Exercise-Regulated Gene Differential Expression In The Hippocampal Region of C57BL/6J Mice Ameliorates Methamphetamine Dependence

Yue Li
Kunming Medical University
Guo-Fen Re
Kunming Medical University
Yu Zhao
Kunming Medical University
Shenyue KongDe
Kunming Medical University
Jun-Hong Mao
Kunming Medical University
Kun-Hua Wang
Kunming Medical University
Yi-Qun Kuang (yq610433@hotmail.com)
Kunming Medical University

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Abstract

Background: Methamphetamine (METH) is the highly addictive psychoactive drug which could harm to individual health and lead to great social problems. Various approaches have been adopted to address these problems, but relapse rates remain high. Recently, it has been found that comprehensive treatment combined with scientific and appropriate exercise intervention can improve 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 reduce METH relapse. This study is to investigate the effects and regulated genes expression related to running exercise in METH addicted mice.

Method: We used male C57BL/6J mice to construct METH addiction model and performed running exercise intervention, conditional place preference (CPP) was used to measure the effects of running intervention on METH addict mice. RNA sequencing(RNA-seq) and transcriptome analysis was performed on mice hippocampus, functions and differential expressed genes (DEGs) significantly regulated by exercise intervention in METH addict mice were analysed and noted.

Results: The results showed that days of CPP preference was shortened to day 3 in METH addict mice given moderate exercise intervention, compared to preference to day 6 in METH addict mice without exercise. In addition, hippocampal transcriptome analysis revealed 12 DEGs significantly regulated by exercise intervention. By performing Gene ontology and KEGG analysis, function of immune responses was significant enriched in METH addiction mice with exercise. The expression of 12 differential expressed genes was verified by qRT-PCR, which showed that relative mRNA expression of DEGs was consistent with the RNA sequencing results.

Conclusion: Running intervention can promote the recovery of METH addiction in mice, and the 12 candidate DEGs from mice hippocampus could use for further research on regulation mechanisms of exercise in METH addiction mice.

Introduction

Methamphetamine (METH), also known as deoxyephedrine, is a kind of highly addictive psychoactive drug. It is commonly known as ice, because its pure appearance resembles rock sugar [1]. METH is one of the most widely abused drugs in the world, it 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 emotional state and cognitive function of individuals, thus decline 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 area reconstruction, etc. Although these methods have certain efficacy, they have some problems. The effect of drug substitution therapies is temporary due to its addictive nature, other therapies are difficult to implement due to poor patient compliance and lack of standard efficacy evaluation system [2, 4]. Therefore, new treatments are urgently needed for drug addicts. Recent studies

found that scientific and appropriate exercise intervention on the basis of the above treatment methods can improve the psychological state of drug addicts, strengthen their physical fitness, and promote their physical and mental rehabilitation. Long-term regular exercise has a significant effect on improving METH withdrawal symptoms and reducing METH relapse [4]. As an important part of limbic system, hippocampus plays an important role in short-term memory, long-term memory and spatial memory processing. Some reports confirmed that METH caused significant decrease in short-term and long-term spatial memory in rats, the hippocampal volume changed significantly after METH treatment, which also caused astrocyte proliferation [5]. 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[6]. 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 drug environment is a measure of drug reward effect[7, 8]. Based on the above studies, we can conclude that exercise can significantly reduce the degree of addiction to methamphetamine. CPP training was used to detect methamphetamine 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, looking for genes that vary significantly between sample groups. RNA-seq transcriptome analysis is becoming increasingly popular in molecular phenotypic studies[9]. RNA-seq has the potential to quantify low-expression genes, revealing subtle changes in gene expression [10]. METH and exercise may lead to gene changes. We screened differentially expressed genes by sequencing, which provided new ideas for future molecular phenotype studies. In this study, we explore the influence of running exercise on METH dependence of C57BL/6J mice by establishing METH addiction mice model. It showed that exercise could interfere with cue memory established by drug and environment, and reduced drug-induced CPP after withdrawal. By performing analysis of differential gene expression of hippocampal and selected functions and candidate genes significantly related to exercise intervention, this study could explore a new method for drug abstinence treatment.

Materials And Methods

Animals

Male C57BL/6J mice (6-week-old, 18-22g) were purchased from Chongqing Tengxin Biotechnology Co., LTD. Before the experiment, in order to adapt to the surrounding environment, they were reared in separate cages for 2 weeks. The room temperature is 22 ± 2ºC with a dark light cycle of 12 hours (light from 07:00 to 19:00) and free access to food and water [11]. The animals were divided into control group and METH administration group by random number method. After the METH mice model was established, METH model group and control group were randomly divided into exercise group and non-exercise group. Each group contained 10 mice. All experiments 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.

Animal experimental instruments

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

Reagent

The drug of methamphetamine salt came from the Public Security Department of Yunnan Province, it was dissolved in 0.9% saline to reach the concentration of 75%, then performed intraperitoneal (*i.p*) injection 0.2 mL to mice at the dose of 5mg/kg, according to literature[12, 13] and preliminary laboratory basis. The control group was given the same volume of saline.

