

Moderate-intensity interval training (MIT) can protect rats against Methamphetamine –induced injuries

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Abstract

Methamphetamine (METH) can cause neurotoxicity and increase the risk of neurodegenerative disorders such as Alzheimer disease and Parkinson disease. This study aimed to investigate the effect of moderate-intensity interval training (MIT) on gene expression and antioxidant status of the hippocampus of METH-dependent rats. Twenty-eight male Wistar rats were randomly divided into four equal groups (n=7): saline, METH, MIT, and METH+MIT. METH was injected intraperitoneally at 5 mg/kg for 21 days. The MIT (intermittent running) was performed on the treadmill 5 days a week for 8 weeks. Morris Water Maze test was performed to measure learning and memory. Then, the hippocampal tissue was extracted to evaluate changes in gene expression and biochemical enzymes. The data were analyzed using one-way and two-way ANOVA methods at $P < 0.05$. The results showed that METH injection significantly reduced spatial memory and antioxidant enzymes and increased the expression of α -synuclein (α -syn), cyclin-dependent kinase 5 (CDK5), tau and phosphorylated tau (p-Tau) genes compared to the saline group. MIT significantly increased spatial memory and antioxidant enzymes. However, it reduced α -syn, CDK5, tau and p-tau expression. Thus, METH caused neural damage, and MIT could protect the neural system against METH-induced insults in male rats.

Introduction

Methamphetamine (METH), an illicit psychotropic drug, is abused by some people worldwide. It has been shown to increase dopamine secretion and regulate dopamine transport activity (DAT), thus selectively impairing the dopaminergic pathway in animal models (Prakash et al. 2017). METH consumers show various disorders in memory retrieval, verbal memory, and executive performance tests. Studies demonstrated that METH can cause hippocampal atrophy, neurodegeneration, and impaired memory and learning (Thompson et al. 2004). An animal study showed that hippocampal neurogenesis inhibition due to METH resulted in the development of cognitive and memory impairments (Recinto et al. 2012).

Research has indicated that oxidative stress is involved in altering the function of N-methyl aspartate (NMDA) receptors, inducing cell death, and activating microglia (Panenka et al. 2013). Certain post-translational changes, such as phosphorylation, toxicity uptake, and alpha-synuclein (α -syn) nitrosylation, may lead to the accumulation of α -syn which is secreted by neurons and activates microglial cells. Pathological α -syn and microglia activation by amplifying each other can lead to neuronal injury.

In addition, α -syn has been shown to activate astrocytes through Tol-like receptors (TLRs) 4 in vitro. Dysfunction of synapses surrounded by astrocytes eventually leads to neural damage. It is hypothesized that METH-induced α -syn accumulation may directly lead to mitochondrial damage, myelin sheath destruction, and failure to form synaptic vesicles, and may indirectly lead to the overexpression of cyclin-dependent kinase 5 (CDK5) and glycogen synthase kinase 3 beta (GSK-3 β). The activity of these kinases leads to tau phosphorylation and block autophagy (Rannikko et al. 2015, Sorrentino et al. 2019). Both pathways can synergistically enhance the breakdown of METH-induced neurons (Ding et al. 2020). Accumulation of tau phosphorylation is a feature of several neurodegenerative diseases such as

Alzheimer disease (AD), progressive supranuclear palsy (PSP), and frontotemporal dementia with Parkinsonism-17 (FTDP-17) (Lee et al. 2001).

There are several treatments for the neurotoxic effects of METH, one of them is voluntary exercise (sustained physical activity). It is considered as a physical activity intervention to reduce the destructive effects of METH. METH has been shown to interfere with normal brain function, and physical activity can prevent or reduce the disorder. Koji et al. (2017) stated that α -Syn accumulation is the main symptom of Parkinson's disease (PD). They found that endurance training reduced α -Syn levels that subsequently cause the destruction of dopaminergic neurons and cell death (α -Syn-mediated cell death) (Koo and Cho 2017).

Minakaki et al. (2019) demonstrated that treadmill exercise increases activity and stability during walking, and improves dopaminergic and α -Syn homeostasis without stimulating the brain autophagy-lysosomal pathway (ALP) (Minakaki et al. 2019). Currently, there is no study regarding the effect of intermittent exercise on α -syn, CDK5, tau, and p-Tau gene expression in METH-treated rats. Therefore, the present study aimed to investigate the effect of MIIT on gene expression and antioxidant status of the hippocampus of METH-dependent rats.

Materials And Methods

Animals

Twenty-eight male Wistar rats with an average weight of 200 ± 20 g were purchased from the Animal Farm of Kerman University of Medical Sciences (KMUS). They were kept under the sleep-wake cycle (12 hours of light and 12 hours of darkness) and a humidity of 40-60% and a temperature of $21 \pm 2^\circ\text{C}$ ($n=4$ per cage). The rats were randomly divided into four groups of 7, including saline, METH, moderate-intensity interval training (MIT), and METH + MIT. All procedures for the treatment of animals were conducted in accordance with the Ethics Committee of Kerman University of Medical Sciences, Kerman, Iran (Ethics Code: IR.KMS.REC.1399.436). All experiments were done in a blind manner.

