MEDICAL SCIENCE MONITOR

ANIMAL STUDY

e-ISSN 1643-3750
© Med Sci Monit, 2018; 24: 3789-3803
DOI: 10.12659/MSM.910550

Received: 2018.04.12
Accepted: 2018.05.02
Published: 2018.06.05

Effect of Sirtuin-1 on Synaptic Plasticity in Nucleus Accumbens in a Rat Model of Heroin Addiction

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

BCEF Baijuan Xia
BF Yixin Li
BF Rongrong Li
CE Dan Yin
DE Xingqiang Chen
BF Jie Li
AG Wenmei Liang

Department of Histology and Embryology, Guizhou Medical University, Guiyang, Guizhou, P.R. China

Corresponding Author: Wenmei Liang, e-mail: weimeiliang1@163.com
Source of support: This work was supported by the National Natural Science Foundation of China (No. 81260207), the Foundation of the Science and Technology Department of Guizhou Province (No. [2012]3141, [2015]7321) ,the Foundation of Guizhou Educational Committee (No. [2011]48), and the United Foundation Project of the Guiyang Science and Technology Bureau (No. [2016]001-6, [2017]5-15)

Background: Synaptic plasticity plays an important role in the process of addiction. This study investigated the relationship between synaptic plasticity and changes in addictive behavior and examined the expression of synaptic plasticity-associated proteins and genes in the nucleus accumbens (NAc) region in different rat models.

Material/Methods: Heroin addiction, SIRT1-overexpression, and SIRT1-silenced rat models were established. Polymerase chain reaction gene chip technology, immunohistochemistry, Western blotting, and transmission electron microscopy were used to detect changes in synaptic plasticity-related gene and protein expression, and changes in the ultrastructure of synapses, in the NAc.

Results: Naloxone withdrawal symptoms appeared in the SIRT1-overexpression group. In the SIRT1-silenced group the symptoms were reduced. Immunohistochemistry and Western blotting results showed that FOXO1 expression decreased in the heroin addiction (HA) group but increased in the SIRT1-silenced group ($p<0.05$). The expression of Cdk5, Nf-κB, PSD95, and Syn was enhanced in the HA group ($p<0.05$) and further increased in the SIRT1-overexpression group but were reduced in the SIRT1-silenced group ($p<0.05$). The number of synapses increased in the HA group ($p<0.05$) along with mitochondrial swelling in the presynaptic membrane and obscuring of the synaptic cleft.

Conclusions: SIRT1 and other synaptic plasticity-related genes in NAc are involved in the regulation of heroin addiction. SIRT1 overexpression can increase behavioral sensitization in the NAc of rats, and SIRT1 silencing might ease withdrawal symptoms and reduce conditioned place preferences.

MeSH Keywords: Heroin • Neuronal Plasticity • Nucleus Accumbens

Full-text PDF: https://www.medscimonit.com/abstract/index/idArt/910550

3891 4 7 37

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

Background

Drug addiction threatens human health and social development globally, and the abuse of addictive substances has become an important medical and societal problem worldwide [1,2].

Addiction is defined as uncontrollable drug seeking and compulsive drug use. If drug availability is restricted and drug use is consequently stopped, withdrawal symptoms occur [3]. Due to its strong water-solubility and fat-solubility, heroin can easily pass through the blood-brain barrier to enter the central nervous system and induce a strong response; thus, it is more addictive than other drugs. Because the addictive mechanism of heroin is not yet clear, effective prevention and treatment approaches for heroin addiction are still lacking. Addiction is a pathological learning and memory formation process [4]. As the neurobiological basis for learning and memory [5], synaptic plasticity plays an important role in the occurrence and progression of addictive behaviors. Studies have confirmed that drug addiction is associated with the reward system in the brain [6]. The nucleus accumbens (NAc) is a central component of the brain's reward system and is involved in cognitive activities as well as learning and memory-related behaviors. *Silent mating type information regulation 2-homolog 1* (SIRT1) is an important member of the third class of the histone deacetylase family known as sirtuins (silent information regulator 2 proteins), which regulate gene transcription through substrates, and are thus involved in the regulation of physical and pathological processes [7]. However, the role of SIRT1 in addiction has received little attention.

In the present study, adeno-associated viruses (AAV) were transfected into the NAc to establish rat models of heroin addiction, SIRT1 overexpression, and SIRT1 silencing. The expression of synaptic plasticity-related genes was detected by a polymerase chain reaction (PCR) array, and the localization and expression of synaptic plasticity-related proteins in the NAc of rats in each group was detected by immunohistochemistry and immunoblotting. The expression of synaptic plasticity-related proteins was detected by immunohistochemistry and immunoprecipitation. The ultrastructure of synapses was observed by transmission electron microscopy. This study aimed to elucidate the possible role of SIRT1 in the mechanisms of heroin addiction and the effect of SIRT1 expression on synaptic plasticity in the NAc. Overall, our study should aid in identifying new targets for detoxification and drug abuse treatment.

Material and Methods

Experimental animals

Sixty wild-type adult, male, specific-pathogen-free Sprague-Dawley rats weighing 180–220 g were provided by Guizhou Medical University Experimental Animal Center, China (License number: SYXK (Guizhou) 2012-0001). Animal husbandry and experimental procedures were performed in accordance with the experimental animal use and management guidelines of Guizhou Medical University Experimental Animal Committee. During the experiment, all animals were allowed free access to water and food, and the ambient temperature was 18–24°C.

Reagents

The reagents and analysis systems used were as follows: heroin (purity 92.09%, provided by the Guizhou Public Security Bureau), pentobarbital sodium (Sigma, USA), naloxone hydrochloride (Chongqing YaoPharma, China), mouse monoclonal anti-SIRT1 (Abcam, USA), rabbit polyclonal anti-Cdk5, rabbit polyclonal anti-NF-κB (Bioss, China), rabbit monoclonal anti-FOXO1 (Abcam, USA), rabbit polyclonal anti-Cbp (Bioss, China), rabbit monoclonal anti-PSD95 (Abcam, USA), rabbit monoclonal anti-Syn (Abcam, USA), mouse monoclonal anti-beta-actin (Abcam, USA), SuperScript III RT reverse transcription kit (Invitrogen, USA) Sybr qPCR mix (Invitrogen, USA), Plasmid Mini kit (TransGen Biotech, Beijing, China), gel extraction kit (Omega), Trans2K Plus II DNA Marker (TransGen, Beijing, China), DNA primers (Invitrogen, USA), DNA sequencing (Invitrogen, USA), restriction endonuclease EcoRI (NEB, USA), T4 DNA Ligase (NEB, USA), Plasmid Maxi Kit (Qiagen, Germany), CPT High-efficiency Transfection Kit (Virotherapy Technologies, Wuhan, China).

Packaging of rAAV9-rSirt1 and rAAV9-rSirt1 shRNA

A recombinant plasmid containing the target gene (pAAV-rSirt1-IRES-ZsGreen) and a plasmid with the strongest gene-silencing effect (pAAV-ZsGreen-shRNA-rSirt) were constructed and transfected into 293AAV cells. The constructs produced a high-titer AAV virus containing the target gene (i.e., rAAV9-rSirt1) and a virus containing the target gene-silencing sequence (i.e., rAAV9-rSirt1 shRNA).

Intracranial micro-injection of AAV into the rat NAc

The rats in the SIRT1-overexpression group and SIRT1-silenced group were weighed, anesthetized with an intraperitoneal injection of pentobarbital sodium (50 mg/kg), and secured on a rat brain stereotaxic instrument. The coordinates of the NAc were determined per *The Rat Brain in Stereotaxic Coordinates* [8]. Bregma was set as 0, and the coordinates of the NAc were set as 1.0 mm anterior to bregma, 1.5 mm lateral from the midline, and 7 mm below the dura mater. Using a microinjector, 1 µl AAV was injected into the NAc of both hemispheres. After surgery, the rats received antibiotics and were allowed to recover for 2 weeks.

