



# Messenger RNA expression profiles and bioinformatics analysis of mouse hippocampi during exercise alleviates methamphetamine dependence via mRNA profile change in hippocampi

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**Background:** Methamphetamine (METH) is a highly addictive, psychoactive drug which can harm individual health and lead to great social problems. Various approaches have been adopted to address the problems arising from METH addiction, but relapse rates remain high. Recently, it has been found that comprehensive treatment combined with scientific and appropriate exercise interventions can improve the mental state and physical fitness of drug addicts and promote their physical and mental rehabilitation. Long-term, regular exercise improves the symptoms of METH withdrawal and reduces METH relapse. This study aimed to investigate the effects and regulated gene expression related to running exercise in METH-addicted mice.

**Methods:** Male C57BL/6J mice were used to construct a METH addiction model. We performed a running exercise intervention and used conditioned place preference (CPP) to measure the effects of the running intervention on the METH-addicted mice. We also performed RNA sequencing (RNA-seq) and transcriptome analysis on the mice hippocampi, and the functions and differentially expressed genes (DEGs) that were significantly regulated by exercise intervention in the METH-addicted mice were analyzed and noted.

**Results:** The results showed that days of CPP were shortened to 3 days in METH-addicted mice that underwent moderate exercise intervention, compared to 6 days in METH-addicted mice that went without exercise intervention. In addition, hippocampal transcriptome analysis revealed 12 DEGs significantly regulated by exercise intervention. By performing Gene Ontology (GO) and the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis, it was revealed that the function of immune responses was significantly enriched in the METH-addicted mice undertaking exercise. The expression of 12 DEGs was verified by real-time quantitative reverse transcription polymerase chain reaction (qRT-PCR), which showed that relative messenger RNA (mRNA) expression of DEGs was consistent with the RNA-seq results.

**Conclusions:** A running intervention can promote the recovery of METH addiction in mice, and the 12 candidate DEGs from the mouse hippocampus can be used for further research on the regulatory mechanisms of exercise in METH-addicted mice.

**Keywords:** Methamphetamine (METH); exercise; conditional place preference; hippocampal transcriptome; drug dependence

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## Introduction

Methamphetamine (METH), also known as deoxyephedrine, is a highly addictive psychoactive drug. Due to its transparent white appearance resembling ice or rock sugar, METH is commonly known as ice (1). One of the most widely abused drugs in the world, METH activates the reward system of the brain and produces highly reinforcing effects that lead to abuse and dependence (2). In addition, METH abuse can bring various harmful changes to the emotional state and cognitive function of individuals, thus resulting in a decline in their physical health and quality of life (3). At present, treatment methods for METH addiction mainly focus on drug substitution therapy, cognitive behavioral therapy, health education, psychological therapy, and residential rehabilitation. Although these methods have certain efficacy, they also have some problems. The effect of drug substitution therapies is temporary due to the addictive nature of drugs, the implementation of other therapies is difficult due to poor patient compliance, and there is a lack of a standard for evaluating system efficacy (2,4). Therefore, new treatments are urgently needed for drug addicts. Recent studies have found that scientific and appropriate exercise interventions based on the above treatment methods can improve the psychological state of drug addicts, strengthen their physical fitness, and promote their physical and mental rehabilitation (5-7). Long-term regular exercise has been shown to exert a significant ameliorative effect on METH withdrawal symptoms and reduces METH relapse (4). As an important part of the limbic system, the hippocampus plays an important role in short-term memory, long-term memory, and spatial memory processing. In some previous studies, METH caused a significant decrease in the short- and long-term spatial memories of rats, and the hippocampal volume changed significantly after METH treatment, which also caused astrocyte proliferation (8). Therefore, the hippocampus is crucial to the study of the potential rewarding properties of METH and memory, as well as the recovery of psychostimulant-seeking behaviors (9). Conditioned place preference (CPP) is a classical (Pavlovian) conditioned reflex process in which one environment is paired with a drug injection and a different environment is

paired with a control injection. In the context of subsequent drug and control pairings, increased preference for the drug environment is a measure of the drug-reward effect (10,11). Based on the above studies, we conclude that exercise can significantly reduce the degree of addiction to METH. We used CPP training to detect METH preference in mice. Transcriptome sequencing is a powerful technique that can be used to study the molecular changes behind differences in physiological conditions and disease progression through the identification of genes that vary significantly between sample groups. Currently, RNA sequencing (RNA-seq) transcriptome analysis is becoming increasingly popular in molecular phenotypic studies (12). It has the potential to quantify low-expression genes, revealing subtle changes in gene expression (13). Both METH and exercise may lead to gene changes. In this study, we screened differentially expressed genes (DEGs) by sequencing, which provided new ideas for future molecular phenotype studies. We explored the influence of running exercise on the METH dependency of C57BL/6J mice by establishing a METH addiction mouse model. We showed that exercise could interfere with cue memory established by the drug and the environment and reduce drug-induced CPP after withdrawal. By performing an analysis of the DEGs of hippocampal and selected functions and the candidate genes significantly related to exercise intervention, this study explored a potential new method for drug abstinence treatment. We present the following article in accordance with the ARRIVE reporting checklist (available at <https://atm.amegroups.com/article/view/10.21037/atm-22-450/rc>).

## Methods

### *Animals*

Male C57BL/6J mice (6 weeks old, 18–22 g weight) were purchased from Chongqing Tengxin Biotechnology Co., Ltd. (Chongqing, China). Before the experiment, the mice were reared in separate cages for 2 weeks for environmental adaptation. The room temperature was 22±2 °C with a dark/light cycle of 12 hours (light from 7:00 am to 7:00 pm) and free access to food and water (14). The animals were divided into a control group and a METH-administration group

by random number method. After the METH mice model was established, the METH model group and the control group were randomly divided into an exercise group and a non-exercise group. Each group contained 10 mice. All experimental procedures were approved by the Institutional Animal Care and Use Committee of Kunming Medical University and carried out in accordance with the relevant laws and regulations in China [ethics review approval No. kmmu20211261. Animal laboratory facility Permit Number: SYXK (Dian) K-2020-0006]. All the procedures were conducted according to the Chinese Guidelines for the Care and Use of Laboratory Animals.

