 group rats had lower GLUT3 protein expression (Fig. 6d, e) and GLUT4 protein expression (Fig. 6d, f)
311 in both the hippocampus and prefrontal cortex than that of SNI-NCP rats.
312
313 **Discussion**
314 Clinical studies have demonstrated that the majority of children and adolescents who suffer from
315 chronic pain also experience cognitive impairment and mood disorders [38-42], but the underlying
316 mechanisms remain largely unclear. Some studies have proposed neuroplasticity, competing limited
317 resources, or dysregulated neurochemistry to explain the potential mechanisms involved in pain-related
318 cognitive impairment [43,9]. In this study, we found that chronic pathologic pain during adolescence
319 resulted in the impairment of emotion-related cognition (anxiety-like behaviors and social interaction
320 deficits), working memory and spatial memory loss (novel object recognition test and Y maze test) in
321 adult rats, which was further supported by the finding that the SNI-CP group had reduced glucose uptake
322 and transport in the PFC and hippocampus and inhibited ^{13}C enrichment of $GABA_2$ and Glu_4 in the PFC
323 and HIP. Our results offer greater evidence of chronic pain, not peripheral nerve injury or transient pain,
324 mediating cognitive impairment and mood disorders, which could be attributed to energy deficiency and
325 disorder of neurotransmitters.

In addition to pain sensory symptoms, neuropsychological functioning is affected by SNI [25], and the complex forebrain network is considered the substrate for negative affective states and cognitive impairments [44]. However, in young animals that do not have a mature immune system, tactile allodynia and microglial activation are not evident and do not develop until the rats are at least 4 weeks old at the time of SNI induction [45,46]. Here, we screened pain and no pain animals in 4-week-old SNI rats and tested whether behavioral function in adulthood was influenced by chronic pain. We found that the adolescent rats with chronic pain exhibited anxiety and depression-like behaviors and cognitive impairment on Day 56 after SNI surgery. Interestingly, no behavioral abnormalities were observed in adult SNI-NCP animals who showed initial mechanical hypersensitivity immediately after surgery that returned to baseline thresholds within one week.

The brain is an organ with the most abundant energy metabolism in the human body. The high energy consumption in the brain dominantly derived from glucose metabolism makes it vulnerable to impaired energy metabolism [22]. Deficits in glucose metabolism heavily affect human brain health, especially cognitive function [47]. Substantial evidence has also shown that in aging subjects, performance deficits on a series of cognitive tasks during training are due to insufficient cerebral glucose supply [48,49]. Previous studies proposed that decreased regional glucose metabolism was closely correlated with cognitive impairments [50]. ^{18}F-FDG PET imaging revealed that patients with cognitive impairments exhibited significantly lower metabolism in the right cerebellar posterior lobe, left cerebellar anterior lobe, bilateral thalamus and left limbic lobe and hypometabolic changes in the temporoparietal and posterior cingulate cortices [51]. Individuals with regional brain glucose hypometabolism have an increased risk of incident cognitive dysfunctions compared to those with normal glucose metabolism [48]. Impaired brain glucose metabolism compromises transmembrane ion transport, vesicle recycling,

348 neurotransmitter release and synaptic signaling, leading to hyperexcitability, excitatory-inhibitory
349 imbalance and functional impairment of cortical networks, which further compromises the brain's energy
350 efficiency [52]. Insufficient neuronal glucose and mitochondrial energy generation compromise the
351 clearance of neurotoxic proteins from the brain. Energy deficits and neurotoxic protein accumulation
352 mutually aggravate one another in a vicious cycle, accelerating the development of cognitive dysfunction
353 [53]. It was demonstrated that glucose metabolism was contralaterally reduced in the dorsal prefrontal
354 cortex and primary motor cortex in patients with chronic pain who were diagnosed with complex regional
355 pain syndrome (CRPS) [21]. In SNL rats, regional cerebral glucose metabolism was decreased in the
356 thalamus, left primary somatosensory cortex and right cerebellum [54]. In the present study, chronic pain
357 impaired glucose metabolism in the PFC and hippocampus, with no difference between left and right
358 glucose metabolism, leading to more profound task-associated depletion of local brain glucose correlated
359 with impaired cognitive performance. Increased local glycolytic metabolism is key to supporting the
360 energy demands of the Na^+/K^+ ATPase and promoting enhanced cellular excitability and synaptic
361 plasticity.