Establishment of animal models

CPP experiment is generally divided into three stages: pre-test, experiment and test. The model was established according to literature[12], which is the same as previous studies in our laboratory. Pretest phase (day 1): The mice (METH + exercise group, METH + sedentary group, saline + exercise group, saline + sedentary group) were moved into the examination room one hour in advance and kept in darkness to reduce noise and minimize animal pressure. Before formal training, the partition was removed, and the experimental animals were put into the experimental box to move freely for 15 minutes, and their stay time in each box was 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 be familiarized with experimental equipment, reducing novelty and stress, and natural preference (unconditioned place preference) can be recorded 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 (out of 43 mice used in the current study, 3 were excluded). Adaptation training (Day 1: put the mice into the box for free movement to adapt to the box environment, for 20 minutes; Training phase (Day 2-10): METH group mice were intraperitoneally injected with METH and placed in the drug box (black box), which was taken out 30 minutes later. Mice in 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. METH group and saline group 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 alternated four times. Test phase: After eight days of training, the animals were placed on the aisle without any drug treatment, and the partitions were removed. The animals could shuttle freely in 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:am-7:00pm).

Exercise plan

After the exercise intervention was established, the model was given moderate intensity aerobic treadmill exercise [14, 15]. CPP test was conducted every day before the exercise intervention until there was no significant difference between CPP test and pretest score for 2 consecutive days, indicating that CPP disappeared. The exercise group received treadmill training: the speed was 10 m/min, 0° inclination, and the acceleration was 8m/min, 45 min/d, lasting for 7 days in total [16]. All treadmill sessions are scheduled from 9:00-11:30 a.m. 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).

Organization Preparations

The mice were sacrificed for cervical dislocation, whole brains were taken out of the head and placed on an ice plate, the bilateral hippocampus was carefully dissected. The mice were quickly frozen in liquid nitrogen for 1 hour then transferred to the -80ºC refrigerator for preservation. Three repeat samples from each group were sent to Lianchuan Biological Co., LTD for reference transcriptome sequencing to identify differentially expressed genes.

Hippocampal transcriptome analysis

The expression levels of gene were evaluated by Fragments Per Kilobase of transcript per Million fragments mapped (FPKM). The standard of identifying differential expressed genes (DEG) were expression fold change equal to or more than 2 and the false discovery rate lower than 0.01. Functional analysis of DEG was performed by Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment.

Real-Time Quantitative Reverse Transcription PCR (qRT-PCR)

Total RNA from the hippocampus was extracted using Trizol regent. Reverse transcription was performed according to the Goldenstar RT6 cDNA Synthesis Kit Ver 2(Optimus Biotechnology Co., LTD. Wuhan, China) protocol. qRT-PCR was performed using 2× T5 SYBR Green I Fast qPCR Mix (Optimus Biotechnology Co., LTD.). Reference gene and target gene sequence of primer 3.0 plus design of Hangzhou Bori FQD-96A are shown in Table 1. The conditions of PCR were as follows: 95 °C for 30 s, 40 cycles of 95 °C for 5 s, 60 °C for 30 s, and 72 °C for 30 s. The melting curve analysis was executed at 95 °C for 15 s, 60 °C for 1 min, and 95 °C for 15 s. The relative mRNA expression of DEG was calculated using the $2^{-\Delta\Delta CT}$method.

Statistical analysis

All experiments were repeated three times. CPP score is calculated by subtracting the time of non-accompanied box from accompanied box to reflect the degree of addiction of mice [17]. Laboratorial data were represented as the mean ± the standard error of the mean (SEM). Differences between control and treatment groups were assessed using t-test with GraphPad Prism 8.0.1 software (GraphPad Software Inc., San Diego, USA). A value of p less than 0.05 was considered statistical significance.

Results

Exercise intervention accelerate withdrawal of METH addict mice

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

Differential expressed gene screening

The hippocampus tissues of mice were collected, three repeated samples were taken from each group. RNA was extracted from samples and used for sequencing and transcriptome analysis. There were four groups: Saline, Saline + Running, METH, METH + Running. According to the transcriptome data, FPKM > 10, $P < 0.05$, FC > 2 was used as screening criteria. The heat map showed the differential expression of genes in the hippocampal region of the four groups of mice, indicating that exercise could cause METH addiction mice change gene expression of hippocampus tissues (Fig. 3A). There were 30 genes (14 up-regulated and 16 down-regulated) in the mice with METH addiction screened out. Since merely exercise also leads to gene changes, it is necessary to exclude the exercise-induced gene changes regardless of addiction. The volcano map showed up-regulation and down-regulation of DEGs between METH and METH + Running group, 16 prominent up and down regulated DEGs were noted (Fig.4A). Venn diagrams showing the overlap of 3 genes overlapping between Saline + Running vs Saline and METH + Running vs METH were needed to be excluded as Running had no effect on these genes (Fig. 3B). Finally, we excluded genes with large differences among the repeated sample of same group, and screen out 12 genes that significantly affect by METH addiction with exercise and needed to be verified (Table 2). *Gm12918*, *Pcdhgb1*, *Dagla*, *Mgll*, *RPL30-PS9* and *Fos* were highly expressed, while *Pip5k1c*, *Stxbp1*, *Lrrc4b*, *Mapt*, *Napg* and *Hnrnpa3* were lowly expressed (Fig.4A).

Validation of candidate genes related to running in METH addiction mice

Real-Time Quantitative Reverse Transcription PCR (qRT-PCR) was used to verify the authenticity of the results of RNA-seq. The most significant 12 genes were screened from the DEGs of exercise-methamphetamine addiction (Table 1), and qRT-PCR verified that the relative mRNA expression was

consistent with the RNA sequencing results (Fig. 4B). The 8 genes *Gm12918, Pcdhgb1* and *Fos* were highly expressed, while *Pip5k1c, Stxbp1*, *Lrrc4b, Mapt* and *Napg* were lowly expressed compared with sedentary group. The candidate genes related to running exercise in addiction mice could use for further analysis.