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

Generation of rat model of heroin addiction and drug withdrawal

Rats in the heroin addiction (HA) group received an intraperitoneal injection of heroin solution twice/day (8 am and 3 pm) for 9 days, with a daily increase in the dose administered. The dose on the first day was 3 mg/kg, and the increase each day was 3 mg/kg. On the 9th day, the dose of heroin administered was 27 mg/kg. In the saline controls (SC), the rats were injected with a matching amount of normal saline for 9 days. On the 10th day, rats in both the SC group and the HA group were injected intraperitoneally with a solution of naloxone at a dose of 5 mg/kg to induce acute onset of withdrawal symptoms (in the HA group rats). Withdrawal symptoms were observed within 30 min. The criteria of Maldonado [9] and others were used to score withdrawal symptoms.

PCR array procedure

Rats in the HA group and SC groups were killed after anesthesia, and the NAc was quickly dissected out on ice and frozen in liquid nitrogen before being stored at –80°C. The samples were analyzed by PCR array. The main steps were as follows: RNA was extracted using an RNeasy kit (Qiagen) and tested for purity. After gel electrophoresis, cDNA was synthesized using an RT2 First Strand Kit, and real-time PCR was conducted with the RT2 SYBR Green Mastermix kit (Qiagen) to detect changes in the expression of 84 synaptic plasticity-associated genes. Results were determined using the ΔΔCt method [10]. The ΔΔCt of each gene in the SC group, HA group, and SIRT1-overexpression group was calculated as follows: ΔΔCt=ΔCt (HA group)–ΔCt (SC group), ΔΔCt=ΔCt (SIRT1-overexpression group)–ΔCt (SC group). The difference in expression in genes between the HA group and the SC group was measured by 2-ΔΔCt. A difference in the 2-ΔΔCt value ≥2 or ≤0.5 was considered to be significantly different for the comparison between the SC and HA groups [10].

Conditioned place preference (CPP) model

This experiment consisted of 3 phases: pre-test period, training period, and test period. The rats were trained in groups, requiring a total of 13 days of continuous training. The difference between the time for which a rat stayed in the white chamber during the test period and during the pre-test period was used as the CPP score.

Western blot analysis

Rats were killed and the location of the NAc was determined per *The Rat Brain in Stereotaxic Coordinates* [8]. The NAc tissue was quickly dissected out on ice. Different percentages of sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) gels were prepared according to the sizes of the target proteins. The samples were denatured, loaded onto the gels, electrophoresed, and transferred to cellulose acetate ultrafiltration membrane membranes. The membranes were blocked in 5% skim milk for 2 h. The membranes were then incubated with primary antibodies (SIRT1 (1: 3000); Cdk5 (1: 5000); NF-κB (1: 500); Syn (1: 50000); FOXO1 (1: 5000); PSD95 (1: 4000)) at 4°C overnight. The secondary antibodies used were horseradish peroxidase (HRP) goat anti-mouse IgG (1: 5000) or HRP goat anti-rabbit IgG (1: 5000). Finally, the membranes were placed in a gel imager, the chemiluminescence method was used for detection, and images were captured.

Immunohistochemistry

After anesthesia, the rats were quickly perfused with normal saline and then 4% paraformaldehyde for fixation. Brain tissue was collected and then routinely dehydrated, cleared, paraffin-embedded, and sectioned at 4 μm. Slides were stained by the streptavidin-peroxidase (SP) method, and cells positive for SIRT1, Cbp, Cdk5, FOXO1, NF-κB, and Syn were identified. The dilutions of the primary antibodies were as follows: SIRT1 (1: 500), Cbp (1: 200), Cdk5 (1: 400), NF-κB (1: 200), Syn (1: 600), and FOXO1 (1: 300). In the negative control sample, the primary antibody was replaced by phosphate-buffered saline (PBS). After staining, the average optical density values for the positive cells were measured with Image Pro Plus software.

Transmission electron microscopic observations

A 1-mm^3 piece of NAc tissue was collected and fixed in prechilled 3% glutaraldehyde fixative. After the samples were prepared for transmission electron microscopy (TEM), ultrathin 60-nm sections were cut and double-stained with 1% uranyl acetate and lead citrate for observation.

Statistical analysis

Data are presented as mean ± standard error. Statistical analyses were conducted using Statistical Package for the Social Science (SPSS) software. A one-way analysis of variance (ANOVA) was used for comparisons between groups and differences with $P<0.05$ were considered to be statistically significant.

Results

pAAV-rSirt1-IRES-ZsGreen transduction results

As the gene-carrying virus contained ZsGreen green fluorescence, in the pre-test period, NAc tissue was collected at 1 and 2 weeks after transduction and subjected to immunofluorescence staining to determine the efficiency of transduction. The

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

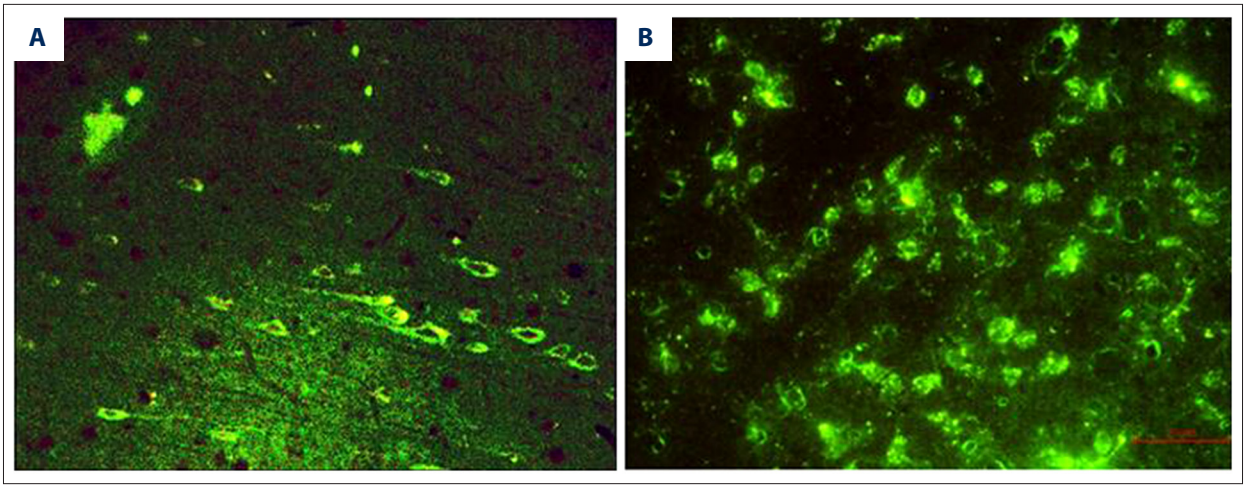


Figure 1. Expression of pAAV-rSirt1-IRES-ZsGreen after transfection in the nucleus accumbens (NAc). (**A**) NAc of rats 1 week after virus transfection. (**B**) NAc of rats 2 weeks after virus transduction.

results showed that the amount of AAV in the cells at 2 weeks post-transduction was significantly greater than the amount during the first week (Figure 1).

As shown in Figure 2, the Western blot analysis showed that the expression of SIRT1 protein in the NAc after AAV transduction was significantly higher than that in the SC group ($P<0.05$), and the expression at 2 weeks after transduction was higher than that at 1 week after transduction ($P<0.05$). Therefore, the HA model was determined to be established from the second week after surgery.

PCR array results

The expression levels of 23 synaptic plasticity-associated genes were altered in the NAc of the rat model of the HA group (Table 1). The expression levels of 24 synaptic plasticity-associated genes were altered in the NAc of rats overexpressing SIRT1 (Table 2).