Drugs

Saline solution (0.9% injection) and METH hydrochloride (5 mg/kg once daily for 21 days) (purity <96% of Kerman National Drug Center, Iran) were injected intraperitoneally (IP) (García-Cabrerizo et al. 2018, Ghazvini et al. 2016).

Exercise protocol

The exercises with moderate intensity (60% of maximum speed) were performed on a rodent treadmill at an inclination of 0° for 8 weeks, 5 days a week. The duration of training in each session was 36 minutes, including 6 minutes of warm-up with an intensity of 20% of maximum speed, 6 minutes of cooling with an intensity of 20% of maximum speed, and the main program of two sections of medium frequency with an intensity of 60% and 30% of the maximum speed (4 cycles) (Yazdanparast Chaharmahali et al. 2018).

Tissue sampling

After the last training session, the rats were anesthetized with CO₂ gas. Hippocampal tissue samples from the rats were isolated and immediately transferred to a -70°C freezer for biochemical and molecular tests.

Learning and memory test (Morris Water Maze)

The maze comprised a black circular tub (80 cm in height) filled with water (22±1°C) located in a room with special cues on the walls. A platform (8 cm in diameter) was located in the water (1.5 cm below the water surface). In the learning phase, the animals were trained 4 times a day for 4 days to find the platform in the middle of one of the quarters of the maze. Each time the rat was randomly placed in water at one of the four quadrants (north, south, east, or west). If they failed to find the platform, they would be placed on it for 10 s to associate its location with the spatial cues of the room. One day after the last learning trial, the animals underwent a probe trial to test their spatial memory. On the fifth day, the rats were placed in a non-platform pool to check their spatial memory. For this purpose, the percentage of time spent in the target quadrant was recorded and analyzed. In both stages, the movements of the rats in the water were recorded by a camera positioned above the center of the water maze, and the data were collected by a computer equipped with the water maze software (Veschsanit et al. 2021).

Biochemical tests

Malondialdehyde (MDA) measurement

MDA is a compound that can be evaluated as a lipid peroxidation index. The levels of MDA were tested by the thiobarbituric acid (TBA) method. The absorbance of products was measured at 535 nm (Buege and Aust 1978).

Glutathione peroxidase (GPx) and superoxide dismutase (SOD) activity

The activities of GPx and SOD were measured by using the Randox kits (UK; Cat NO.RS504, UK; Cat NO.SD125, respectively). The SOD activity was detected at the wavelength of 560 nm, and the GPx level was detected at the wavelength of 340 nm (Paglia and Valentine 1967).

Estimation of catalase (CAT) activity

CAT activity in the hippocampus was determined as described by Sinha (1972) with little modifications. The CAT activity was expressed as units/mg protein (Sinha 1972).

Total antioxidant capacity (TAC)

Hippocampal total antioxidant capacity was evaluated according to the method of Benzie and Strain (1996). In this method, the ability to eliminate added hydrogen peroxide was assessed. The remaining

H₂O₂ is measured using enzymatic reaction converting 3,5-dichloro-2-hydroxyl benzenesulfonate to a colored product determined at 532 nm (Benzie and Strain 1996).

Quantitative RT-PCR

Hippocampal tissue (50 mg) was lysed for RNA extraction using Trizol solution (Yektatajhiz, Cat No:YT9066) (Unique Tajhiz Azma Company) and completely homogenized with a tissue homogenizer. RNase –DNase-free was used to remove DNA contaminants. All the samples were measured with a Picodrop device (Picodrop Limited, Hinxton, United Kingdom) to measure RNA concentrations with wavelengths of 260/280 and 230/280. cDNA synthesis was performed using the cDNA Synthesis Kit (Yektatajhiz, Cat No: YT4500) and based on the cDNA synthesis protocol in the kit. By adding the RNase inhibitor to eliminate contamination, cDNA synthesis was performed in a PCR device manufactured by Analytik Jena. The expression level of the relevant genes was determined by real-time PCR (qRT-PCR) using Real Q Plus 2x Master Mix Green enzyme produced by the company (Ampliqon SYBR green Master Mix High ROX, Cat No: A323402, Denmark) (Real-time PCR of Rotor Gene Q model made by QIAGEN company). The temperature protocol was: initial denaturation at 95°C for 15 minutes, followed by 40 consecutive cycles of denaturation at 95°C for 10 seconds, 60°C for 20 seconds, and 72°C for 20 seconds. The primer sequences were designed by Primer-BLAST (NCBI) online software, and the gene (Gapdh) was used as an internal control gene (Table 1). Data analysis was performed based on threshold cycle comparison (CT). The amplification curve of each PCR reaction was normalized with the amplification curve of the corresponding Gapdh reference gene. The CT difference obtained from the tested and control samples was calculated, and the ratio of the target gene to the reference gene was calculated using the CT-2 formula.