GO and KEGG enrichment analysis

To explore the biological functions of differentially expressed genes, GO and KEGG enrichment analysis were conducted by comparison each two groups among Saline, Saline + Running, METH and METH + Running (Fig.5 and Fig.6). The enrichment of GO showed that METH addiction mice have the function of drug binding, branch involved in labyrinthine layer morphogenesis, whereas running exercise prominent enriched for neutrophil chemotaxis, response to glucocorticoid and cellular response to interleukin-1 (Fig.5). These functions were found in both METH + Running vs METH and Saline + Running vs Saline. In addition, the enrichment of KEGG enrichment also showed running exercise have the function of IL-17 siganling pathway, Salmonelia infection, TNF signaling pathway, etc. These pathways were prominent and have higher rich factors in METH + Running vs METH than others (METH + Running vs Saline Running, Saline running vs Saline, Saline vs METH) (Fig.6). These results showed that running exercise play the significant role in immune responses of METH addiction mice.

Discussion

METH use is a serious global public health problem with significant mental and medical consequences, including psychosis, dependence, overdose death, cognitive, socio-economic and legal consequences [18]. 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, what's more, no drug has yet been identified as an effective treatment for the disease. Therefore, it is necessary to find a more effective and lasting new treatment for METH addiction[3].Literature has shown 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 [19].On this basis, further effects of exercise with 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 [20].In addition, some scholars have also made 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 [21]. 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, with overall positive feedback for continued abstinence, such as tobacco, alcohol and marijuana use. In recent years, there have been clinical studies on methamphetamine. Methamphetamine users who participated in physical exercise programs showed 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 [18]. Many studies have been done on exercise-based interventions for METH abuse management [22]. Physical exercise reduces the extent, duration and frequency of drug use in individuals with drug addiction during initiation, addiction after prolonged use, and during withdrawal and relapse [23]. Moderate aerobic exercise, for example, is effective in reducing METH use by reducing depression and anxiety and controlling drug cravings in METH addicted individuals[24-26]. In addition, exercise intervention had a positive effect on METH withdrawal [27, 28]. Similarly, it has been confirmed in animal studies that METH self-dose is significantly reduced in mice that voluntarily running on wheels [29-31]. 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 [16, 32]. In this study, moderate intensity forced running was used to intervene the mice in the acute withdrawal period of METH addiction. Behavioral experiments showed that running could effectively alleviate the condition location bias of METH addiction and accelerate the recovery of METH addiction in mice. DEGs were screened by RNA-seq in the hippocampal region of mouse brain, and the expression of DEGs was further verified by qRT-PCR. Running exercise could change the gene regulation in addiction mice, these genes were closely related to the function of immune responses. There is no relevant report so far, and the experimental design is innovative and of reference significance. Clinical experiments showed that compared with those who did not exercise, METH users who exercised could improve their emotional state and general health status, enhance their cognitive function, reduce the recurrence rate and continuous abstinence, thus improving the overall quality of life of METH users[33].Exercise-based interventions or combination therapy are promising as a useful tool for managing METH addiction[18].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 quit addiction[3].

Conclusion

Exercise could relieve the symptom of addiction in mice with METH abuse. By analyzing RNA-seq transcriptome data on mice hippocampus, 12 DEGs significantly regulated by exercise was noted, which could use for further research on regulation mechanisms. Exercise intervention is expected to be an effective treatment to relieve symptoms of METH for drug abusers.

Abbreviations

METH: Methamphetamine; CPP: Conditional place preference; RNA-seq: RNA sequencing; FPKM: Fragments Per Kilobase of transcript per million fragments mapped; DEG: Differential expressed genes; GO: Gene ontology; KEGG: Kyoto encyclopedia of genes and genomes; qRT-PCR: Real-Time quantitative reverse transcription PCR; *i.p*: Intraperitoneal; BDNF: Brain-derived neurotrophic factor.

Declarations

Consent for publication

Not applicable.

Availability of data and materials

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

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Competing Interest

The authors declare that they have no competing interests.

Author contributions

K.H.W. and Y.Q.K. conceived and designed the experiments. Y.L. and R.G.F. performed sample collection and acquired data. Y.L., R.G.F., and Y.Q.K. analyzed and interpreted data. Y.L., G.F.R. and Y.Z. wrote the initial draft of the paper. All authors reviewed the draft versions and approved the final submission.

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Tables

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	TGATGTCACGCACGATTTCC	20	
Fos F	GTTCGTGAAACACACCAGGC	20	184
Fos R	GGCCTTGACTCACATGCTCT	20	
Gm12918 F	TGAGGTGCCCTACAGTGAGA	20	144
Gm12918 R	TGGACTTGTTTCGCCTCCTC	20	
Dagla F	CTTCGTCAAGCTGAGAGCCA	20	114
Dagla R	AACACTTTTAGACGGCGGGA	20	
Pip5k1c F	CGGCTCTGTCTTCTACGTCA	20	125
Pip5k1c R	TCCGTGGGTTCTGGTTGAGA	20	
Stxbp1 F	CACGATGGACCCCGATCATT	20	240
Stxbp1 R	CTTCGTAAGCACAGCGCATC	20	
Mgll F	ATCCAGAAGGACTACCCCGA	20	98
Mgll 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	GTCTGCAACTCGCCCCTTTT	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	