Observation of withdrawal symptoms

Per the protocol for the generation of a rat model of heroin addiction, rats received heroin in increasing quantities daily for 9 days, and on the 10th day naloxone was administered intraperitoneally to induce withdrawal. The rats in the HA group, SIRT1-overexpression group (AAV-rSirt1 group), and SIRT1-silenced group (AAV-rSirt1 shRNA group) all exhibited obvious withdrawal symptoms, including jumping, rearing, wet-dog shaking, stretching, and teeth chattering. These symptoms were not observed in the SC group. Loose and unformed stools and blepharoptosis were also obvious (Table 3).

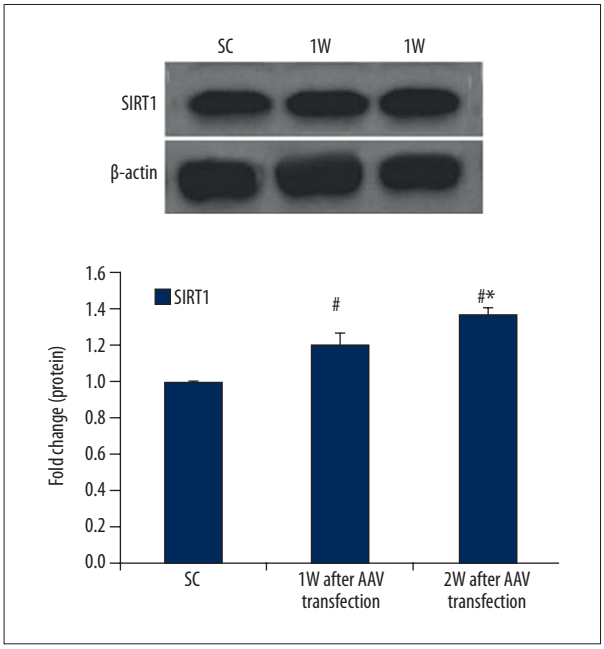


Figure 2. SIRT1 expression after transduction of pAAV-rSirt1-IRES-ZsGreen into the nucleus accumbens.

CPP results

CPP results of rats in each group after training (Figure 3).

Figure 3 shows that the lengths of stay in the drug-associated chamber for rats in the HA group, SIRT1-overexpression group (rSirt1), and SIRT1-silenced group (rSirt1 shRNA) after training were significantly greater than before training ($P<0.05$). The length of stay in the drug-associated chamber for rats in the rSirt1 shRNA group was shorter than that of rats in the HA and rSirt1 groups ($P<0.05$).

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

Table 1. Expression of synaptic plasticity-associated genes in the NAc of rats in the HA group compared with those in the SIRT1 overexpression group.

Gene name	Description	Fractional up- or down-regulation
Arc	Activity-regulated cytoskeleton-associated protein	4.50 ↑
Camk2a	Calcium/calmodulin-dependent protein kinase II alpha	2.46 ↑
Cebpd	CCAAT/enhancer binding protein (C/EBP), delta	2.32 ↑
Egr1	Early growth response 1	2.88 ↑
Egr2	Early growth response 2	4.14 ↑
Egr3	Early growth response 3	3.96 ↑
Egr4	Early growth response 4	2.39 ↑
ΔFosB	Delta FBJ osteosarcoma oncogene B	2.03 ↑
Mapk	Mitogen activated protein kinase	2.53 ↑
Nr4a1	Nuclear receptor subfamily 4, group A, member 2	3.69 ↑
Gria1	Glutamate receptor, ionotropic, AMPA 1	4.21 ↑
Gria2	Glutamate receptor, ionotropic, AMPA 2	2.15 ↑
Grm3	Glutamate receptor, metabotropic 3	2.54 ↑
Grm4	Glutamate receptor, metabotropic 4	2.41 ↑
Inhba	Inhibin beta-A	2.07 ↑
JunB	Jun B proto-oncogene	4.20 ↑
NGF	Nerve growth factor (beta polypeptide)	2.32 ↑
NF-κB	Nuclear factor of kappa light polypeptide gene enhancer in B-cells	2.18 ↑
Nptx2	Neuronal pentraxin 2	2.51 ↑
Pim1	Pim-1 oncogene	2.38 ↑
Ppp3ca	Protein phosphatase 3, catalytic subunit, alpha isoform	2.36 ↑
BDNF	brain derived neurotrophic factor	0.28 ↓
Sirt1	Sirtuin (silent mating type information regulation 2 homolog) 1	2.38 ↑

↑ gene expression increase; ↓ gene expression decrease.

Immunohistochemistry results and image analysis

The average optical density of cells positive for SIRT1, Cdk5, NF-κB, Cbp, and Syn in the NAc region following immunostaining was determined by image analysis (Figure 4). The results showed that the average optical densities for the HA and AAV-rSirt1 groups were significantly higher than those for the SC group ($P<0.05$); however, there was no significant difference between the HA and AAV-rSirt1 groups ($P>0.05$). The average optical density values for the AAV-rSirt1 shRNA group were not significantly different from those of the SC group, but they were significantly lower than those of the HA group ($P<0.05$). The average optical density of FOXO1-positive cells was significantly decreased in the HA and AAV-rSirt1 groups compared with that in the SC group ($P<0.05$), while the average optical densities of positive cells in the AAV-rSirt1 shRNA group were increased, but they were not significantly different from those of the SC group ($P>0.05$).

Western blot analysis

Analysis of the optical densities of bands showed that, compared with rats in the SC group, the levels of SIRT1 protein in the HA and AAV-rSirt1 groups were significantly increased; in the AAV-rSirt1 shRNA group, however, the level was decreased ($P<0.05$). The differences between the AAV-rSirt1, AAV-rSirt1 shRNA, and HA groups were also statistically significant ($P<0.05$). Upon administration of heroin, the levels of FOXO1 protein in each group decreased compared with the SC group. The protein levels of Cdk5, NF-κB, Syn, and PSD95 increased in the HA and AAV-rSirt1 groups, while the levels in the AAV-rSirt1 shRNA group were lower than in the HA group ($P<0.05$) (Figure 5).

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

Table 2. The expression of synaptic plasticity-associated genes in the NAc of rats overexpressing SIRT1 compared to controls.

Gene name	Description	Fractional up- or down- regulation
Arc	Activity-regulated cytoskeleton-associated protein	2.86 ↑
Camk2a	Calcium/calmodulin-dependent protein kinase II alpha	2.09 ↑
Adcy8	Adenylate cyclase 8 (brain)	0.44 ↓
Egr1	Early growth response 1	3.02 ↑
Egr2	Early growth response 2	4.37 ↑
Egr3	Early growth response 3	2.77 ↑
Egr4	Early growth response 4	2.15 ↑
Homer1	Mitogen activated protein kinase 1	2.61 ↑
Nr4a2	Nuclear receptor subfamily 4, group A, member 2	2.72 ↑
Gria1	Glutamate receptor, ionotropic, AMPA 1	2.63 ↑
Gria2	Glutamate receptor, ionotropic, AMPA2	2.32 ↑
Grm3	Glutamate receptor, metabotropic 3	2.44 ↑
Grm4	Glutamate receptor, metabotropic 4	2.13 ↑
Grm5	Glutamate receptor, metabotropic 5	2.01 ↑
ΔFosB	Delta FBJ osteosarcoma oncogene B	2.47 ↑
JunB	Jun B proto-oncogene	4.81 ↑
Klf10	Kruppel-like factor 10	2.11 ↑
NF-κB	Nuclear factor of kappa light polypeptide gene enhancer in B-cells	3.22 ↑
Mmp9	Matrix metallopeptidase 9	2.07 ↑
Ntf3	Neurotrophin 3	5.73 ↑
Ppp1r14a	Protein phosphatase 1, regulatory (inhibitor) subunit 14A	0.48 ↓
Srf	Serum response factor	2.07 ↑
Timp1	TIMP metallopeptidase inhibitor 1	0.41 ↓
Sirt1	Sirtuin (silent mating type information regulation 2 homolog) 1	6.28 ↑

↑ gene expression increase; ↓ gene expression decrease.

Table 3. Comparison of naloxone-induced withdrawal symptoms among groups.