Table 1
The designed rat primers sequences for each gene

Target	Forward Primer	Reverse Primer	TM(c)
α-synuclein	5'-GAGGGAGTCGTTTCATGGAGT-3'	5'-CATTTGTCACTTGCTCTTTGG-3'	55.92
CDK5	5'-AAGGCACCTACGGAAGTGTG-3'	5'-CTGAACTTGGCACACCCTCA-3'	59.35
tau	5'-AAGAAGCAGGCATCGGAGAC-3'	5'-CCTTGGCTTTCTTCTCGTCA-3'	57.30
p-tau	5'-GACCAGGCCGGAGATTACAC-3'	5'-AGCTTGGTCCTCCATGTTTCG-3'	58.62
Gapdh	5'-CAACTCCCTCAAGATTGTCAGCAA-3'	5'-GGCATGGACTGTGGTCATGA-3'	59.40

Statistical Analyses

All the results are expressed as Means ± SD. To evaluate the normal distribution of data, the Kolmogorov-Smirnov test was performed; and to compare the differences between groups, one-way ; two-way and Welch's ANOVA analysis of variance test, Tukey and Dunnett's T3 post-hoc test at p<0.05 were used. The statistical calculations were performed using GraphPad Prism 9.

Results

MIT increase weight of the METH-treated rats

The final weight of the METH group (230.6 ± 8.12) significantly decreased compared to the saline group (257.5 ± 14.15); $q(20) = 4.29$, $p = 0.030$. The METH + MIT (258.4 ± 14.22) and MIT (270.3 ± 23.97) groups had significant weight gains compared to the METH group $q(20) = 6.34$, $p = 0.034$; $q(20) = 4.22$, $p = 0.001$. (Figure 1). One way ANOVA test showed $F(3, 20) = 7$ $p = 0.002$.

MIT improves learning in the METH treated rats

To measure learning in the first 4 days of the MWM test, the total distance traveled to find the hidden platform was assessed. Our data did not show any significant difference between the groups, but in the first 4 days of the learning phase, significant differences were observed within the groups. There was a significant difference in the saline group on the first day with days 2, 3, and 4 ($P = 0.009$, $P = 0.010$, and $P < 0.001$, respectively). Furthermore, the METH group had a significant difference on the first day with days 3 and 4 ($P = 0.003$ and $P < 0.001$, respectively). The METH + MIT group demonstrated a significant difference on the first day with days 3 and 4 ($P = 0.014$ and $P = 0.002$); in addition, the second day also showed a significant difference with days 3 and 4 ($P = 0.024$ and $P = 0.005$, respectively). Finally, the first day of the MIT group indicated a significant difference with days 2, 3, and 4 ($P = 0.004$, $P = 0.026$, and $P = 0.004$, respectively) (Figure 2A).

MIT improves memory in the METH treated rats

There was a significant difference in the percentage of distance traveled (2.40 ± 0.66) $q(28) = 8.41$, $p < 0.001$, time spent (26.01 ± 6.70), $q(28) = 9.52$, $p < 0.001$, and frequency (4.43 ± 0.98), $q(24) = 7.96$, $p < 0.001$ in the target quadrant for the METH compared with the saline group [(5.28 ± 0.90), (55.13 ± 10.98), (7.85 ± 1.12), respectively]. MIT ameliorated distance (3.76 ± 1.18), $q(28) = 3.97$, $p = 0.042$, time (44.35 ± 7.90), $q(28) = 5.99$, $p = 0.001$, and frequency (6.57 ± 0.97), $q(24) = 4.97$, $p = 0.009$). In addition, a significant difference was found in the percentage of distance, time spent and frequency between MIT [(4.01 ± 1.05), $q(28) = 4.71$, $p = 0.012$, time (48.74 ± 8.45), $q(28) = 7.43$, $p < 0.001$, and frequency (6.85 ± 1.34), $q(24) = 5.64$, $p = 0.003$] and METH groups (Fig. 2B, C, D).

MIT ameliorates hippocampal antioxidant status in the METH treated rats

The results of the statistical analysis on biochemical parameters of rat hippocampal tissue showed a significant difference between the groups. The MDA level in the METH (0.041 ± 0.009) group was significantly increased compared with the saline group (0.028 ± 0.001), $q(24) = 5.92$, $p = 0.002$. MIT (0.022 ± 0.003) markedly decreased the MDA level compared to the METH group $q(24) = 8.30$, $p < 0.001$. METH injection significantly decreased the levels of antioxidant enzymes GPx (5.46 ± 0.32), $q(24) = 14.45$, $p < 0.001$, SOD (0.051 ± 0.005), $q(24) = 13.90$, $p < 0.001$) CAT (10.92 ± 1.24), $q(24) = 5.50$, $p = 0.004$) and TAC (3.07 ± 0.32), $q(24) = 9.93$, $p < 0.001$) in the treated group compared to saline [(7.91 ± 0.46), (0.074 ± 0.004), (14.29 ± 1.32) and (4.17 ± 0.27), respectively]. MIT improved this reduction [(6.63 ± 0.48), $q(24) = 6.88$,

$p < 0.001$); (0.65 ± 0.002) , $q(24) = 8.01$, $p < 0.001$); (12.83 ± 1.31) , $q(24) = 3.11$, $p = 0.15$); (3.35 ± 0.31) , $q(24) = 2.48$, $p = 0.31$), respectively)] (Figure 3).