(F: Forward Primer, R: Reverse Primer)

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

Gene symbol	Description	Function
Gm12918	predicted gene 12918	Unknown
Pcdhgb1	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.
Dagla	diacylglycerol lipase, alpha	Required for axonal growth during development and for retrograde synaptic signaling at mature synapses (By similarity);
Pip5k1c	phosphatidylinositol-4-phosphate 5-kinase, type 1 gamma	Participates in a variety of cellular processes such as vesicle mediated transport
Stxbp1	syntaxin binding protein 1	May participate in the regulation of synaptic vesicle docking and fusion, possibly through interaction with GTP-binding proteins
Mgll	monoglyceride lipase	Hydrolyzes the endocannabinoid 2-arachidonoylglycerol, and thereby contributes to the regulation of endocannabinoid signaling, nociperception and perception of pain.
Rpl30-ps9	ribosomal protein L30, pseudogene 9	Unknown
Lrrc4b	leucine rich repeat containing 4B	Regulates the formation of excitatory synapses.
Mapt	microtubule-associated protein tau	Promotes microtubule assembly and stability, and might be involved in the establishment and maintenance of neuronal polarity.
Napg	N-ethylmaleimide sensitive fusion protein attachment protein gamma	Required for vesicular transport between the endoplasmic reticulum and the Golgi apparatus
Hnrnpa3	heterogeneous nuclear ribonucleoprotein A3	Plays a role in cytoplasmic trafficking of RNA.
Fos	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, cell proliferation and differentiation.