Withdrawal signs	SC	HA	AAV-rSirt	AAV-Sirt1 ShRNA
Jumping	2.8±0.84	3.4±1.14	3.6±1.14	3.2±0.837
Rearing	6.2±1.095	7.4±1.14	7.8±1.095$^{\#}$	6.2±1.304
Face washing	3.2±0.837	10.6±2.074$^{\#}$	12.2±1.304$^{\#}$	7.6±1.342$^{\#*}$
Wet dog shaking	1.2±0.837	18±1.871$^{\#}$	22.6±2.302$^{\#}$	15.4±1.14$^{\#*}$
Stretch	1.6±0.548	7.2±1.483$^{\#}$	7.2±1.304$^{\#}$	5.4±1.14$^{\#*}$
Teeth chatting	2±0.707	6.6±1.949$^{\#}$	8.6±1.14$^{\#}$	5.6±0.894$^{\#}$
Blepharoptosis	None	With	With	With
Diarrhea	None	With	With	With

* $P<0.05$ compared with the SC group; $^{\#}$ $P<0.05$ compared with the HA group.

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

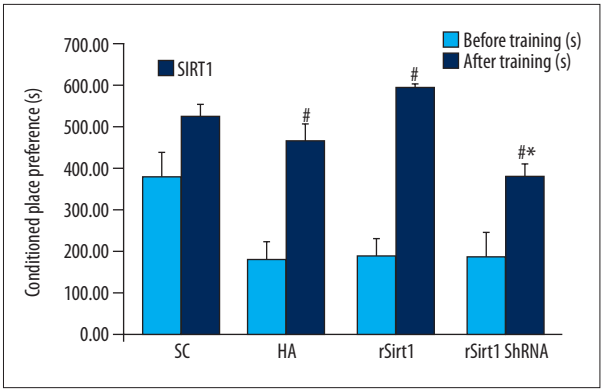
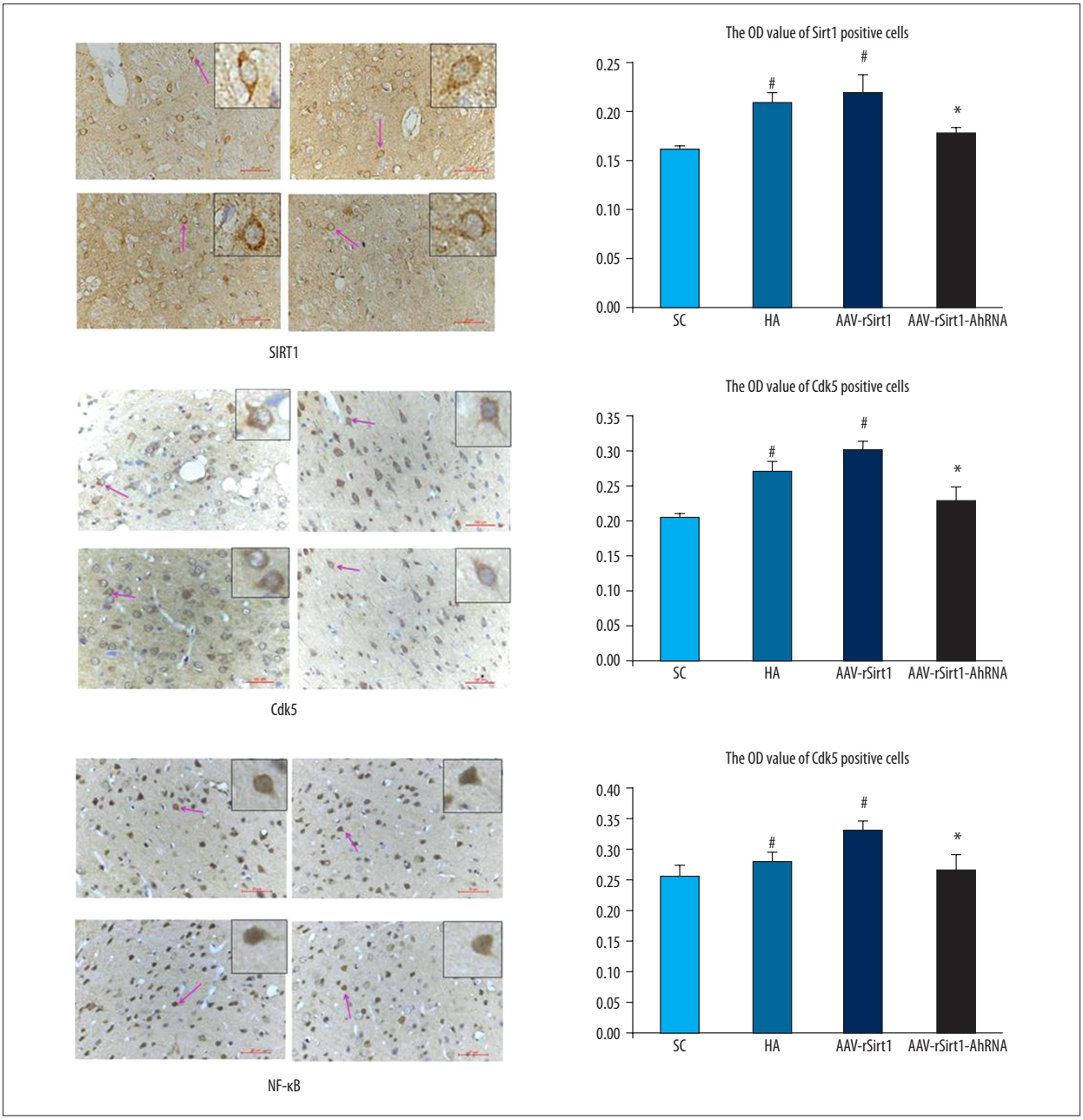


Figure 3. Length of stay of rats in the drug-associated chamber before and after training. # *P*<0.05 compared with the pre-training session; * *P*<0.05 compared with the heroin-addicted group.



Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

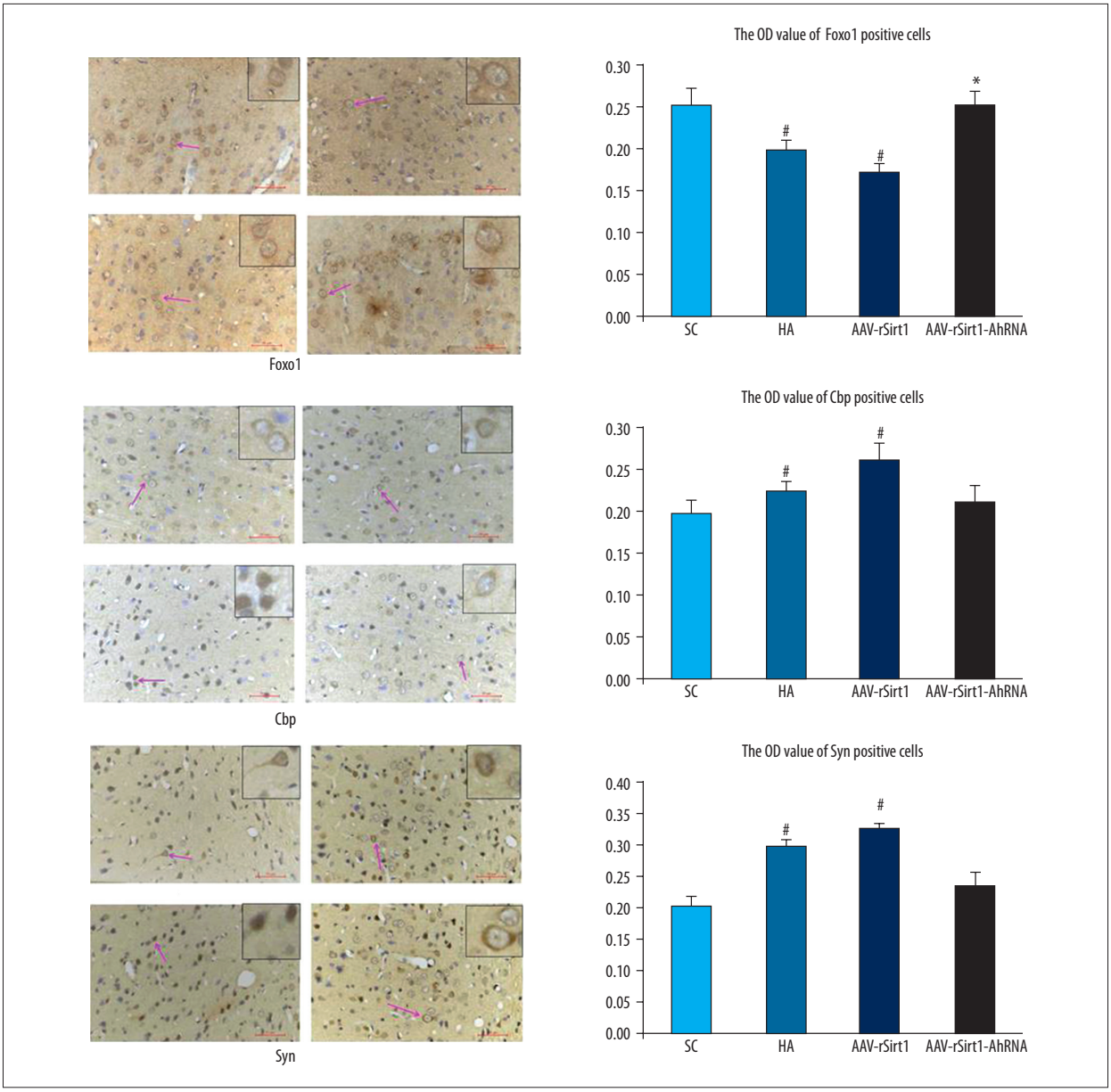


Figure 4. Cells positive for SIRT1, Cdk5, NF-κB, FOXO1, Cbp, and Syn in the NAc region (×400) and their optical density values (**A**) Saline control (SC) group; (**B**) Heroin-addicted (HA) group; (**C**) AAV-rSirt1 group; (**D**) AAV-rSirt1 shRNA group. # $P<0.05$ compared with the SC group; * $P<0.05$ compared with the HA group.

Transmission electron microscope (TEM) results

Using a TEM (×80 000), the mitochondria in neurons in the NAc region of HA rats were found to be swollen and to have broken ridges, and the endoplasmic reticulum was slightly expanded. The number of synapses was increased compared with the SC group. The number of synapses in the SIRT1-overexpression group was increased further, and the synaptic cleft was not clearly defined. The number of synapses was also decreased in the group in which SIRT1 was silenced, and the mitochondria were swollen and had damaged membranes (Figure 6).

Electron microscopic (×160 000) observation of the NAc region in the SC group showed that the neurons had clear cell membranes and that there were numerous mitochondria with clear ridges in the presynaptic enlargement. In addition, many synaptic vesicles containing electro-dense particles were observed. There were electron-dense materials attached to the postsynaptic membrane. In the HA group, the synaptic clefts were unclear and the mitochondria were slightly swollen, with ruptured ridges. In the AAV-rSirt1 group, the mitochondria in the presynaptic membrane were significantly swollen, while in the AAV-rSirt1 shRNA group the structure of the synaptic

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

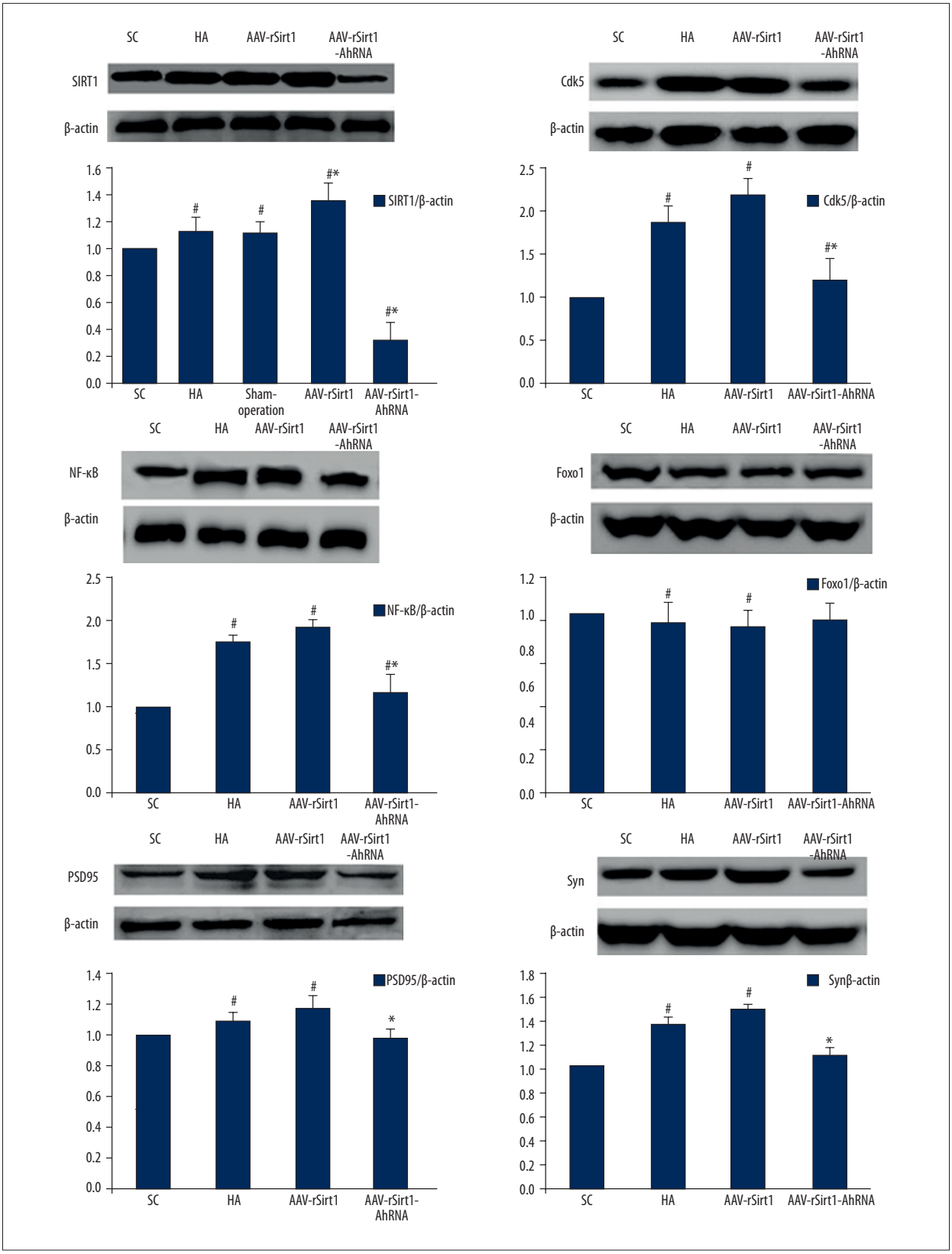


Figure 5. Detection of SIRT1, Cdk5, FOXO1, NF-κB, Syn, and PSD95 protein levels in each group by immunoblotting. # $P<0.05$ compared with the saline control group; * $P<0.05$ compared with the HA group.