362 The developing rat brain undergoes a series of functional and anatomic changes that affect its rate
363 of cerebral glucose utilization. The cellular expression of glucose transporter proteins is rate limiting for
364 cerebral glucose utilization during early postnatal development in rats [55]. Glucose transport across the
365 plasma membrane is the most upstream and one of the most important processes in cellular glucose
366 metabolism, and evidence indicates that GLUT3 and GLUT4 are altered in cognitive development
367 [56,57], which could also explain the cellular machinery fueling the transmembrane transport of
368 glutamate through glycolytic enzymes and glucose transporters in a model of chronic pain. The increase
369 in GLUT3 expression is further seen in the brain regional variation in the GLUT3 expression pattern

with maturation, which coincides with the increased need for the supply of fuel to meet these demands [58]. Our results show that chronic pain in adolescent rats decreased both GLUT3 and GLUT4 expression in the hippocampus and mPFC. Demonstrations of the decreased glucose transport and glycolysis provide further confirmation of cognitive impairment associated with chronic pain.

Glucose is required to provide precursors for neurotransmitter synthesis. Glucose metabolism provides fuel for physiological brain function through the generation of ATP, the foundation for neuronal and nonneuronal cellular maintenance, as well as the generation of neurotransmitters [28]. A proposed mechanism of chronic pain is dysregulation between the main inhibitory (GABA) and excitatory (glutamate) neurometabolites of the central nervous system [59]. GC J's studies showed that pain-related plasticity in the BLA and synaptic inhibition of mPFC pyramidal cells were glutamate driven, which impairs mPFC function and produces cognitive decision-making deficits [60]. Pain-related inhibition of mPFC neurons depends on mGluR1-mediated endogenous activation of GABA(A) receptors [61]. At the same time, hippocampal glutamatergic synapses were significantly impaired after peripheral nerve injury [62]. Human brain imaging studies have revealed consistent cortical and subcortical networks that are activated by pain, including the primary somatosensory cortex (S1), secondary somatosensory cortex (S2), anterior cingulate cortex (ACC), insula, prefrontal cortex (PFC), thalamus and cerebellum [11]. Findings from studies using proton magnetic resonance spectrometry show increased glutamate levels and decreased neuronal marker N-acetyl aspartate in the frontal cortices of patients with chronic back pain and fibromyalgia. In our study, we found that the dynamics information of Glu_4 (FC_L, FC_R, TC_L, TC_R, HIP, THA, MID, and CE), $GABA_2$ (FC_L, FC_R, TC_L, TC_R, HIP, THA, and CE), Glx_3 (FC_L, FC_R, TC_L, TC_R, HIP, and THA) and Gln_4 (FC_L, FC_R, TC_L, TC_R, HIP, THA, and CE) were significantly reduced in SNI-CP rats. This can quantitatively be observed by considering the relationship between

neurotransmitter glutamate-glutamine cycling and glucose metabolism determined over a large range of neural activities.

Furthermore, lactate acts as a signaling molecule in the brain linking metabolism, substrate availability, blood flow and neuronal activity [63]. It can modulate various neuronal functions, including excitability, plasticity and memory consolidation [64], and homeostatic functions [65]. Unfortunately, the present study is an *in vitro* study, the lactate concentration and its metabolism are very unstable [66,67], and its concentration is significantly increased due to post-mortem changes. To decrease these consequences, the head focused microwave method [67] was utilized to fix the brain and immediately cease the metabolism. Thus, the changes of lactate kinetics in the brain regions were not studied in this study.

In conclusion, this study confirmed that animals experience chronic pain in adolescence with induced anxiety and social deficits, as well as recognition and spatial memory defects. GABA and glutamate neurometabolites in the mPFC and hippocampus were impacted by glucose transporters and metabolism, which could be the key parameters of cognitive impairment associated with chronic pain in adolescence. Our findings suggest that elevated glucose metabolism in neurons should be considered a new effective strategy for the treatment of cognitive impairment in patients with chronic pain.

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Author Contributions

Yuanyuan Fang, Chang Chen, Jie Wang, and Zongze Zhang designed the study; Yuanyuan Fang,

Qi Zhong, Lirong Wang, Zhu Gui, and Jinpiao Zhu performed the experiments; Yuanyuan Fang, Chang Chen, Jie Wang, and Fuqiang Xu contributed to the data. Yuanyuan Fang, Chang Chen, Jie Wang, Zongze Zhang, and Anne Manyande wrote the manuscript. The content of this manuscript has been reviewed, read, and agreed upon by all designated authors.

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Availability of data and materials

All raw data and materials during the current study are available from the corresponding author upon reasonable request.

Compliance with Ethical Standards

All animal procedures were carried out in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals and were approved by the Animal Ethics Committee of Zhongnan Hospital of Wuhan University (Ethics approval number: ZN2021097).

Conflict of interest

All authors claim that there are no conflicts of interest.

Consent to Participate Not applicable.