Figures

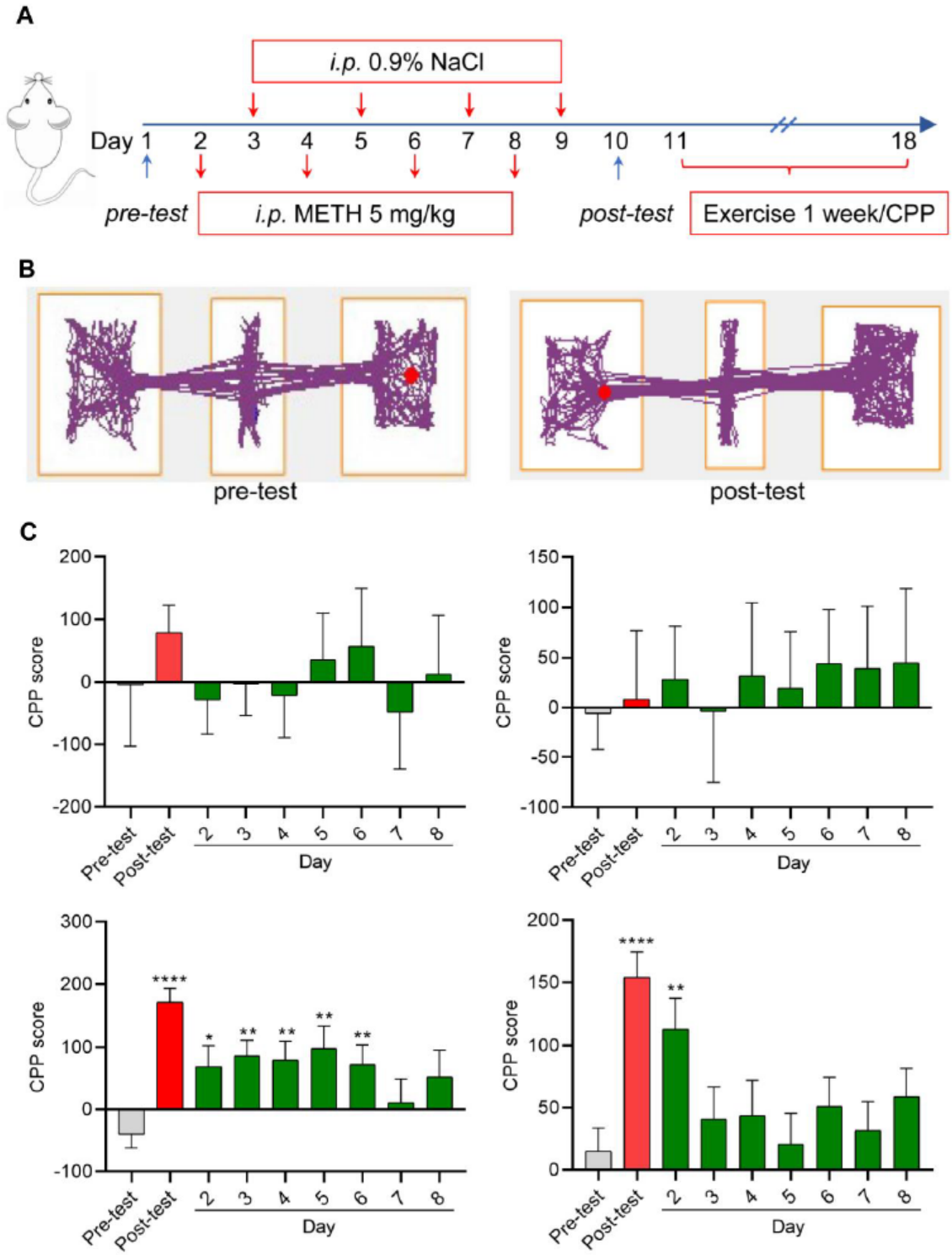

Figure 1

Effects of running on METH-induced addiction behaviors in mice. (A) The protocol of animal experimental design. Day 1 is the pre-test phase, excluding mice with obvious preference; From the 2, 4, 6 and 8 day, Mice of METH group and 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 the 3, 5, 7 and 9 day, mice of each group were injected with control agent (saline) and placed in the non-accompanied drug box (black and white box) at the same time as before. In this way, four experiments were conducted alternately; On day 10, CPP was tested to test the addiction of mice. Day 11-18 was the exercise intervention phase, in which moderate intensity aerobic treadmill exercise was administered and CPP tests were performed. (B) The schematic of CPP preference test. In pre-test METH was not injected intraperitoneally in mice, the movement track and time of mice in black and white box and black box were similar without obvious preference. Post-test is the track chart of mice addicted to METH after intraperitoneal injection, mice obviously preferred black box (accompanied by drug box). (C) The effect of exercise on METH addicted mice. Mice of Saline, Saline + Running, METH and METH + Running group were tested by CPP during the time of pre- and post-exercise. Data were compared to pre-test and presented by means + SEM. ****P < 0.0001, ** *P < 0.001, ** P < 0.01, and * P < 0.05.

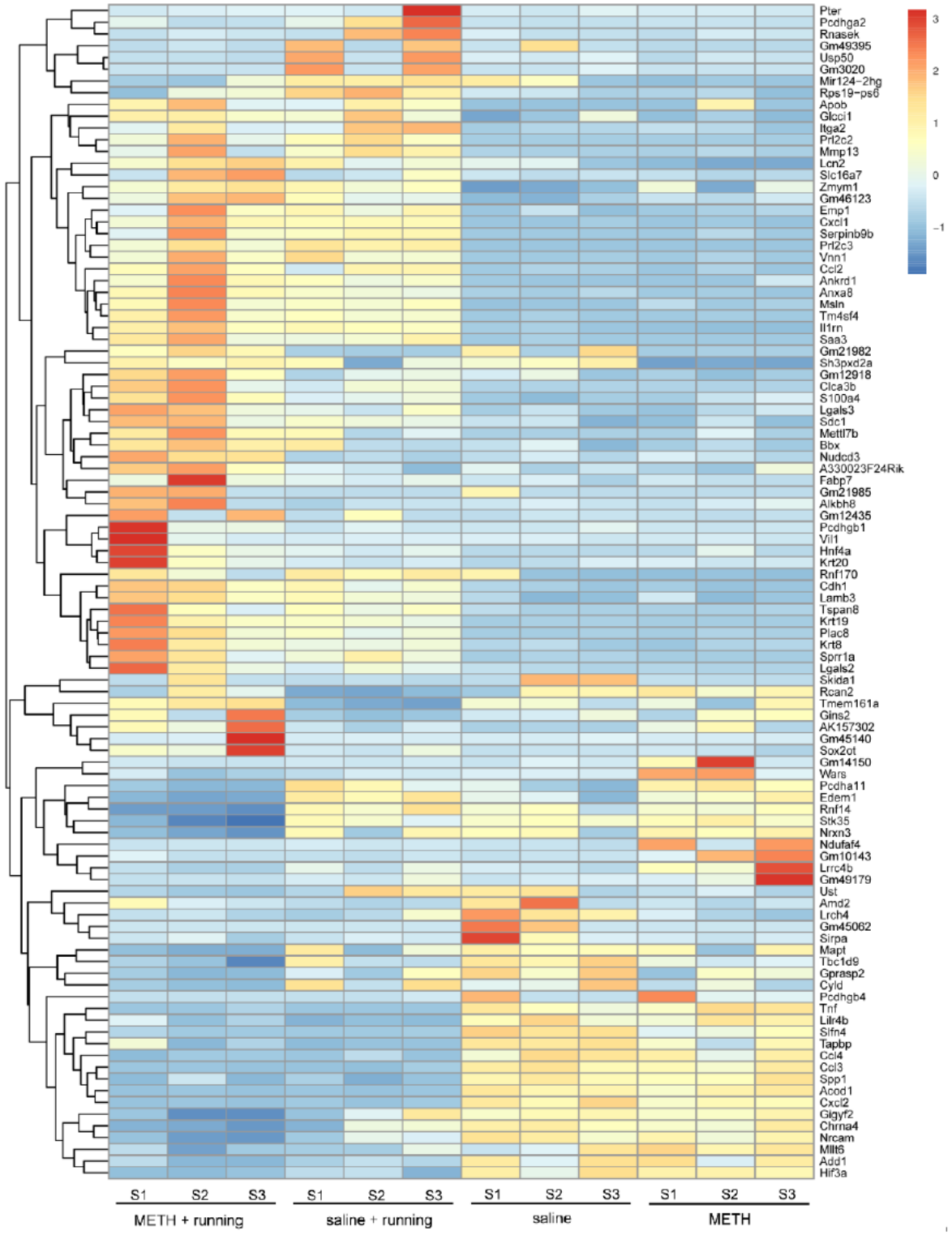

Figure 2

Differential expression of genes (DEGs) in the hippocampal region. Heat map showed the differential expression of genes in the hippocampal region among the four groups of mice, each group has three repeated samples. S represents sample.