MIT changes gene expression of the hippocampus in the METH treated rats

There was a significant elevation in CDK5 gene expression in the METH group (2.4 ± 0.67) compared to the saline group (1.0 ± 0.02), $t(6.02) = 5.50$, $P = 0.008$). Moreover, the decline in CDK5 expression in METH + MIT (1.16 ± 0.14) and MIT (0.90 ± 0.08) groups compared to the METH group was statistically significant, ($t(6.52) = 4.78$, $P = 0.01$) and $t(6.18) = 5.87$, $P = 0.005$, respectively) (Fig. 4A).

The α -synuclein gene expressed a higher level in the METH group (2.92 ± 0.57) compared to the saline group (1.0 ± 0.03), $t(6.03) = 8.80$, ($p < 0.001$), and MIT (1.49 ± 0.30) $t(8.98) = 5.85$, $p = 0.001$) diminished this elevated level. In addition, a significant difference was found between the MIT (0.83 ± 0.15) and METH+MIT groups ($t(9.29) = 5.22$, $p = 0.003$) (Figure 4B).

Increased expression of Tau (1.60 ± 0.40) and p-Tau (1.87 ± 0.52) genes were significant in the METH group compared to the saline group (1.0 ± 0.02), $t(6.05) = 3.92$, ($p = 0.038$); $t(6.03) = 4.39$, $p = 0.02$), respectively); moreover, the decrease in Tau (1.03 ± 0.12 , 0.95 ± 0.10) and p-Tau (1.16 ± 0.10 , 0.94 ± 0.08) expressions in METH + MIT and MIT groups compared to the METH group was statistically significant [$t(7.12) = 3.60$, ($p = 0.043$); $t(6.78) = 4.20$, $p = 0.021$); $t(6.51) = 3.53$, ($p = 0.047$); $t(6.34) = 4.63$, $p = 0.018$), respectively] (Figures 4C, D).

Discussion

Our results showed that METH administration in rats led to spatial memory impairment, reduced antioxidant enzymes, and increased the expression of α -syn, CDK5, Tau, and p-Tau genes. MIT significantly increased spatial memory and antioxidant enzymes, but significantly decreased the expression of the aforementioned genes.

In accordance with our result, Izawa et al. (2006) showed that METH reduced memory and induced confusion and amnesia, and its long-term use destroyed dopaminergic and serotonergic nerve endings in the brain (Izawa et al. 2006). In addition, Sertani et al. (2011) reported a decrease in spatial and non-spatial memory due to the shrinkage of the hippocampus and cell death of neurons by METH (Cerretani et al. 2011).

Hay et al. (2017) and Berchtold et al. (2010) reported the beneficial effects of exercise on spatial memory in mice, which are consistent with the present study. MIT training appears to have some effect on improving spatial memory in mice taking METH (Berchtold et al. 2010, He et al. 2017).

Our results demonstrated that antioxidant enzymes were reduced by METH; in other words, the antioxidant defense of hippocampal tissue against oxidative stress was greatly reduced. Kakita et al. (2002) showed that the use of METH in animal models causes oxidative stress and neurotoxicity due to the production of reactive oxygen species (Kakita et al. 2002).

Hamakawa et al. (2013) reported that 3 weeks of exercise reduces the level of free radicals, which are associated with a decrease in oxidative damage and the resulting movement disorders (Hamakawa et al. 2013). In particular, Ogunovsky et al. (2005) demonstrated that moderate-intensity exercise (1 hour of swimming per day for 8 weeks) increases both antioxidant capacity and resistance to oxidative stress in the body. Note that over-training does not induce oxidative damage in the brain and does not cause loss of memory (Ogonovszky et al. 2005).

MIT can increase the resistance of hippocampal neurons to METH-induced damage by increasing antioxidants in neurons. These neuroprotective mechanisms of exercise activity provide a new therapeutic perspective and an important preventive point of view, serving as an effective and strategic method to reduce METH-induced brain complications. Kamilti et al. (2013) stated that exercise increases the basal activity of some antioxidant enzymes and, as direct and indirect antioxidants, neutralizes a variety of oxidizing species and protects cells from oxidative damage (Camiletti-Moirón et al. 2013). These studies are consistent with our findings, and METH in the rats led to oxidative stress, decreased activity of the antioxidant enzymes including TAC, SOD, GPX, and CAT, and increased level of MDA. MIT significantly increased some antioxidant enzyme levels (GPx and SOD) and mitigated the detrimental effects of METH-induced oxidative stress.