### *Animal experimental instruments*

The small animal treadmill platform (Model SA101B; Kunming Baiole Biotechnology Co., Ltd., Yunnan, China) was used to exercise the mice. The CPP experimental system (Model 2A213; Kunming Baiole Biotechnology Co., Ltd.) was composed of three compartments. The size of the CPP box was 80 cm × 30 cm × 25 cm, and the center of the box had a movable door (5 cm × 7 cm) to divide it into symmetrical spaces on both sides (30 cm × 30 cm × 25 cm). One of the inner walls was black with white stripes, with a round hole into a hollow bottom. When the partition was lifted, the mice were free to move between the two sides of the box. An infrared monitoring system recorded the length of time each mouse spent in each compartment and the number of times it crossed through the compartment.

### *Reagent*

The drug of METH salt, which came from the Public Security Department of Yunnan Province, was dissolved in 0.9% saline to reach a concentration of 75%, then administered via an intraperitoneal (i.p.) injection of 0.2 mL to the mice at a dose of 5 mg/kg, in accordance with the literature (15,16) and the preliminary laboratory analysis. The control group was administered the same volume of saline.

### *Establishment of animal models*

A CPP experiment is generally divided into three stages: pre-test, experiment, and test. The model was established according to the literature (15) and was the same model as that used in previous studies in our laboratory. For the pretest phase (day 1), the mice (METH + exercise group,

METH + sedentary group, saline + exercise group, saline + sedentary group) were moved into the examination room 1 hour in advance and kept in darkness to reduce noise and minimize stress. Before formal training, the partition was removed, the experimental animals were placed into the experimental box and allowed to move freely for 15 minutes, with their stay time in each box recorded. After each experiment, feces and urine were removed from the mice, and the floor and walls of each compartment were wiped with 75% ethanol solution to avoid odor affecting the results. At this stage, animals can become familiar with the experimental equipment, reducing the novelty and stress, and natural preference (unconditioned place preference) can be recorded and used to select animals. To eliminate large individual differences, mice that spent more than 600 seconds in a single compartment or shuttled back and forth less than 20 times were excluded (of the 43 mice used in the current study, 3 were excluded). For adaptation training on Day 1, mice were put into the box and permitted free movement for 20 minutes to adjust to the box environment. For the training phase on days 2 to 10, the METH group of mice were injected with METH (i.p.) and placed in the drug box (black box), which was removed 30 minutes later. Mice in the saline group were intraperitoneally injected with the same volume of saline and placed in the accompanying medicine box (black box), which was removed 30 minutes later. The control experiment was conducted at the same time on the second day. The METH group and the saline group of mice were injected with control (saline) and placed in the non-accompanied drug box (black and white box). The experiment lasted for the same time as before. The experiment was alternated 4 times. For the test phase, after 8 days of training, the animals were placed on the aisle without any drug treatment, and the partitions were removed. The animals could shuttle freely between each box. The test was conducted once, and the stay time of the animals in each box was recorded. The long-stay time in the medication cabinet was significantly different from that in the control group, indicating that the mice were mentally dependent on METH. All behavioral experiments were conducted during the light phase of the light/dark cycle (7:00 am–7:00 pm).

### *Exercise plan*

After the exercise intervention was established, the mouse model was given a moderate intensity aerobic treadmill exercise (17,18). The CPP test was conducted every

day before the exercise intervention until there was no significant difference between the CPP test and the pretest score for 2 consecutive days, indicating that the CPP had disappeared. The exercise group received treadmill training at a speed of 10 meters/minute, an inclination of 0°, and an acceleration of 8 meters/minute for 45 minutes/day, lasting 7 days in total (6). All treadmill sessions were scheduled from 9:00 am to 11:30 am. The sedentary group was placed on a treadmill, but did not exercise. The sedentary group and the exercise group were tested daily for CPP (Figure 1A).

### Organizational preparations

The mice were sacrificed via cervical dislocation immediately after the CPP behavior test. The whole brains were removed from their heads and placed on an ice plate. The bilateral hippocampus of each brain was carefully dissected. The mice were quickly frozen in liquid nitrogen for 1 hour and then transferred to a -80 °C refrigerator for preservation. Three repeat samples from each group were sent to Lianchuan Biological Co., Ltd. (Zhejiang, China) for reference transcriptome sequencing to identify DEGs.

### Hippocampal transcriptome analysis

The expression levels of genes were evaluated by fragments per kilobase of transcript per million reads/fragments mapped (FPKM). The standard for identifying DEGs was an expression fold change  $\geq 2$  and a false discovery rate (FDR) lower than 0.01. A functional analysis of DEGs was performed using Gene Ontology (GO) and the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis.

### Real-time quantitative reverse transcription polymerase chain reaction (qRT-PCR)

Total RNA from the hippocampus was extracted using Trizol reagent (phenol/guanidine isothiocyanate). Reverse transcription was performed according to the Goldenstar RT6 cDNA Synthesis Kit v.2 (Optimus Biotechnology Co., Ltd., Wuhan, China) protocol. Real-time qRT-PCR was performed using 2× T5 SYBR Green I Fast qPCR Mix (Optimus Biotechnology Co., Ltd., Wuhan, China). The reference gene and target gene sequence of primer 3.0 plus design of Hangzhou Bioer FQD-96A (Hangzhou Bioer Tech., Zhejiang, China) are shown in Table 1. The

conditions of PCR were as follows: 95 °C for 30 seconds, 40 cycles of 95 °C for 5 seconds, 60 °C for 30 seconds, and 72 °C for 30 seconds. The melting curve analysis was executed at 95 °C for 15 seconds, 60 °C for 1 minute, and 95 °C for 15 seconds. The relative messenger RNA (mRNA) expression of DEGs was calculated using the  $2^{-\Delta\Delta CT}$  method.

### Statistical analysis

All experiments were repeated 3 times. The CPP score was calculated by subtracting the time spent in the non-accompanied box from that spent in the accompanied box to reflect the degree of addiction of mice (19). The laboratory data were represented as the mean  $\pm$  the standard error of the mean (SEM). The tests were in line with normal distribution, so the differences between the control and treatment groups were *t*-tested using GraphPad Prism 8.0.1 software (GraphPad Software Inc., San Diego, CA, USA). A P value of less than 0.05 was considered statistically significant.