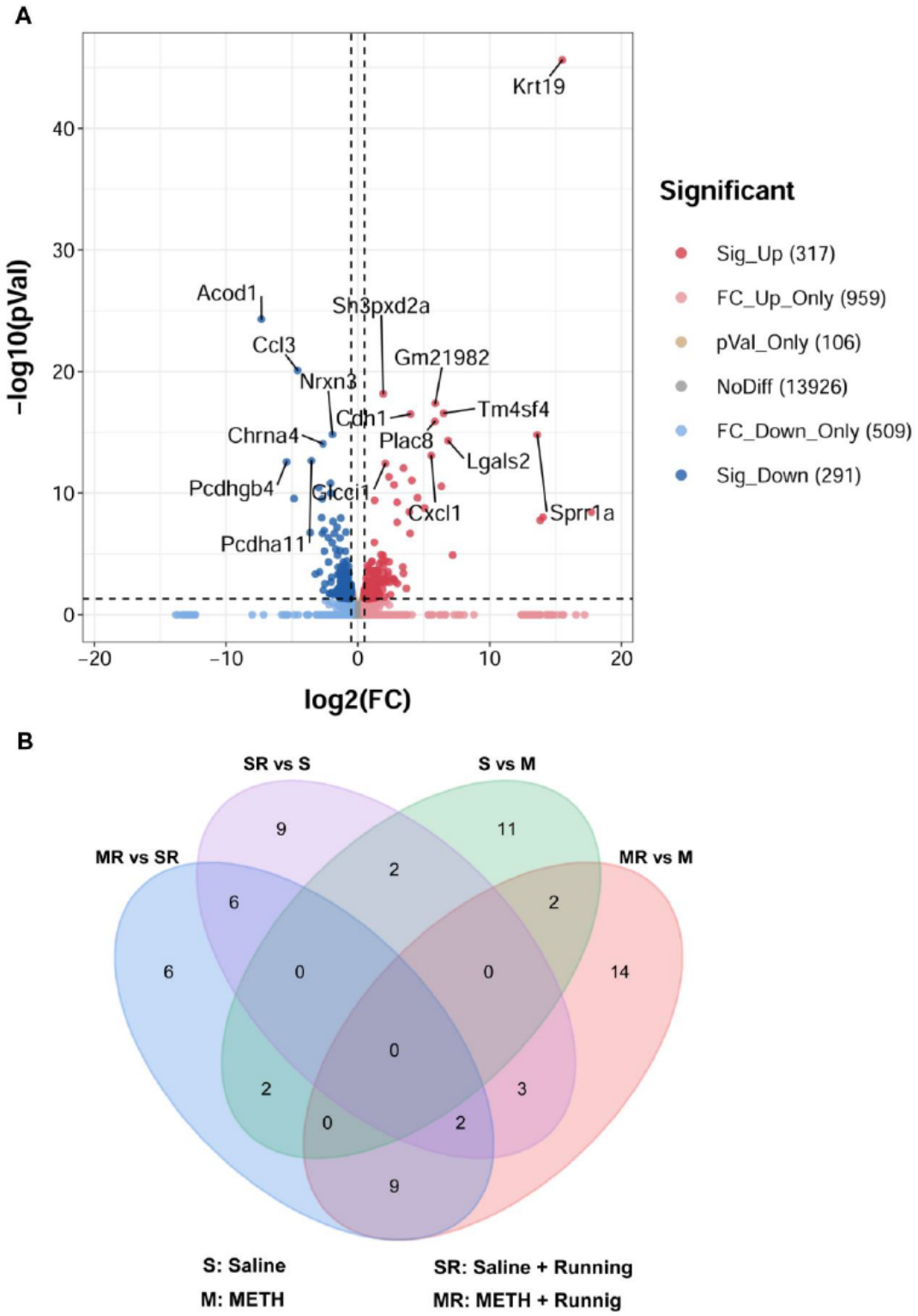

Figure 3

Identification of DEGs. (A) The volcano map showed up-regulation and down-regulation of differential expressed genes (METH, METH + Running). Screening criteria: FPKM > 0.5, |log2FC| > 0.5, P > 0.05. (B) Venn diagrams showed the number of unique and overlap differential expressed genes by comparison each two groups among saline, saline + running, METH and METH + running.