The results of gene expression indices indicated that α -syn, CDK5, Tau and p-Tau levels increased with METH, and MIT decreased their expression, suggesting the positive effect of exercise on the indices. METH-induced α -syn accumulation directly leads to mitochondrial damage, myelin sheath degeneration, and failure to form synaptic vesicles, and can indirectly lead to the overexpression of CDK5, tau phosphorylation, and autophagy blockage. Both pathways can synergistically enhance the breakdown of neurons (Ding et al. 2020).

It appears that METH injection results in the complex formation of CDK5 with P25, and the activity of this kinase is greatly increased, eventually leading to excessive Tau phosphorylation. MIT training possibly reduces the formation of this complex. Tau toxicity in Alzheimer disease is due to its deposition in the soma or dendrites of neurons or its high phosphorylation. It has been shown that CDK5 is activated by α -syn, and eventually, this kinase leads to tau phosphorylation. Various factors such as the toxic effect of β -amyloid deposition on the brain tissue and disruption of message transduction pathways due to hyperphosphorylation of tau protein are involved in the development of this disease. Ko et al. (2017) stated that endurance training increases Sirtuin 1 (SIRT-1) expression, which leads to increased mitochondrial biogenesis and reduced oxidative stress by the activation of peroxisome proliferator-activated receptor-gamma coactivator 1-alpha (PGC-1 α), and may improve mitochondrial function and autophagy process (Koo and Cho 2017). This study is consistent with the present study. It is possible that the level of PGC-1 α in MIT training has increased; one of the limitations of the present study is the lack of PGC-1 α measurement.

Conclusion

It seems that METH causes neurotoxicity in the hippocampal tissue, as well as decreased spatial memory and antioxidant enzymes by increasing oxidative stress at the cellular level. Increased α -syn, CDK5, Tau and p-Tau expression possibly leads to neuronal injury, and MIT may be effective against METH-induced injuries in male rats.

Declarations

Funding: Kerman University of Medical Sciences (KUMS).

Conflicts of interest/Competing interests: The authors hereby state that there is no conflict of interest in the present study.

Availability of data and material: The data will be available based on reasonable request.

Code availability: Not applicable.

Authors' contributions: All authors met the standard writing criteria based on the recommendations of the International Committee of Medical Journal Publishers.

Ethics approval: The research project was registered in the Ethics Committee of KUMS (IR.KUMS.REC.1399.436).

Consent to participate: Not applicable.

Consent for publication: We give our consent for the publication of identifiable details, which can include figures and table within the text to be published in the *Metabolic Brain Disease*.