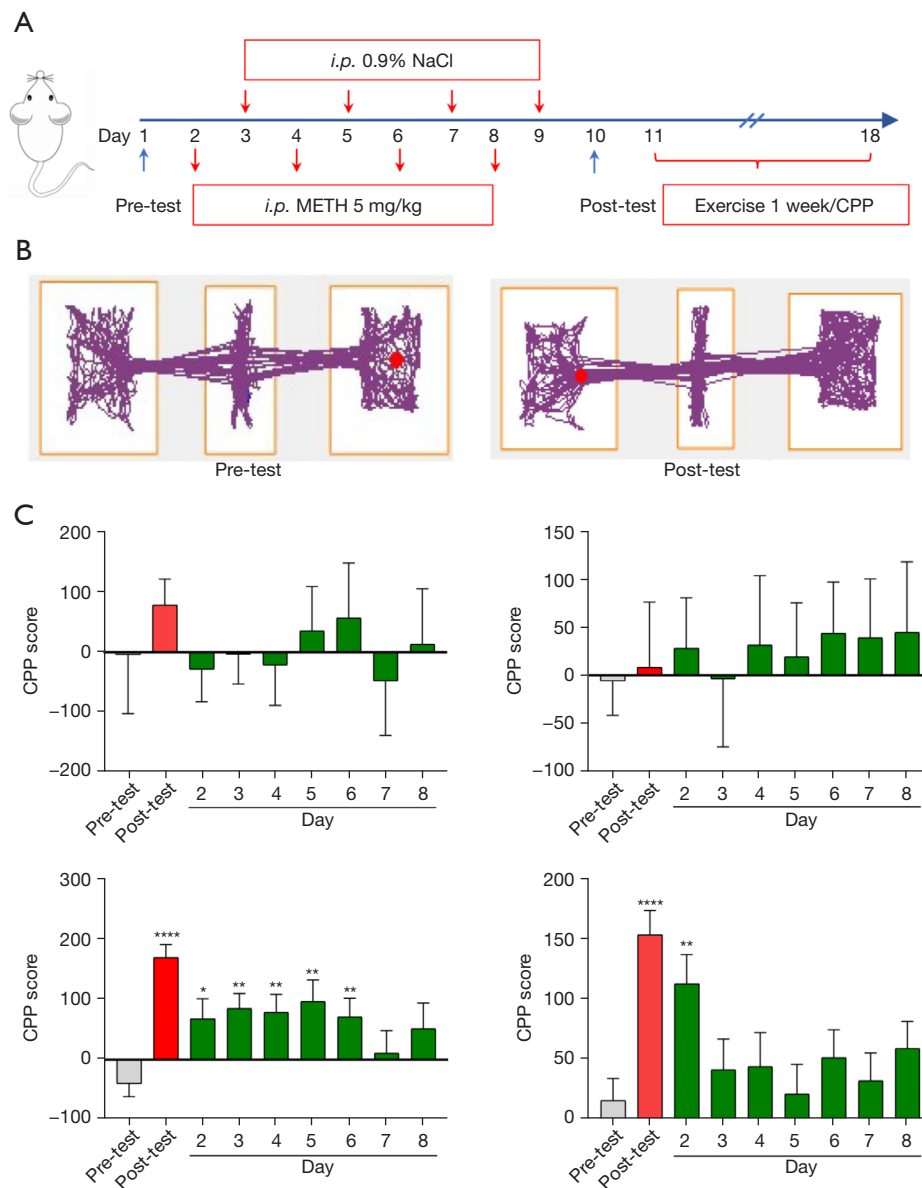
## Results

### Exercise intervention accelerated withdrawal of METH-addicted mice

Male mice received an injection (i.p.) of METH for the addiction model and performed exercise intervention, and the CPP was used to detect the preference of mice. The results showed that exercise could shorten the abstinence time of the METH group of mice (Figure 1B). For the saline and saline + running group, a CPP preference was not found among the pre-test and post-test days. The CPP preference of the METH-addicted mice who underwent exercise intervention lasted to day 3, while the CPP preference of the METH-addicted mice of the sedentary group lasted until day 6 (Figure 1C). These results suggested that exercise can accelerate the recovery of METH addiction in mice.

### DEG screening

The hippocampus tissue of the mice was collected, and 3 repeated samples were taken from each group. Then, RNA was extracted from the samples and used for sequencing and transcriptome analysis. There were 4 groups: Saline, Saline + Running, METH, and METH + Running. These DEGs were thoroughly screened for suitable candidate genes.



**Figure 1** Effects of running on METH-induced addiction behaviors in mice. (A) The protocol of the animal experimental design. Day 1 was the pre-test phase, excluding mice with obvious preferences; from days 2, 4, 6, and 8, mice of the METH group and the saline group were injected intraperitoneally with 5 mg/kg 0.2 mL METH or equal volume of saline, then placed in the drug box (black box) and removed 30 minutes later. The control experiment was performed at the same time on days 3, 5, 7, and 9, and the mice of each group were injected with a control agent (saline) and placed in the non-accompanied drug box (black and white box) at the same time as before. In this way, 4 experiments were conducted alternately; on day 10, CPP was tested to assess the addiction of the mice. Days 11 to 18 comprised the exercise intervention phase, in which moderate-intensity aerobic treadmill exercise was administered and CPP tests were performed. (B) The schematic of the CPP preference test. In the pre-test phase, METH was not injected intraperitoneally in the mice, and the movement track and time of mice in the black and white box and the black box were similar, without obvious preference. In the post-test phase, the tracking chart showed that the mice addicted to METH after intraperitoneal injection preferred the black box (accompanied by the drug box). (C) The effect of exercise on the METH addicted mice. Mice of the Saline, Saline + Running, METH, and METH + Running groups were tested using CPP during the time of pre- and post- exercise. Data were compared to the pre-test phase and presented as means  $\pm$  SEM. \*\*\*\*,  $P < 0.0001$ ; \*\*,  $P < 0.01$ ; and \*,  $P < 0.05$ . METH, methamphetamine; CPP, conditional place preference; SEM, standard error of the mean.