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

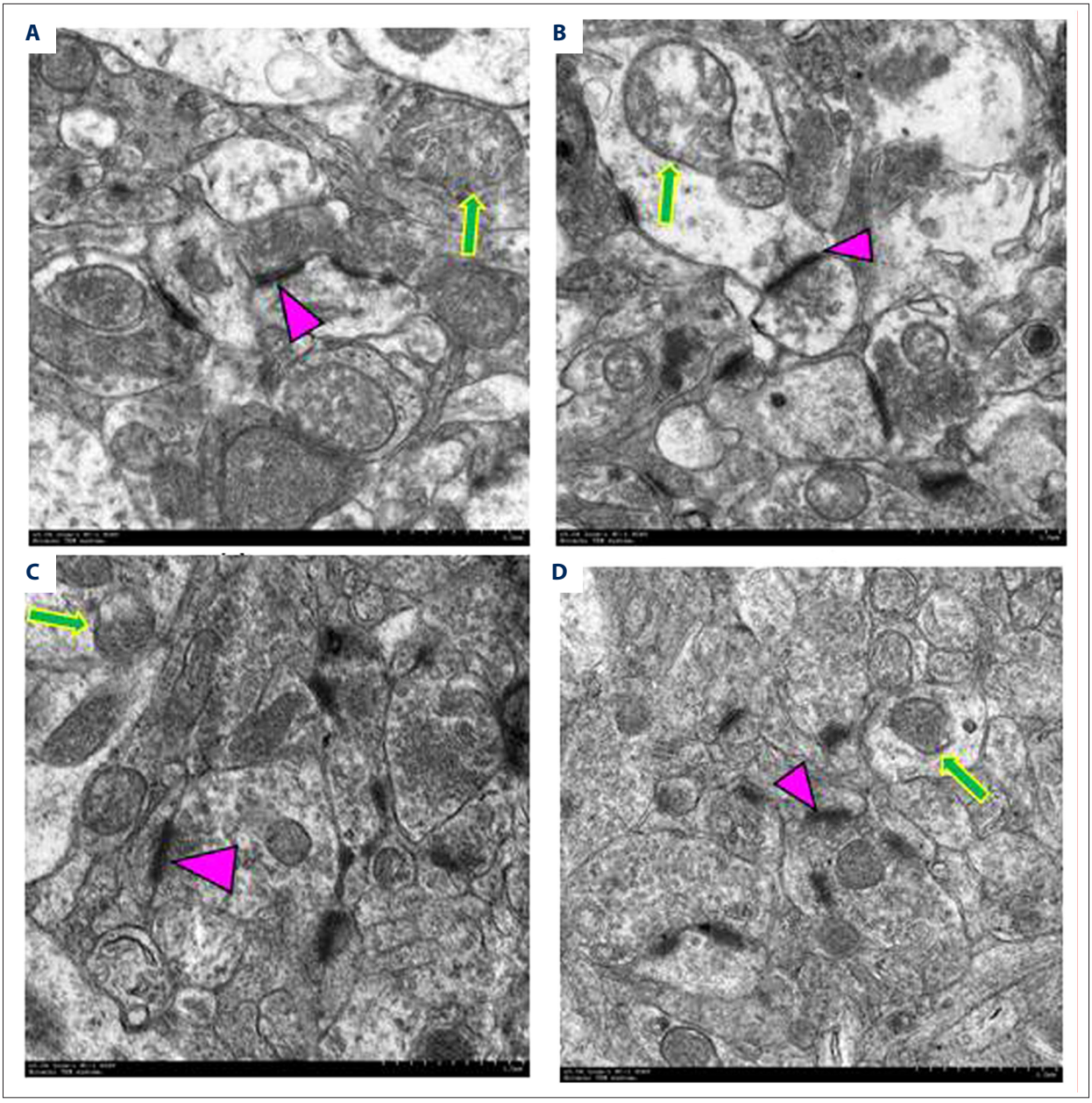


Figure 6. Mitochondria (↑) and synapses (↓) in the nucleus accumbens of each group.

cleft was well-defined but the mitochondrial membranes in the presynaptic terminal were damaged and had ruptured ridges (Figure 7).

Synaptic structure indices were calculated, and the results show that for the HA, SIRT1-overexpression, and SIRT1-silenced groups, the synaptic clefts were wider than those for the SC group, and the postsynaptic densities were thicker, especially in the SIRT1-overexpression group (Table 4).

Discussion

Long-term use of addictive drugs can cause damage to multiple systems in the body, among which adaptive changes in the nervous system are the most profound, ultimately leading to physical and psychological dependence. Once the drug is withdrawn, addicts exhibit a series of acute physiological and psychological discomfort symptoms, i.e., "withdrawal syndrome" [11]. To escape withdrawal symptoms, addicts often show compulsive drug-seeking behavior and relapse [12]. Drug addiction is a chronic, recurrent brain disease caused by interaction between the drugs of abuse and the brain reward

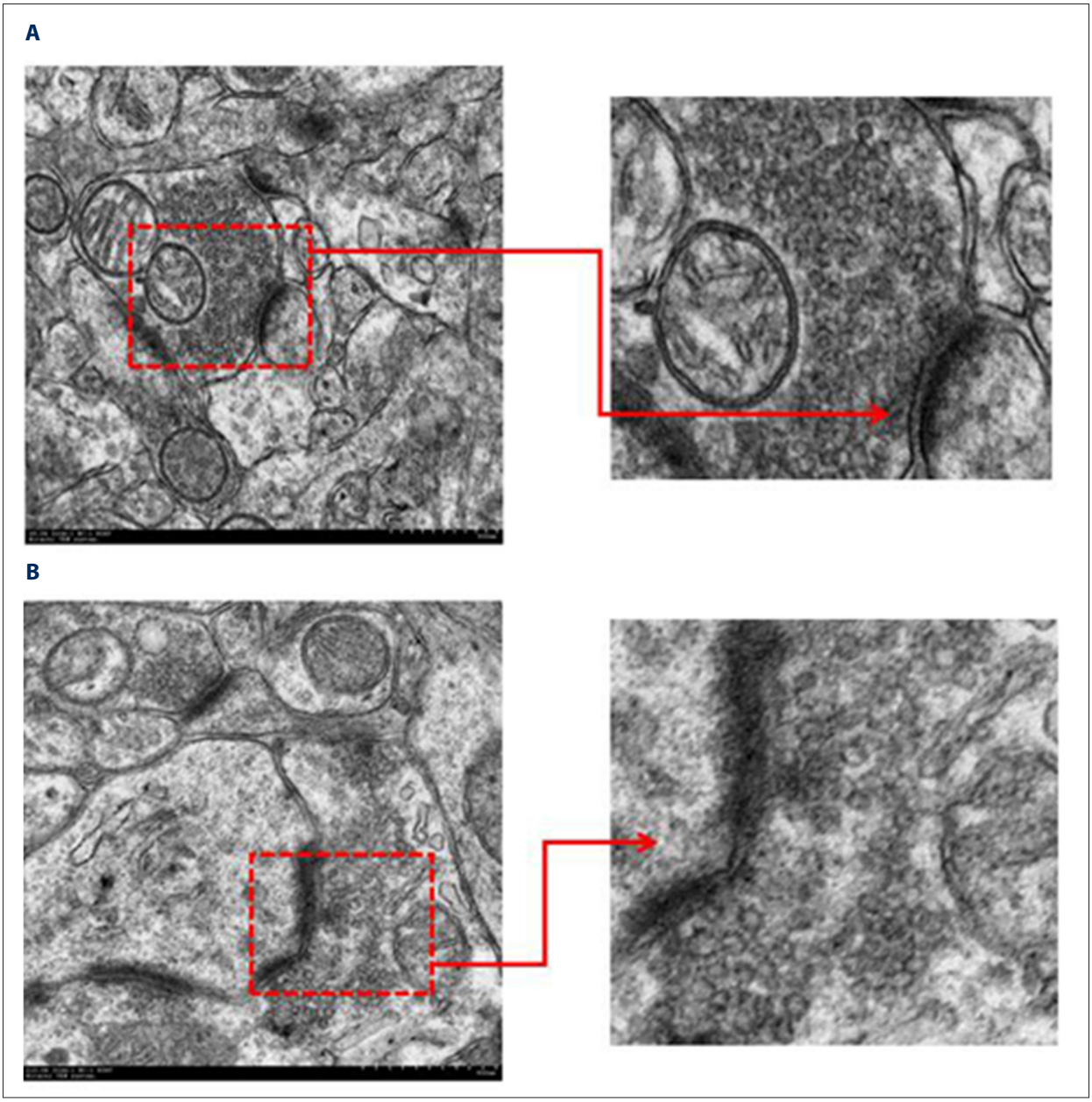
Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

system, which mainly presents as compulsive drug use and a persistent desire to use drugs. It is also an extreme memory-formation process [4]. This reward-related memory is very intense and often leads to relapse. Synaptic plasticity is one of the molecular mechanisms involved in learning and memory [5].