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Figures

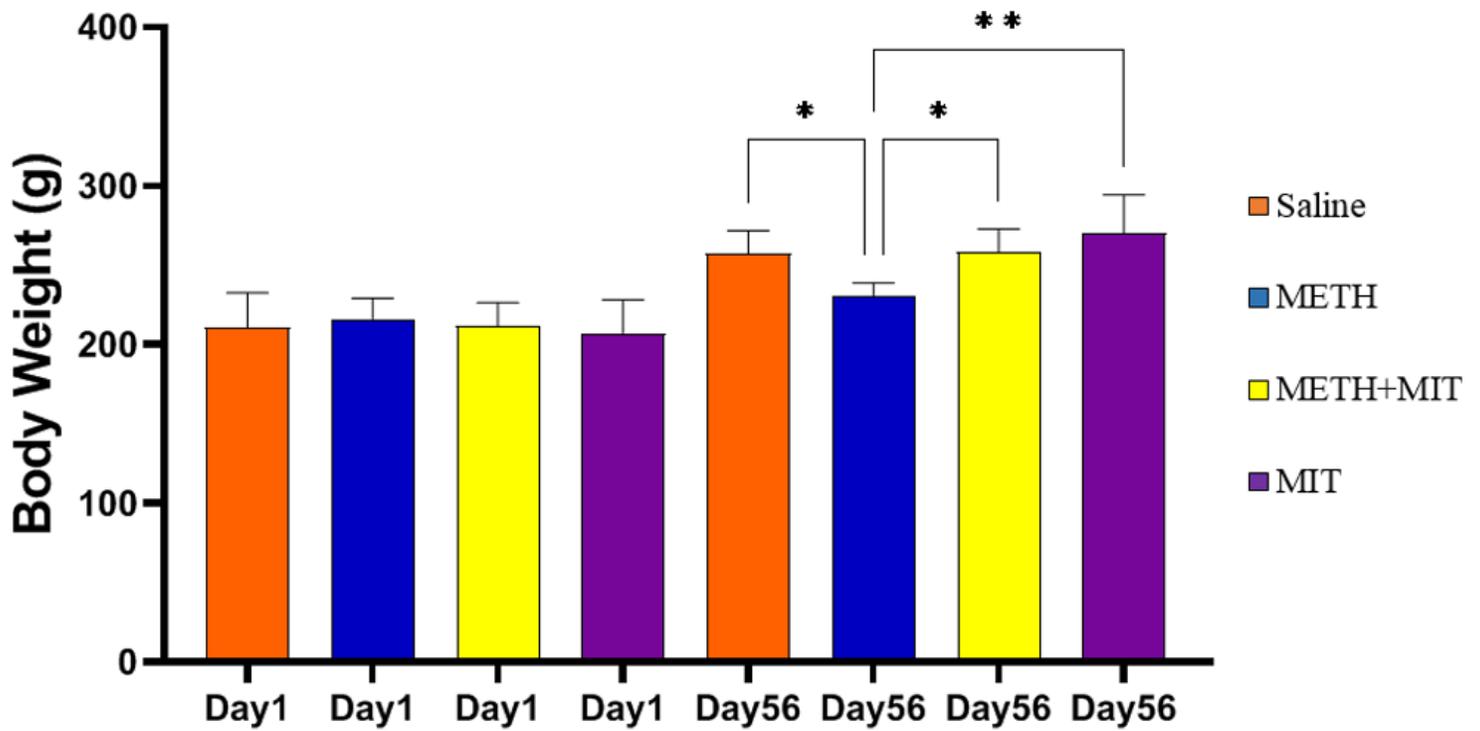


Figure 1

Bodyweight (g), the animals subjected to METH and MIT show significant differences in body weight compared with the saline group after 56 days. * Significant with saline (P=0.030) * Significant with METH (P=0.034); ** significant with METH (P=0.001). METH: methamphetamine; MIT: moderate-intensity interval training.