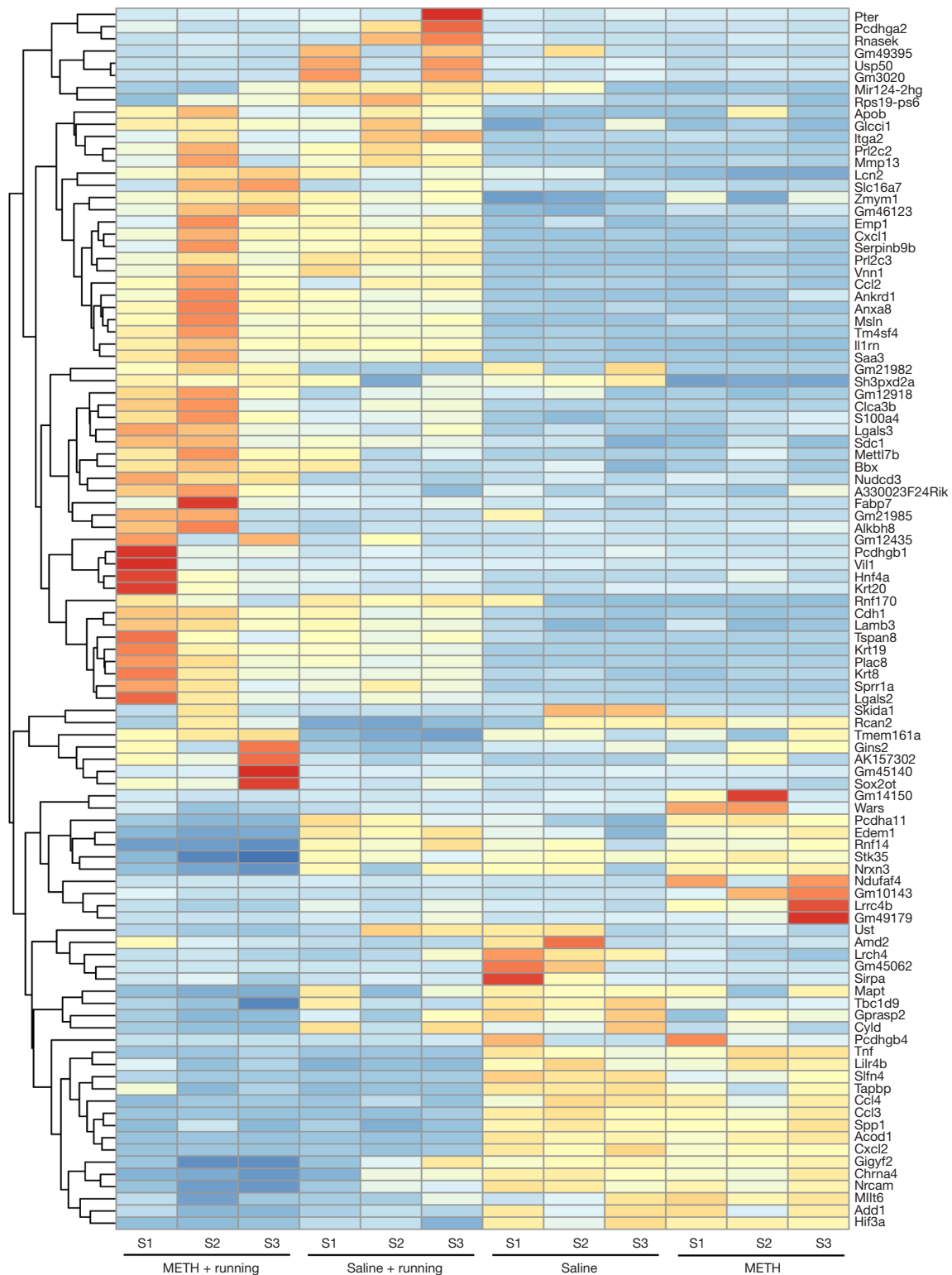
**Table 1** Primers of target genes for qRT-PCR

Primer number	Primer sequences	Bases	Fragment size
Beta-actin F	TGCTGTCCCTGTATGCCTCTG	21	131
Beta-actin R	TGATGTCACGCACGATTTC	20	
Fos F	GTTTCGTGAAACACACCAGGC	20	184
Fos R	GGCCTTGACTCACATGCTCT	20	
Gm12918 F	TGAGGTGCCCTACAGTGAGA	20	144
Gm12918 R	TGGACTTGTTCGCCTCCTC	20	
Dagla F	CTTCGTCAAGCTGAGAGCCA	20	114
Dagla R	AACACTTTTAGACGGCGGGA	20	
Pip5k1c F	CGGCTCTGTCTTCTACGTCA	20	125
Pip5k1c R	TCCGTGGGTCTGTTGAGA	20	
Stxbp1 F	CACGATGGACCCCGATCATT	20	240
Stxbp1 R	CTTCGTAAGCACAGCGCATC	20	
MgII F	ATCCAGAAGGACTACCCCGA	20	98
MgII R	AAGTAGGTTGGCCTCTCTGC	20	
Rpl30-ps9 F	TACGTGCTGGGCTACAAACA	20	81
Rpl30-ps9 R	TGGACAGTTGTTGGCAAGGA	20	
Lrrc4b-1 F	TGTCAACACCCGCTACCTGA	20	129
Lrrc4b-1 R	CCCACCTCGATCTTTCGCAC	20	
Mapt-1 F	GTGTGGCTCGTTAGGGAACA	20	174
Mapt-1 R	CTGAAGGTCAGCTTGTGGGT	20	
Napg-1 F	GTCTGCAACTCGCCCTTTT	20	223
Napg-1 R	ATTCCCGTCTCCTCATCTCCT	21	
Hnrnpa3-1 F	GGTGGATGCTGCAATGTGTG	20	104
Hnrnpa3-1 R	AATGGGCACCAGGCTTTACA	20	
Pcdhgb1 F	GCTTTTTCCAGCACCCATGA	20	231
Pcdhgb1 R	GCAGAACAAAGGCACCAGGA	20	