Currently, the NAc is hypothesized to be the main nucleus involved in the generation of natural reward and drug reward effects [13]. It also plays an important role in the behavioral changes associated with addiction and is the target of many addictive drugs [14]. Naloxone is an opioid receptor antagonist that competes with various opioid receptors and blocks the function of opioids. Thus, naloxone is commonly used for the withdrawal and detoxification of opioids [15,16]. The CPP experiment is the most convenient and widely-used experimental method to evaluate the psychological dependence potential of drugs in drug addiction research [17].

SIRT1 is the most characteristic subtype of the sirtuin family – the type with the highest homology with the yeast Sir2 protein [18]. Studies have shown that SIRT1 expression in brain tissue is significantly higher than in other organs. It is also the most widely-studied protein in the human sirtuin family [19].

In addition to its direct effect on histones, SIRT1 interacts with a variety of non-histone proteins and performs a variety of



Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

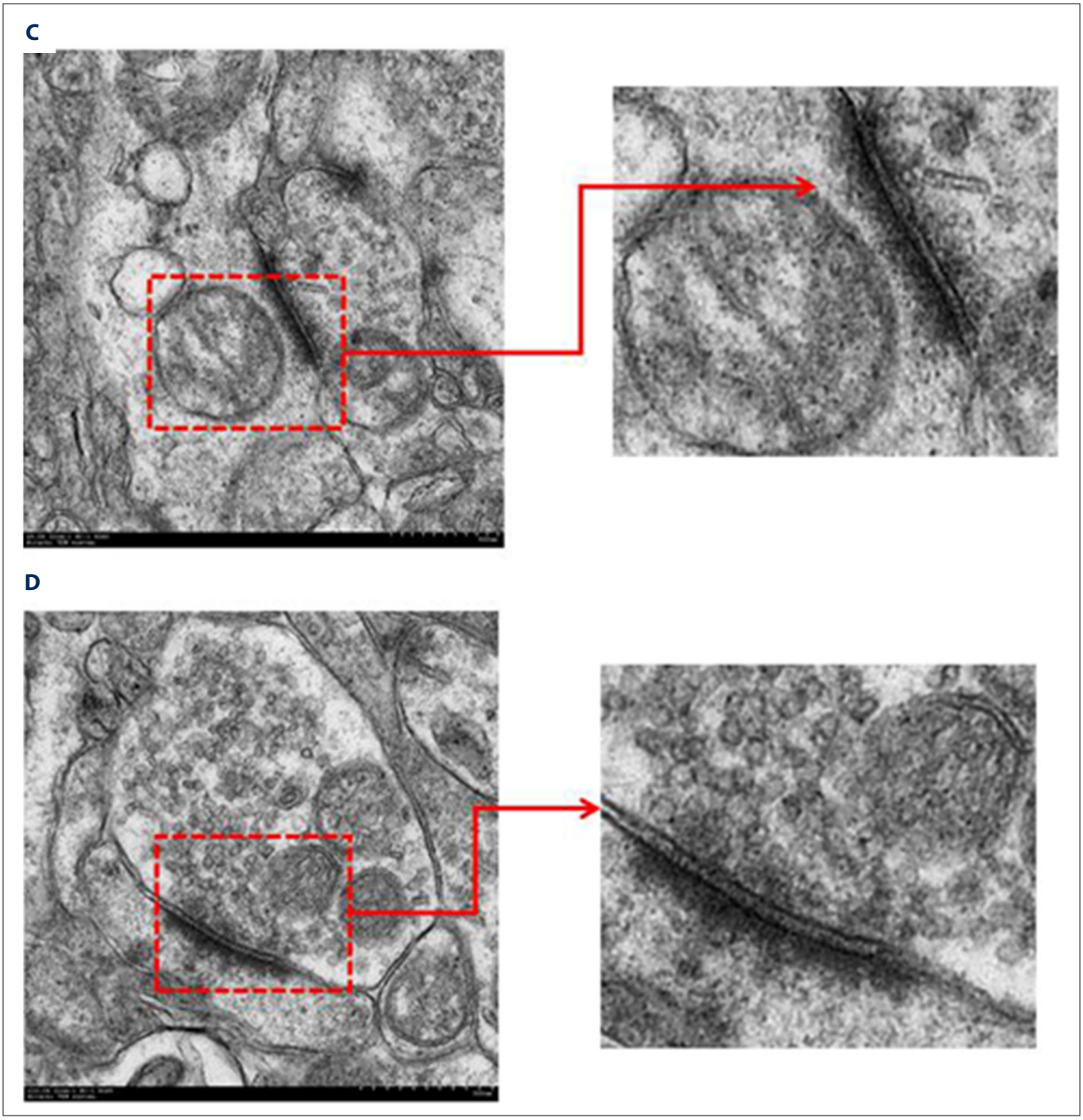


Figure 7. (**A–D**) Ultrastructure of synapses in the nucleus accumbens region of each group.

Table 4. Number of synapses and indices of synapse structures in the NAc region of rats.

Group	Number of synapses	Index of synapse	
		Thickness of synaptic cleft (nm)	Thickness of postsynaptic density (nm)
SCG	4.5±0.35	15.38±2.27	42.82±5.37
HAG	6.73±1.02#	19.34±3.68#	48.07±4.51#
rSirt1	8.42±0.94#	20.15±1.54#	48.96±3.29#
rSirt1 ShRNA	5.35±0.67*	19.11±3.53#	46.35±4.35#*

# P<0.05 compared with the SC group; * P<0.05 compared with the HA group.

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

functions. The non-histone proteins it regulates include p53, Ku70, FOXOs, NF-κB, Smad7, AR, E2F1, Tat, PGC-1α, NcoR, and p300. Some of these proteins are also closely related to synaptic plasticity.

In the present study, AAVs containing the target gene SIRT1 or the silencing shRNA of SIRT1 were transfected into the NAc region by micro-injection. A rat model of HA was then generated, and rat models of SIRT1 overexpression and SIRT1-silencing were also successfully established. Heroin was shown to induce a significant CPP, and the withdrawal reaction was also prominent. The rats in the SIRT1-overexpression group stayed for a significantly longer time in the drug-associated side of the chamber, and showed stronger CPP, indicating that heroin-treated rats were stimulated by environmental cues to exhibit behaviors of drug-desire and showed conditional preferences for the drug-associated chamber. Thus, the CPP model was successfully established and showed that rats form a psychological dependence on heroin. In addition, the incidence of withdrawal symptoms was significantly increased after naloxone injection. In rats in the SIRT1-silenced group, CPP was induced, but their stay in the drug-associated chamber was significantly shorter than that of the rats in the overexpression group. Drug-related learning and memory is involved in drug addiction and relapse [20], suggesting that the memories associated with heroin can be affected and altered by the expression of SIRT1.

In general, it is believed that changes in gene expression induced by addictive drugs in critical brain regions, such as the NAc and striatum, play important roles in long-term addictive behaviors and neural plasticity [21,22]. Therefore, regulation of the expression of addiction-related genes in key brain regions is critical. To identify possible target genes, we used PCR array technology to examine 84 synaptic plasticity-related genes in the NAc region in rats with heroin addiction and SIRT1-overexpression. The results show that the expression of 23 genes was altered in the HA group, of which 13 were immediate-early response genes (IEGs). The levels of 24 genes were altered in the SIRT1-overexpressing rats. These genes also included IEG family members, and 6 genes involved in synaptic long-term potentiation (LTP) and long-term depression (LTD). The results of this study indicate that HA induces expression changes in IEGs and synaptic plasticity genes associated with LTP and LTD, indicating that SIRT1 participates in the process of learning and memory by regulating synaptic plasticity.