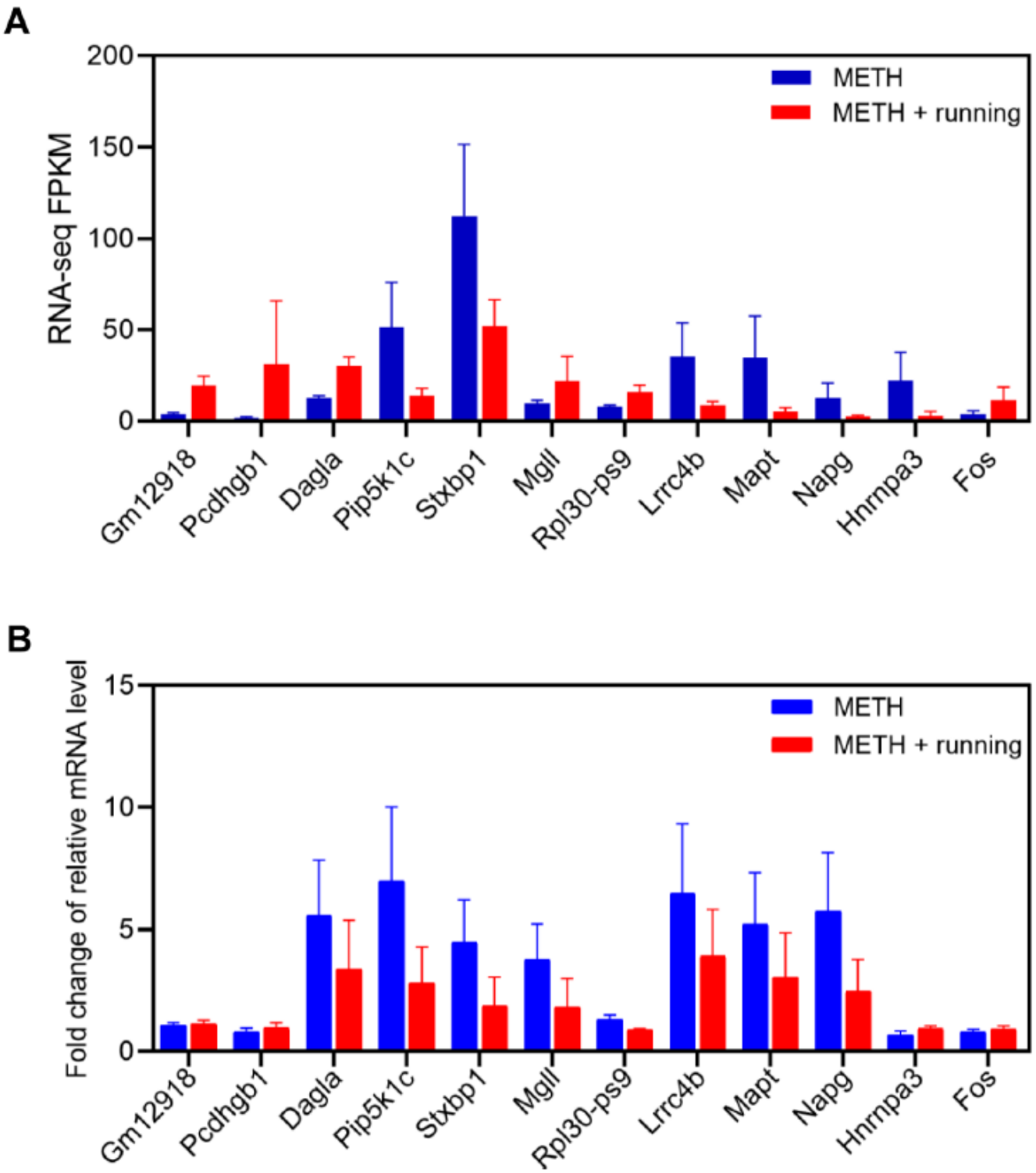

Figure 4

Quantitative PCR verification of the DEGs. (A) According to the transcriptome data, FPKM > 10, P < 0.05, FC > 2 was 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 12 genes were measured by qRT-PCR in METH and METH + running group. The results were calculated and compared using the 2−ΔΔCTmethod.