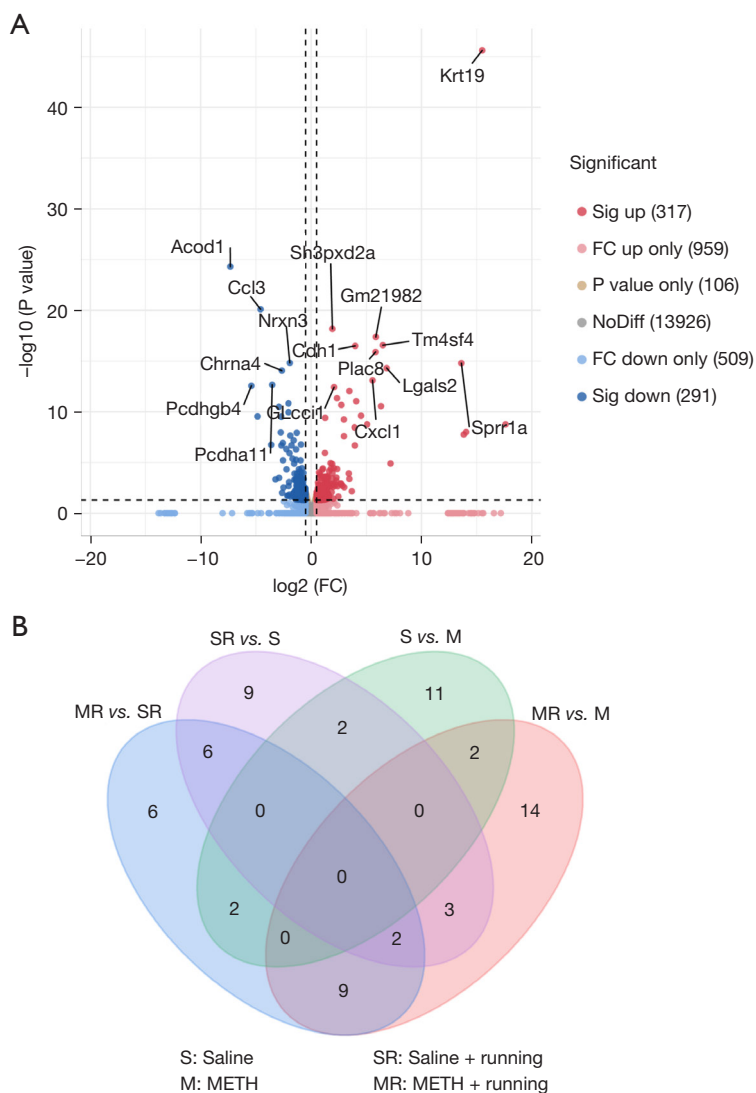
qRT-PCR, quantitative real-time reverse transcription polymerase chain reaction; F, forward primer, R, reverse primer.

Data of the DEGs were downloaded from the Lianchuan Bio-cloud platform, and Excel software (Microsoft Corp., Redmond, WA, USA) was adopted to take FPKM >0.5,  $|\log_2FC| > 0.5$ , and  $P < 0.05$  as the screening parameters. Of 608 genes detected by RNA-seq, DEGs (317 significant up and 291 significant down) were determined. The heat was used to exercise map showed the differential expression of genes in the hippocampal region of the 4 groups of mice, indicating that exercise could cause the gene expression of hippocampus tissues in METH-addicted

mice to change (Figure 2). To further screen out genes with large differences, we took FPKM >10 as the screening criterion for further screening. There were 30 genes (14 up-regulated and 16 down-regulated) screened out in the mice with METH addiction. Since exercise alone also leads to gene changes, it was necessary to exclude the exercise-induced gene changes, regardless of addiction. The volcano map showed up-regulation and down-regulation of DEGs between the METH and METH + Running groups, and 16 prominent up- and down-regulated DEGs



**Figure 2** DEGs in the hippocampal region. The heat map shows the differential expression of genes in the hippocampal region among the 4 groups of mice, with 3 repeated samples for each group. S represents sample. METH, methamphetamine; DEGs, differentially expressed genes.



**Figure 3** Identification of DEGs. (A) The volcano map showed up-regulation and down-regulation of the DEGs (METH, METH + Running). Screening criteria: FPKM >0.5,  $|\log_2FC| >0.5$ , and  $P < 0.05$ . (B) Venn diagrams show the number of unique and overlapping DEGs by comparison of each of the 2 groups among the saline, saline + running, METH, and METH + Running groups. METH, methamphetamine; DEGs, differentially expressed genes; FPKM, fragments per kilobase of transcript per million reads/fragments mapped; FC, fold count.

were noted (Figure 3A). Venn diagrams showed 3 genes overlapping between the Saline + Running, Saline and METH + Running, and METH groups, which needed to be excluded as running did not affect these genes (Figure 3B). Finally, we excluded genes with large differences among the repeated sample of the same group and screened out 12 genes that were significantly affected by METH addiction with exercise and needed further verification (Table 2). The genes *Gm12918*, *Pcdhgb1*, *Dagla*, *Mgll*, *RPL30-PS9*,

and *Fos* were highly expressed, while *Pip5k1c*, *Stxbp1*, *Lrrc4b*, *Mapt*, *Napg*, and *Hnrnpa3* were lowly expressed (Figure 4A).

#### Validation of candidate genes related to running in METH-addicted mice

We used qRT-PCR to verify the authenticity of the results of RNA-seq. The most significant 12 genes were screened from the DEGs of exercise-METH addiction (Table 1), and