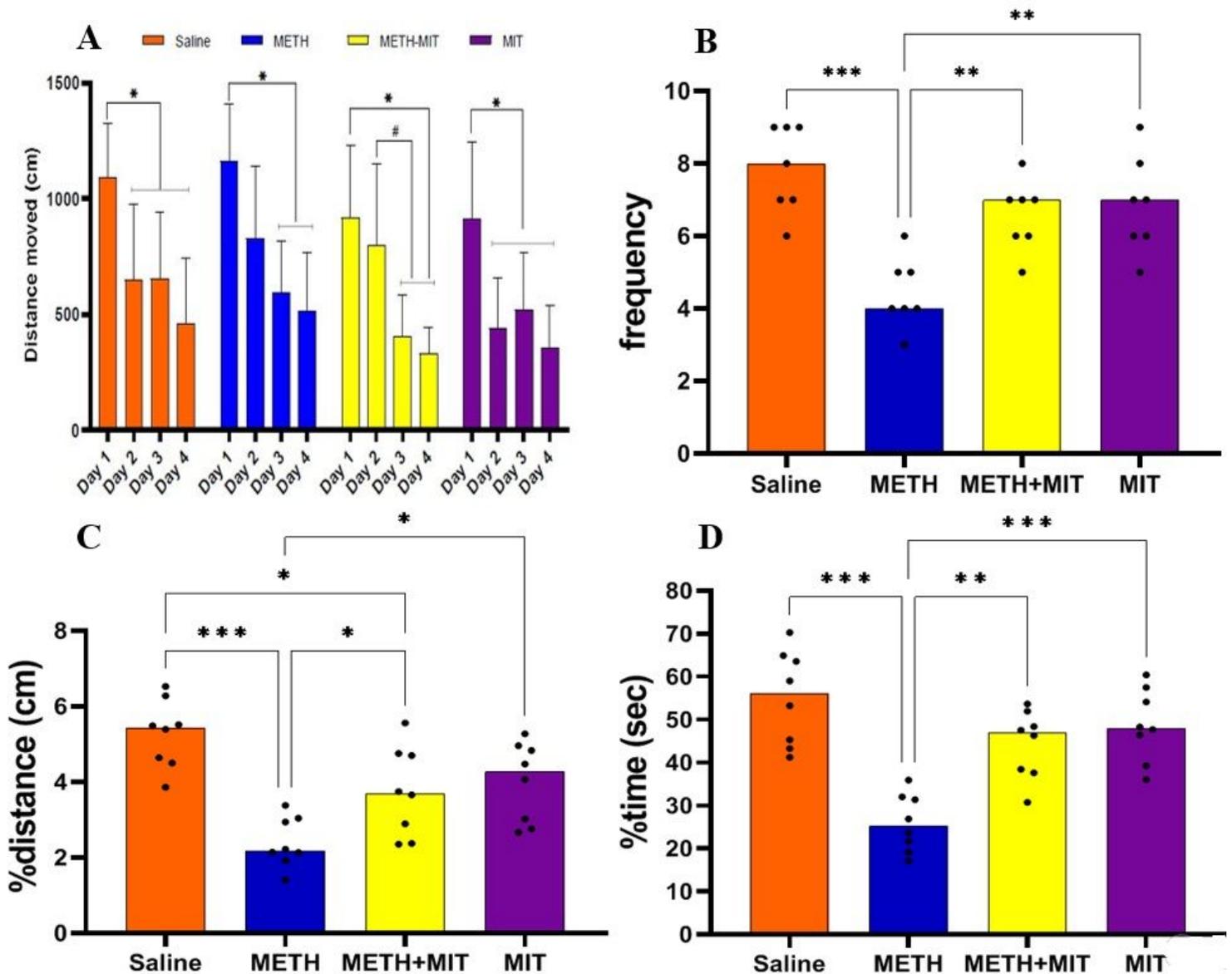


Figure 2

A. The chart displays the learning status of the rats in different groups to find a hidden platform, examined over 4 days. In the first 4 days of the learning phase, significant differences were observed within the groups. (A) Saline group: * shows the level of significance of the first day with second, third, and fourth days ($P=0.009$, 0.01 , and 0.001 , respectively). METH group: * shows the level of significance of the first day with third and fourth days ($P=0.003$, and 0.001 , respectively). METH +MIT group: * shows the level of significance of the first day with third and fourth days ($P=0.014$ and 0.002 , respectively). # shows the level of significance of the second day with the third and fourth days ($P=0.024$ and 0.005 , respectively). MIT group: * shows the level of significance of the first day with second, third, and fourth days ($P=0.004$, 0.026 , and 0.004 , respectively). The frequency (B), percentages of distance traveled (C), and time spent (D) of the animals in the target quadrant in the probe trial in different groups. All the data are presented as Means \pm SD. * shows the level of significance ($P<0.05$); ** shows the level of significance ($P<0.01$); *** shows the level of significance ($P<0.001$).