Consent for Publication

All authors have read the manuscript and agreed for its publication.

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645 **Figure legends**

646 **Fig. 1 Illustration of the experimental design.** SNI surgery was performed on 4-week-old male SD rats
647 at D1. The mechanical paw withdrawal threshold (MPWT) was assessed at D0, D1, D3, D7, D14, D28,
648 D42 and D56. PET/CT and NMR were performed at D56 (n=8). We harvested brain tissue (hippocampus
649 and prefrontal cortex) at D56 for western blot (WB) and immunofluorescence (IF) experiments. Two
650 groups of rats (n=8) were used for behavioral tests from D56 to D63.

651

Fig. 2 Chronic pain in adolescence induces anxiety-like behaviors and cognitive impairment in adulthood. (a) The mechanical paw withdrawal threshold was measured by an electronic Von Frey. **(b)** Representative traces in two groups in the open field test (left panel). Quantification of the time spent in the central zone (right histogram). **(c)** Quantification of the social preference index (SPI) in the social interaction test (left histogram); quantification of the social preference index (SPI) in the social novelty test (right histogram). **(d)** Representative traces in the two groups during the testing phase of the novel object recognition test (left panel). Quantification of the recognition index (RI) (right histogram) during the testing phase. **(e)** Representative tracing heatmap during the testing phase of the Y maze test (left panel). Quantification of novel arm preference (right histogram) during the testing phase. Data are presented as the mean ± SEM. $^{*}p < 0.05$, $^{**}p < 0.001$, $^{***}p < 0.0001$, compared to the SNI-NCP group, n= 8 per group.

Fig. 3 The effect of chronic pain in adolescence on glucose metabolism in adulthood. (a) Representative images of ^{18}F-FDG PET/CT of a rat brain in each group (coronal section, sagittal section and horizontal section, from left to right). **(b)** Quantification of glucose metabolism in different brain regions from the two groups. **(c)** Comparison of SUVs in the left and right brain regions from the SNI-NCP group (left histogram); comparison of SUVs in the left and right brain regions from the SNI-CP group (right histogram). Data are presented as the mean ± SEM. $^{*}p<0.05$, compared to the SNI-NCP group, n= 4 per group.

Fig. 4 The effect of chronic pain in adolescence on cerebral metabolic kinetics in adulthood. (a) Examples of NMR spectra for total metabolites ($^{12}C+^{13}C$, upper panel) and ^{13}C-labeled metabolites ($2*^{13}C$, lower panel) from the hippocampus in the two groups. Note: Asp: aspartate; Cre: creatine; GABA: γ-aminobutyric acid; Gln: glutamine; Glu: glutamate; Glx: glutamine + glutamate; NAA: N-acetylaspartate; Subscript number: ^{13}C labeled positions in different metabolites. **(b)** The difference in metabolites between the two groups in the heat map; the statistical *t* value of the metabolites in different brain regions between the two groups is illustrated by the color of every grid in the heat map in which the grid with a white origin was considered statistically significant between the two groups ($p<0.05$). **(c-f)** The enrichment of Glu_4 **(c)**, $GABA_2$ **(d)**, Glx_3 **(e)** and Gln_4 **(f)** in different brain regions in the two groups. Values represent the mean ± SEM. $^{*}p<0.05$, $^{**}p<0.001$, compared to the SNI-NCP group. n= 8 per group.

Fig. 5 Comparison of metabolites between the left and right cortex (FC, TC, PC, and OC) in the SNI-NCP group and the SNI-CP group. (a) Comparison of metabolites between the left and right cortex (FC, TC, PC, and OC) in the SNI-NCP group. **(b)** Comparison of metabolites between the left and right cortex (FC, TC, PC, and OC) in the SNI-CP group. Data are presented as the mean ± SEM. n= 8 per group. Note: FC: frontal cortex; TC: temporal cortex; PC: parietal cortex; OC: occipital cortex.

Fig. 6 The protein expression of GLUT3 and GLUT4 in the hippocampus and prefrontal cortex. **(a)** Fluorescent images showing GLUT3 expression in neurons of the hippocampal CA1 area and prefrontal cortex (scale bars, 50μm). **(b, c)** Quantification of GLUT3 and GLUT4 intensity in the CA1 region of the hippocampus and prefrontal cortex in the two groups. **(d)** WB analysis of GLUT3 and GLUT4 protein expression in the hippocampus and prefrontal cortex in the two groups. **(e, f)** Histograms showing the quantification of GLUT3 and GLUT4 blots in the hippocampus and prefrontal cortex. n= 4 per group. **P*<0.05 versus the SNI-NCP group. Data are presented as the mean ± SEM.