**Table 2** The DEGs significantly related to METH addiction with exercise

Gene symbol	Description	Function
<i>Gm12918</i>	Predicted gene 12918	Unknown
<i>Pcdhgb1</i>	Protocadherin gamma subfamily B, 1	These gene clusters have an immunoglobulin-like organization, suggesting that a novel mechanism may be involved in their regulation and expression. These neural, cadherin-like, cell adhesion proteins most likely play a critical role in the establishment and function of specific cell-cell connections in the brain
<i>Dagla</i>	Diacylglycerol lipase, alpha	Required for axonal growth during development and for retrograde, synaptic signaling at mature synapses (by similarity)
<i>Pip5k1c</i>	Phosphatidylinositol-4-phosphate 5-kinase, type 1 gamma	Participates in a variety of cellular processes, such as vesicle-mediated transport
<i>Stxbp1</i>	Syntaxin binding protein 1	May participate in the regulation of synaptic vesicle docking and fusion, possibly through interaction with GTP-binding proteins
<i>Mgl1</i>	monoglyceride lipase	Hydrolyzes the endocannabinoid 2-arachidonoylglycerol, and thereby contributes to the regulation of endocannabinoid signaling, nociception, and perception of pain
<i>Rpl30-ps9</i>	Ribosomal protein L30, pseudogene 9	Unknown
<i>Lrrc4b</i>	Leucine rich repeat containing 4B	Regulates the formation of excitatory synapses
<i>Mapt</i>	Microtubule-associated protein tau	Promotes microtubule assembly and stability, and might be involved in the establishment and maintenance of neuronal polarity
<i>Napg</i>	N-ethylmaleimide sensitive fusion protein attachment protein gamma	Required for vesicular transport between the endoplasmic reticulum and the Golgi apparatus
<i>Hnrnpa3</i>	Heterogeneous nuclear ribonucleoprotein A3	Plays a role in cytoplasmic trafficking of RNA
<i>Fos</i>	FBJ osteosarcoma oncogene	Has a critical function in regulating the development of cells destined to form and maintain the skeleton. It is thought to have an important role in signal transduction and cell proliferation and differentiation

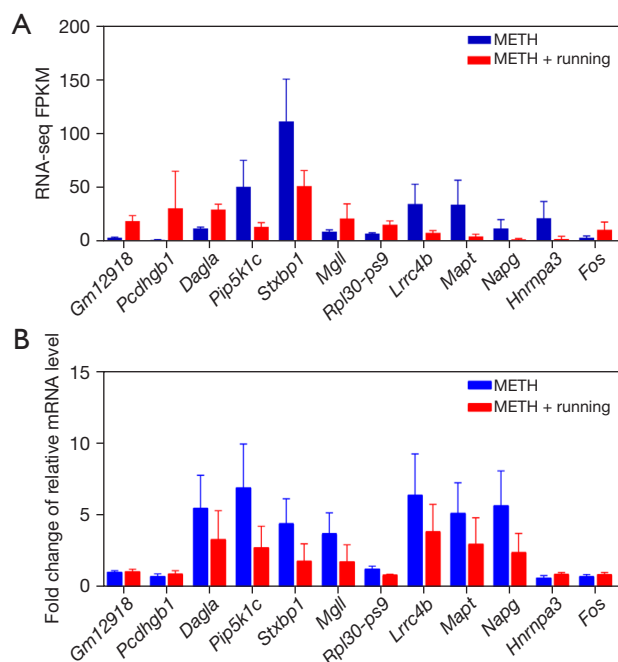
DEGs, differentially expressed genes; METH, methamphetamine; FBJ, Finkel-Biskis-Jinkins; GTP, guanosine triphosphate.

qRT-PCR verified that the relative mRNA expression was consistent with the RNA-seq results (*Figure 4B*). Of the 8 genes, *Gm12918*, *Pcdhgb1*, and *Fos* were highly expressed, while *Pip5k1c*, *Stxbp1*, *Lrrc4b*, *Mapt*, and *Napg* were lowly expressed compared with the sedentary group. The candidate genes related to running exercise in the addicted mice could be used for further analysis.

### GO and KEGG enrichment analysis

To explore the biological functions of DEGs, GO and KEGG enrichment analyses were conducted by comparing each of the two groups Saline and Saline + Running and METH and METH + Running (*Figures 5,6*). The

enrichment of GO showed that the METH-addicted mice exhibited a drug binding function, affecting the branch involved in labyrinthine layer morphogenesis, whereas running exercise enriched neutrophil chemotaxis and glucocorticoid and cellular responses to interleukin-1 (IL-1) (*Figure 5*). These functions were found in both METH + Running versus METH and Saline + Running versus Saline groups. In addition, the KEGG enrichment analysis also showed running exercise impacted the function of the IL-17 signaling pathway, salmonella infection, and tumor necrosis factor (TNF) signaling pathway. These pathways were prominent and there were higher levels of factors in the METH + Running versus METH than in the other groups (METH + Running *vs.* Saline Running, Saline running *vs.*