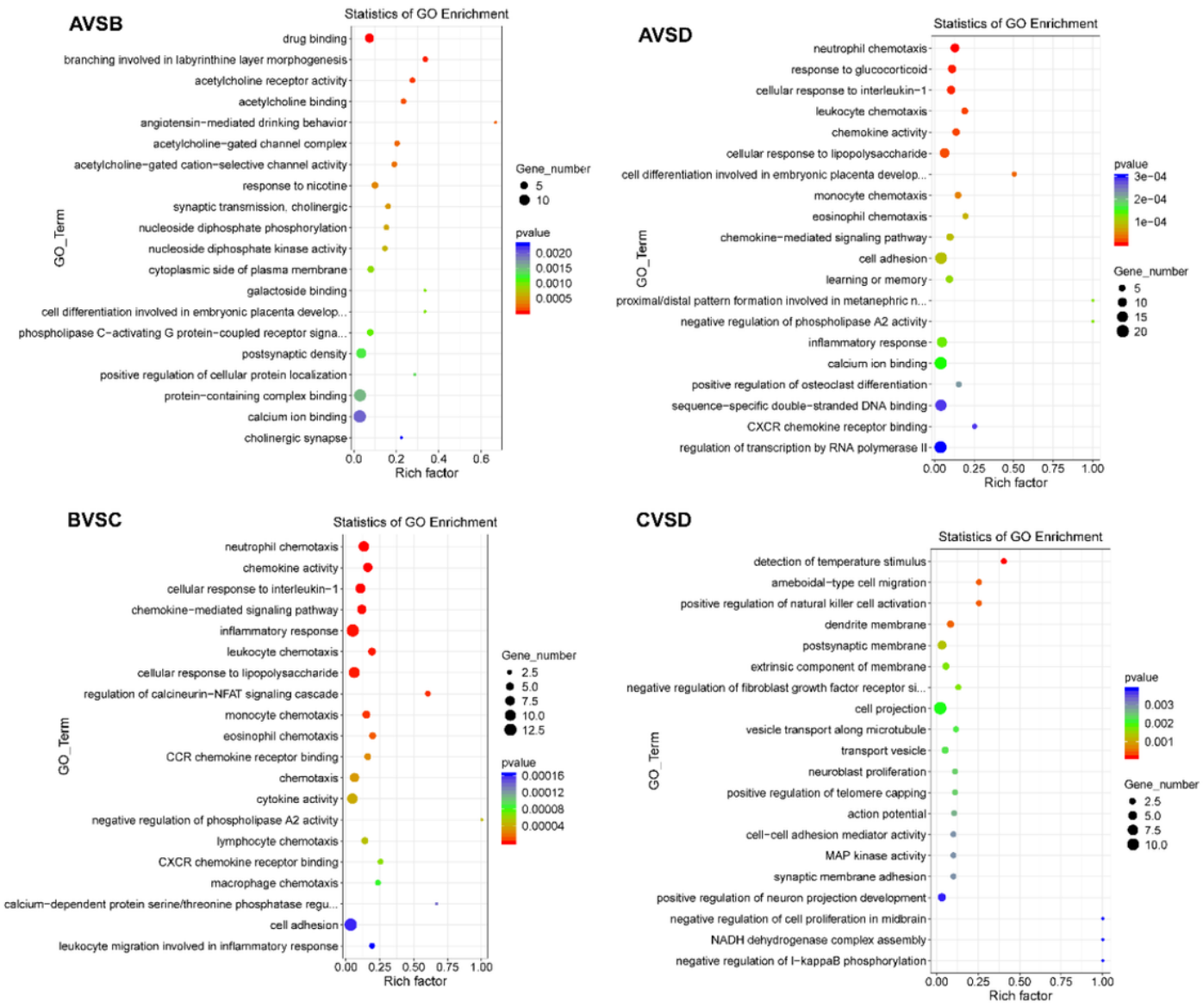

Figure 5

Characterization of top 20 enriched GO biological processes of DEGs. Gene ontology (GO) showed prominent functional terms enriched by comparison with each two groups among saline, saline + running, METH and METH + running.

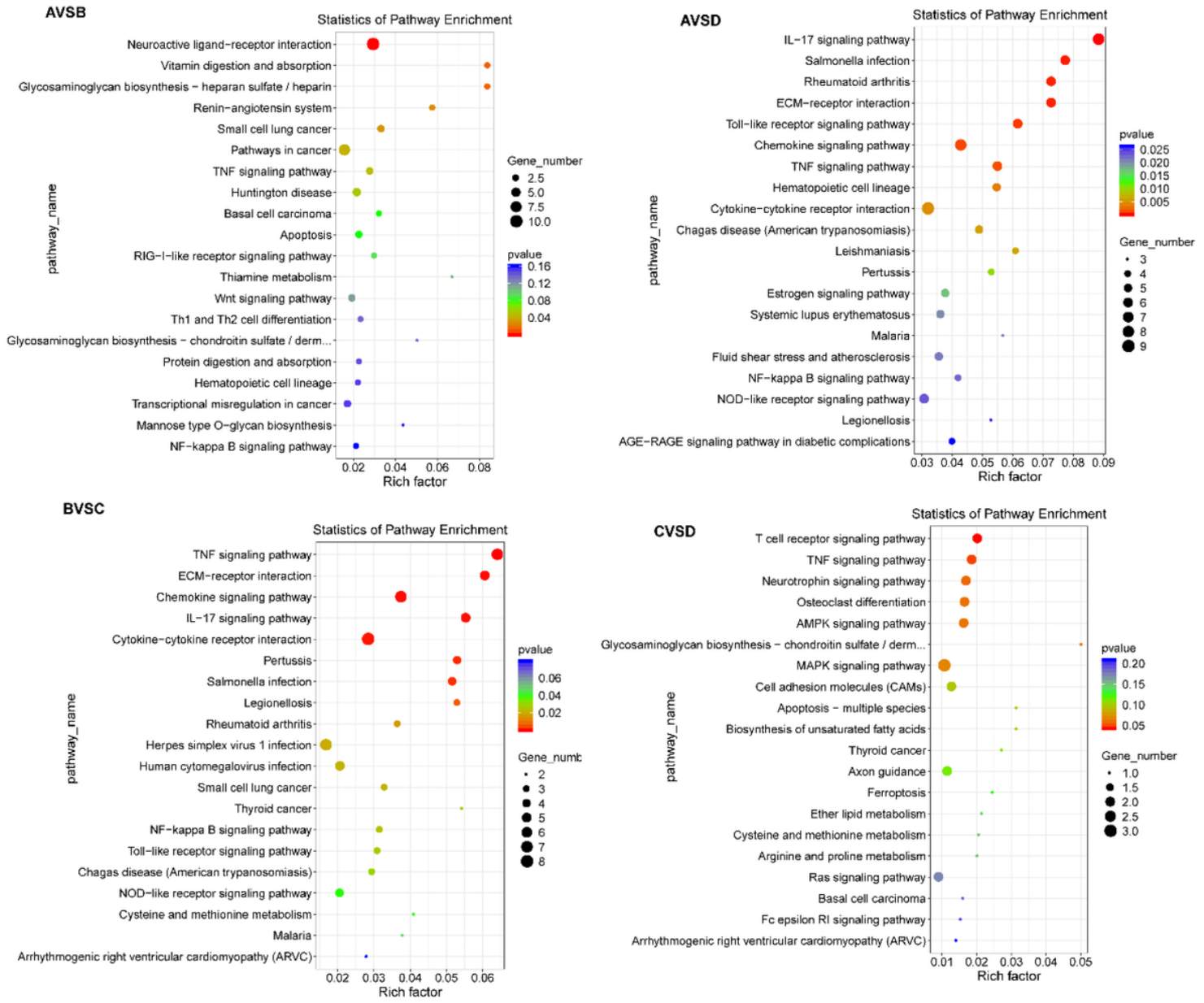

Figure 6

Top 20 enriched KEGG pathways of DEGs. KEGG showed prominent pathways enriched by comparison with each two groups among saline, saline + running, METH and METH + running.