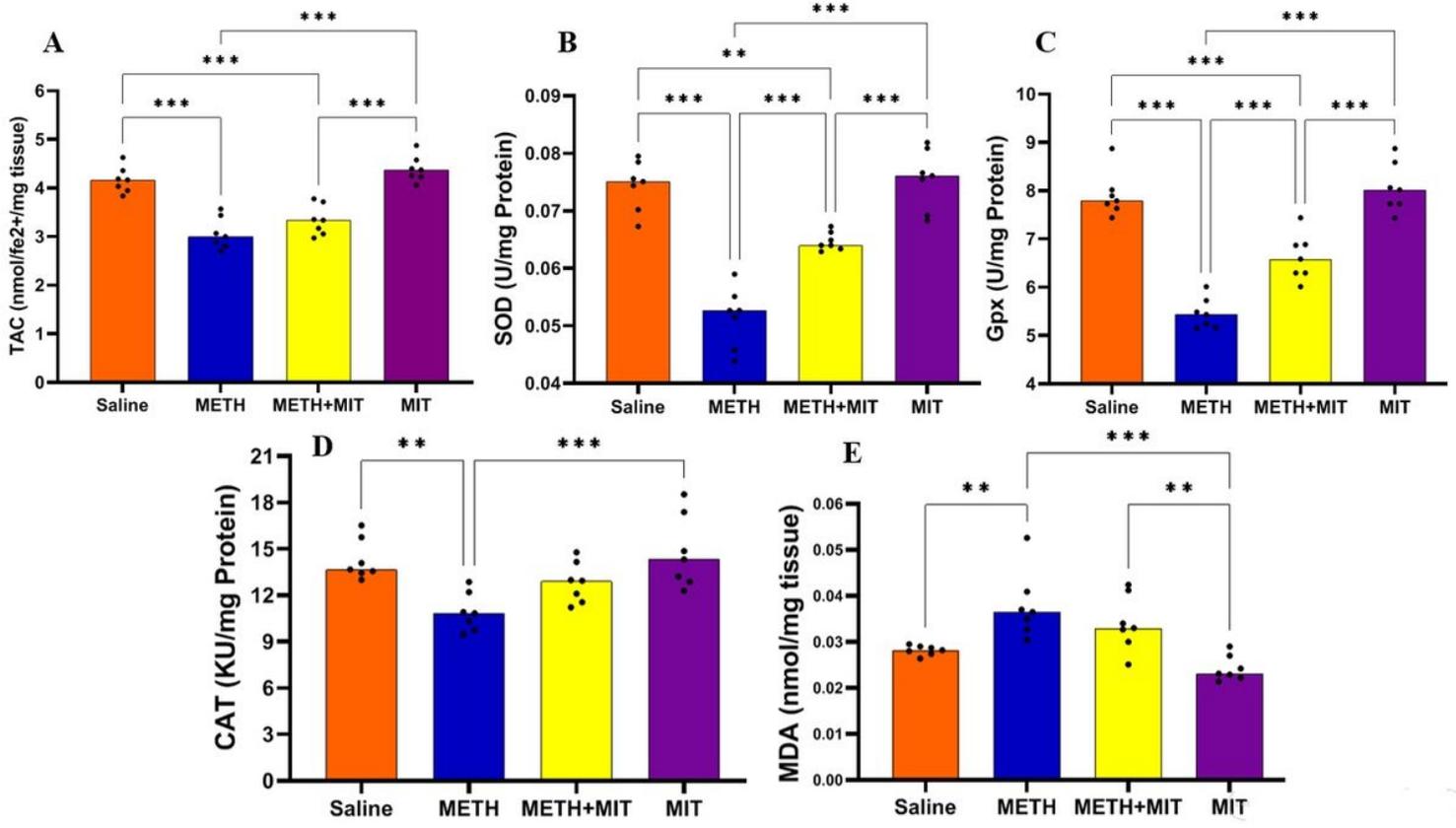


Figure 3

Biochemical parameters of the rat hippocampal tissue treated with METH METH significantly decreased TAC (A), SOD (B), Gpx (C), and CAT (D) levels and increased MDA (E) levels, and exercise (MIT) increased the GPx and SOD. * shows the level of significance (P<0.05); ** shows the level of significance (P<0.01); *** shows the level of significance (P<0.001). TAC: Total antioxidant activity; SOD: superoxide dismutase; GPx: Glutathione peroxidase; CAT: catalase; MDA: Malondialdehyde.

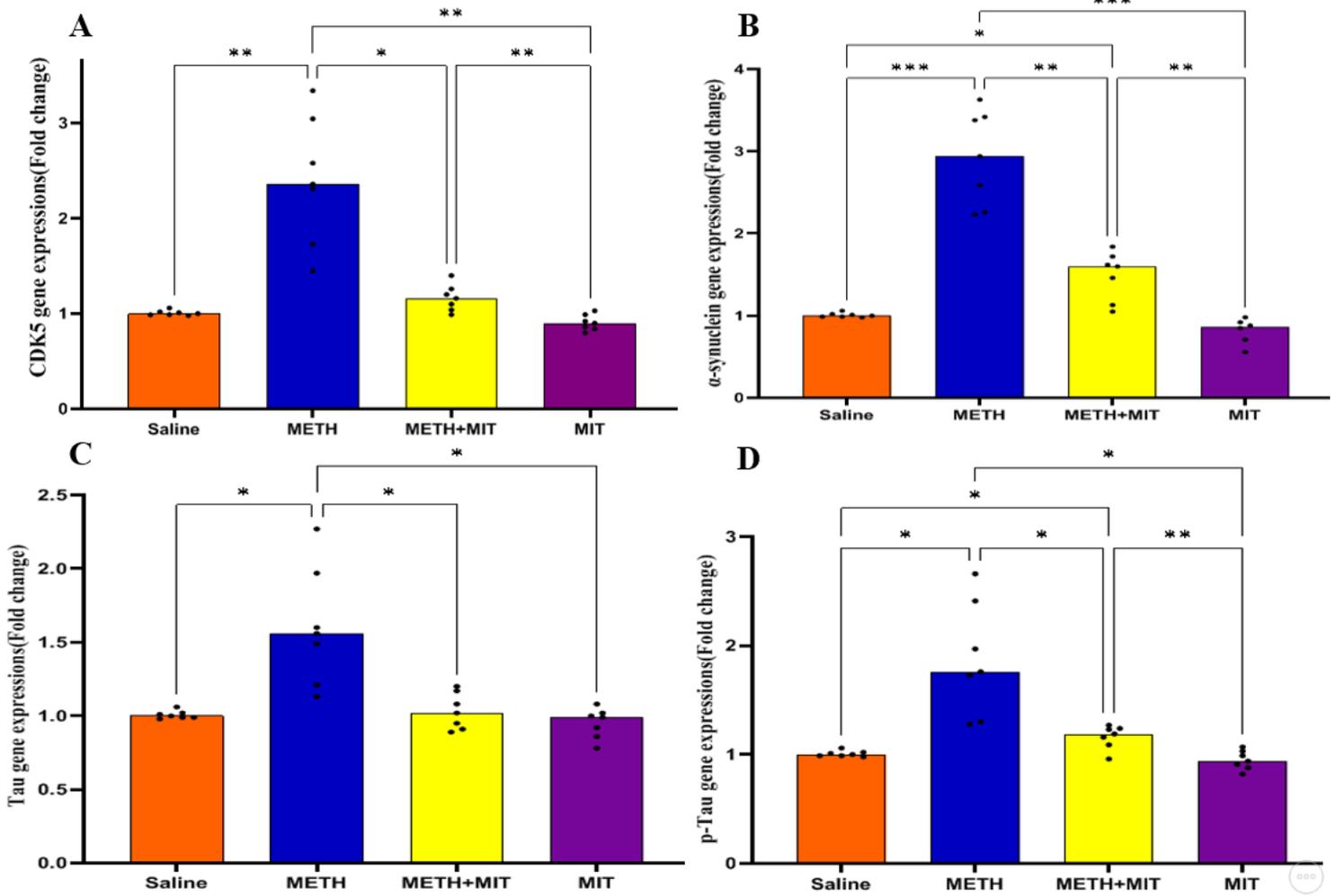


Figure 4

Changes in CDK5 (A), α-synuclein (B), Tau (C) and p-Tau (D) gene expression levels in the rat hippocampus of METH treated rats. * shows the level of significance (P<0.05); ** shows the level of significance (P<0.01); *** shows the level of significance (P<0.001).