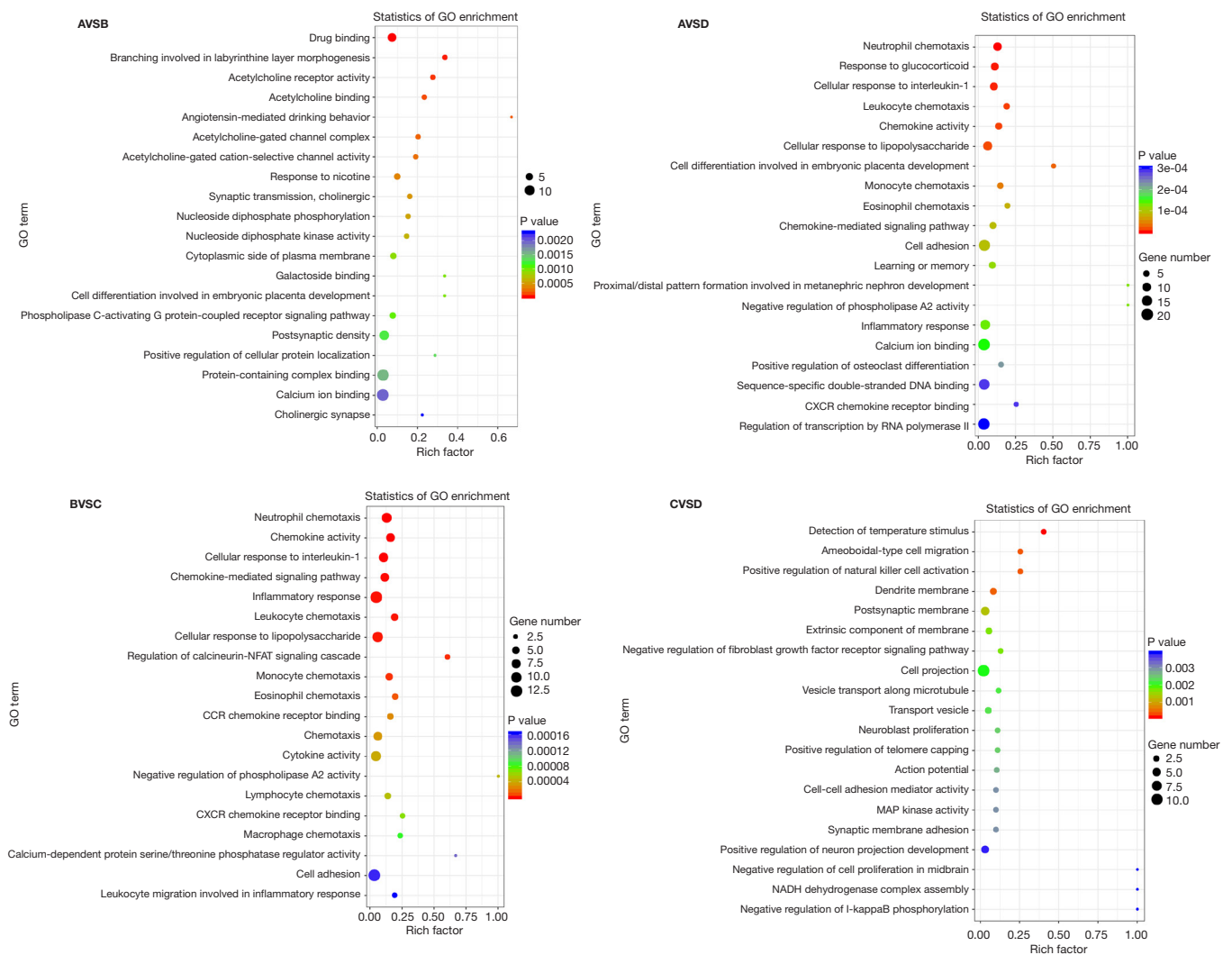
**Figure 4** qPCR verification of the DEGs. (A) According to the transcriptome data, FPKM >10,  $P < 0.05$ , and FC >2 were used as screening criteria. There were 30 genes (14 up-regulated and 16 down-regulated) in the mice with METH addiction screened out. Twelve differentially expressed genes were randomly selected. (B) The relative RNA expression of the 12 genes was measured by qRT-PCR in the METH and METH + running groups. The results were calculated and compared using the  $2^{-\Delta\Delta CT}$  method. RNA-seq, RNA sequencing; FPKM, fragments per kilobase of transcript per million reads/fragments mapped; METH, methamphetamine; qPCR, quantitative polymerase chain reaction; DEGs, differentially expressed genes; FC, fold count; qRT-PCR, quantitative real-time reverse transcription polymerase chain reaction.

Saline, Saline vs. METH) (Figure 6). These results showed that running exercise plays a significant role in the immune responses of METH-addicted mice.

## Discussion

The use of METH is a serious global public health problem with significant mental and medical consequences, including psychosis, dependency, overdose-death, and cognitive decline, as well as socio-economic and legal consequences (20). At present, drug therapy, cognitive behavioral therapy, and psychological

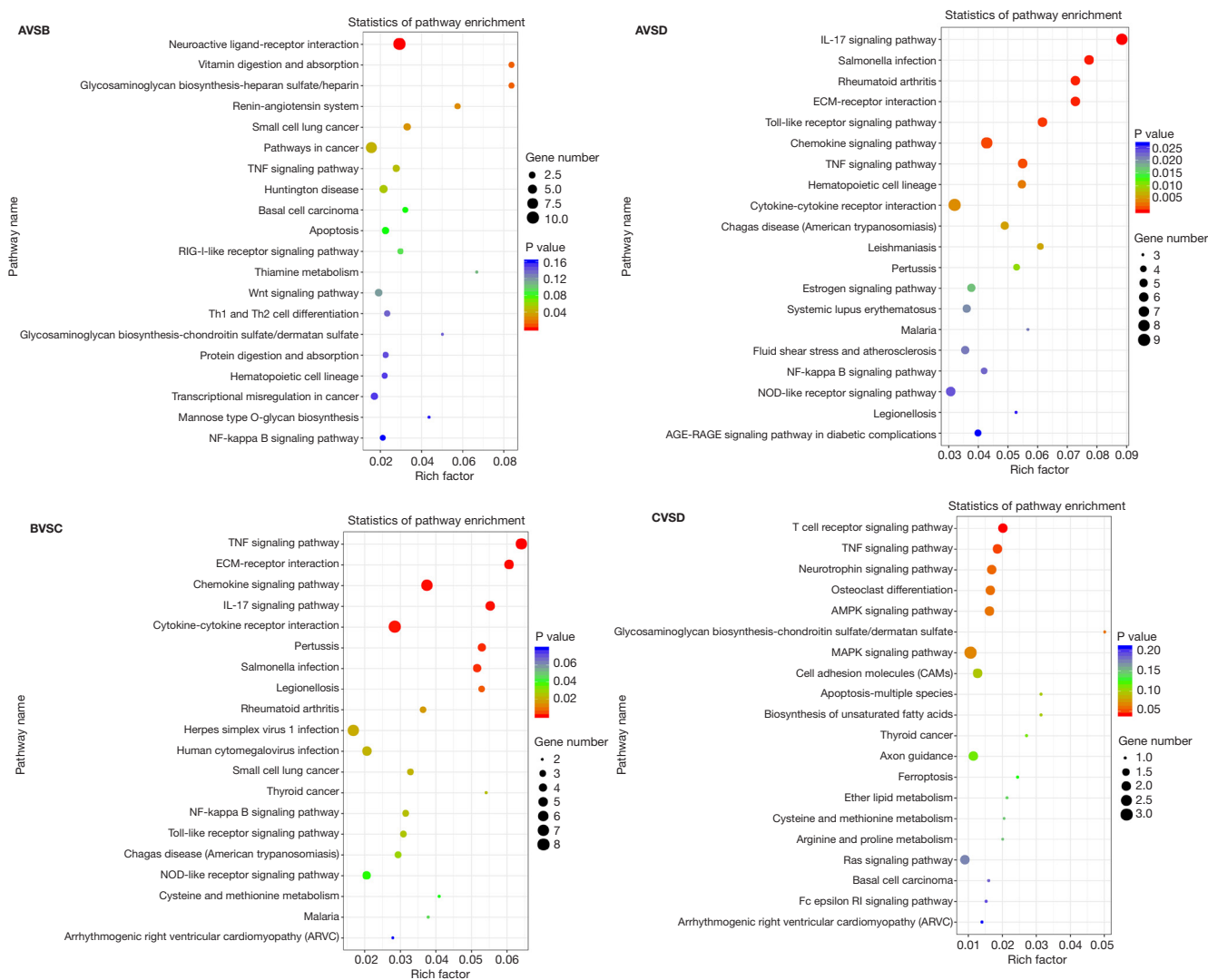
therapy have achieved certain effects in the treatment of METH addicts. However, these therapies also have some prominent negative effects, including high drug development costs and adverse reactions, such as drug dependence. Additionally, no drug has yet been identified as an effective treatment for METH addiction. Therefore, it is necessary to find a more effective and lasting new treatment for METH addiction (3). The literature reveals that voluntary exercise can improve the level of brain-derived neurotrophic factor (BDNF) in the hippocampus, which has an important impact on learning and memory performance (21). On this basis, further effects of exercise (using free-running wheels) on brain plasticity may involve epigenetic modifications of the *BDNF* gene in the hippocampus, and these interactions may determine the ability of exercise to promote long-term changes in the brain to help cope with challenges (22). In addition, some researchers have also designed relevant exercise interventions for opioid addiction, such as long-term exercise or medium- and short-term swimming exercise, which can reduce the reward effect induced by morphine, thus reducing the pursuit of addictive drugs and reducing the risk of relapse after long-term withdrawal (23,24). Therefore, the effectiveness and applicability of physical exercise is feasible in drug rehabilitation programs. In summary, physical activity has been studied for some time as a combination therapy for other substance dependence, such as tobacco, alcohol, and marijuana use, with overall positive feedback for continued abstinence. In recent years, there have been clinical studies on METH. The METH users who have participated in physical exercise programs have shown better health indicators (as measured by significant improvements in aerobic capacity, muscle strength and endurance, body composition, and heart rate variability), reduced symptoms of depression and anxiety, lower relapse rates, and sustained abstinence compared to inactive individuals (20). Many studies have been conducted on exercise-based interventions for METH abuse management (25). Physical exercise reduces the extent, duration, and frequency of drug use in individuals with drug addiction during initiation of use, addiction after prolonged use, and during withdrawal and relapse (26). Moderate aerobic exercise, for example, is effective in reducing METH use by reducing depression and anxiety and controlling drug cravings in METH-addicted individuals (27-29). In addition, exercise intervention has had a positive effect on METH withdrawal (30,31). Similarly, it has been confirmed in animal studies that the



**Figure 5** Characterization of the top-20 enriched GO biological processes of DEGs. GO showed prominent functional terms enriched by comparison of each of the 2 groups among the saline, saline + running, METH, and METH + running groups. AVSB, A versus B; AVSD, A versus D; BVSC, B versus C; CVSD, C versus D; GO, Gene Ontology; CXCR, C-X-C motif chemokine receptor; NFAT, nuclear factor of activated T cells; CCR, chemotactic cytokine receptors; MAP, mitogen-activated protein; DEGs, differentially expressed genes; METH, methamphetamine.

self-administered METH dosage is significantly reduced in mice that voluntarily run on wheels (32-34). Since most drug users lead sedentary lives, studies have reported that rats were subjected to moderate endurance-forced exercise rather than voluntary exercise to mimic the exercise stress conditions found clinically (6,35). In this study, moderate-intensity forced running was used to intervene in the acute withdrawal period of METH-addicted mice. Behavioral experiments have shown that running could effectively

alleviate the condition location bias of METH addiction and accelerate the recovery of METH addiction in mice. Previous studies have reported the effects of METH blocking exercise on the expression of *Bdnf* and *Drd2* genes in the frontal cortex and striatum, but obviously there are few such reports (36). We screened DEGs by RNA-seq in the hippocampal region of the mouse brain, and the expression of DEGs was further verified by qRT-PCR. Running exercise could change the gene regulation



**Figure 6** Top-20 enriched KEGG pathways of DEGs. KEGG showed prominent pathways enriched by comparison of each of the 2 groups among the saline, saline + running, METH, and METH + running groups. AVSB, A versus B; AVSD, A versus D; BVSC, B versus C; CVSD, C versus D; NF, nuclear factor; ECM, extracellular matrix; TNF, tumor necrosis factor; IL, interleukin; MAPK, mitogen-activated protein kinase; KEGG, Kyoto Encyclopedia of Genes and Genomes; DEGs, differentially expressed genes; METH, methamphetamine.

in METH-addicted mice, with these genes closely related to the function of immune responses. There is no relevant report so far, and the experimental design is innovative at this stage. This can lay a preliminary foundation for further verifying the influence of DEGs on related pathways, and also provide reference for our team's subsequent research. Clinical experiments have shown that, compared with those who did not exercise, METH users who exercised were able to improve their emotional state and general health status, enhance their cognitive function, reduce their recurrence rate, and sustain continuous abstinence,

thus improving their overall quality of life (37). Exercise-based interventions or combination therapy are promising as useful tools for managing METH addiction (20). Long-term aerobic exercise has a good effect on METH addicts and can be used as an effective, drug-assisted therapy to help drug addicts overcome addiction (3).

## Conclusions

Exercise could relieve the symptoms of addiction in METH-addicted mice. By analyzing RNA-seq transcriptome data

on mice hippocampus, 12 DEGs significantly regulated by exercise were noted, which could be used for further research on regulation mechanisms. Exercise intervention is expected to be an effective treatment for relieving the symptoms of METH abuse in drug users.

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## Footnote

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**Ethical Statement:** The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All experimental procedures were approved by the Institutional Animal Care and Use Committee of Kunming Medical University and carried out in accordance with the relevant laws and regulations in China [ethics review approval No. kmmu20211261. Animal laboratory facility Permit Number: SYXK (Dian) K-2020-0006]. All the procedures were conducted according to the Chinese Guidelines for the Care and Use of Laboratory Animals.

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