The expression level of SIRT1 in rats with HA was 2.38 times higher than that in rats in the SC group, while the expression levels of SIRT1 in the SIRT1-overexpression group was 6.28 times higher than that in the SC group, further confirming the successful transduction of the AAV. The expression levels of 13 genes were changed in rats in the HA and SIRT1-overexpression groups, among which *Arc*, *EGR1*, *CORR1*, *EGR2*, *EGR3*, *EGR4*, *Nr4a2*, *ΔFosB*, *Jun-B*, and *NF-κB* belong to the IEG family, and *CAMK2A*, *GRIA1*, *GRIA2*, and *MAPK* participate in LTP and LTD. This indicates that SIRT1 is involved in the alteration of expression levels of synaptic plasticity-associated genes caused by HA.

We found that the CPP behaviors of rats overexpressing SIRT1 were enhanced, while those of rats in which SIRT1 was silenced were weakened. In the PCR array analysis, in addition to the genes that were examined at the morphological and molecular biological level in our experiment, other genes were found that are also associated with synaptic plasticity-associated genes regulated by SIRT1.

The rewarding effects of addictive substances leave a clear memory trace in the central nervous system, and when the addict encounters a hint of drug-related information, a relapse may be induced. Because synaptic plasticity is the neurobiological basis for learning and memory, during the development of addictive drug-induced behaviors, synaptic plasticity may be induced. Kasanetz [23] argued that the reason drug-addicted rats lose their self-control over the use of drugs during cocaine self-administration training is alterations in synaptic plasticity in many neurons.

Nuclear factor kappa-light-polypeptide-gene-enhancer in B cells (NF-κB) can transfer information from an active synapse to the nucleus [24], and plays an important role in neuroprotection, learning and memory [25], and synaptic plasticity [26]. In cocaine addiction, NF-κB mediates the reward effects of the addictive drug and induces an increase in the number of synapses in the NAc [27]. Some researchers have injected the NF-κB inhibitor SN50 into the NAc of morphine-addicted rats and found that reduced NF-κB activity impairs the reconsolidation of morphine reward memories [28].

Cyclin-dependent kinase 5 (Cdk5) can induce dynamic changes in the cytoskeleton, regulate the structure and morphology of dendritic spines, and modulate synaptic plasticity [29]. In rat models of drug addiction, morphine administration increases the number of Cdk5-immunoreactive cells in the NAc [30]. The administration of a Cdk5 inhibitor in the dorsal hippocampus promotes self-administration behavior. These results suggest that Cdk5 is an important compensatory mediator involved in restricting heroin use [31], and is also a regulatory molecule for synaptic plasticity, as well as learning and memory, evoked by drug addiction.

FOXO1 is a direct and functional regulator of SIRT1. FOXO1 can bind to a site in the SIRT1 promoter and promote its transcription [32]. SIRT1 can also bind with and deacetylate FOXO1, leading to decreased transcriptional activity. Thus, there is a feedback mechanism between SIRT1 and FOXO1 [33], and this

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

feedback loop may play an important role in the regulation of SIRT1 expression.

Postsynaptic density protein-95 (PSD-95) plays a key role in the signal transduction underlying synaptic plasticity [34,35], and is critical for the generation and maintenance of LTP [36]. Increases in PSD-95 may be the basis of enhancements in synaptic plasticity.

Synaptophysin (Syn) can control release from synaptic vesicles in nerve endings by binding to vesicles, thus participating in the release of neurotransmitters and regulating synaptic transmission efficiency [37].

Our results showed that the expression levels of Cdk5, NF-κB, PSD95, and Syn in the NAc of the HA group were greater than those in the SC group. Immunostaining was also found to be enhanced, which was consistent with the results of the Western blot analysis. In the SIRT1-overexpression group, the expression levels of Cdk5, NF-κB, PSD95, and Syn were further increased, while the expression levels in the SIRT1-silenced group were significantly lower than that in the overexpression group. Alterations in expression of these protein can be regulated by SIRT1, which will lead to changes in synaptic plasticity. The direction of these changes was in line with the behavioral alterations in the rats, including withdrawal symptoms and CPP. The ultrastructural morphological assessment of this study found thickening of postsynaptic densities, which improves the binding of neurotransmitters with their receptors and enhances their biological functions. The increase in the number of synapses makes the morphological changes in synapses more stable. Expression of these proteins was decreased following SIRT1 silencing, but the morphological study found no significant improvement in changes such as mitochondrial swelling and endoplasmic reticulum expansion.

The time period over which this study was carried out was insufficient. We speculate that heroin can increase the expression levels of synaptic plasticity-related proteins in the short term, which can improve the structure of neurons. However, in the long term, excessive activation can cause neuronal degeneration, leading to decreased behavioral sensitivity and memory loss.

Conclusions

In this study, we induced alterations in synaptic plasticity-related genes by regulating the expression of SIRT1. This led to changes in gene transcription and ultimately to changes in drug-seeking behavior or psychological dependence, such as CPP behavior, which is closely related to the development of drug addiction. Genes that have not been fully investigated here will be studied further in future studies.

References:

1. Gould TJ: Addiction and cognition. Addiction Science & Clinical Practice, 2010; 5(2): 4
2. Bolash RB: Trends in opioid analgesic abuse and mortality in the United States. N Engl J Med. 2015;372(16): 1573.
3. Hyman SE, Malenka RC, Nestler EJ: Neural mechanisms of addiction: The role of reward-related learning and memory. Ann Rev Neurosci, 2006; 29(1): 565–89
4. Thomas MJ, Kalivas PW, Shaham Y: Neuroplasticity in the mesolimbic dopamine system and cocaine addiction. Br J Pharmacol, 2008; 154(2): 327–42
5. Grimm JW, Lu L, Hayashi T et al: Time-dependent increases in brain-derived neurotrophic factor protein levels within the mesolimbic dopamine system after withdrawal from cocaine: Implications for incubation of cocaine craving. J Neurosci, 2003; 23(3): 742–47
6. Kauer JA, Malenka RC: Synaptic plasticity and addiction. Nat Rev Neurosci, 2007; 8(11): 844–58
7. Lu L, Dempsey J, Liu SY et al: A single infusion of brain-derived neurotrophic factor into the ventral tegmental area induces long-lasting potentiation of cocaine seeking after withdrawal. J Neurosci, 2004; 24(7): 1604–11
8. Mai J, Majtanik M, Paxinos G et al: The rat brain in stereotaxic coordinates. Rat Brain in Stereotaxic Coordinates. 2013;3(2): 6
9. Maldonado R, Waksman G, Feger J, Roques BP: Naloxone-induced withdrawal syndrome after administration of selective opioid agonists or after activation of the endogenous enkephalinergic system. Prog Clin Biol Res, 1990; 328: 531–34
10. Liao DL, Cheng MC, Lai CH et al: Comparative gene expression profiling analysis of lymphoblastoid cells reveals neuron-specific enolase gene (ENO2) as a susceptibility gene of heroin dependence. Addict Biol, 2014; 19(1): 102–10
11. Cooper ZD, Johnson KW, Pavlicova M et al: The effects of ibudilast, a glial activation inhibitor, on opioid withdrawal symptoms in opioid-dependent volunteers. Addict Biol, 2016; 21(4): 895–903
12. Nestler EJ, Aghajanian GK: Molecular and cellular basis of addiction. Science (New York, NY), 1997; 278(5335): 58–63
13. Soderman AR, Unterwald EM: Cocaine reward and hyperactivity in the rat: Sites of mu opioid receptor modulation. Neuroscience, 2008; 154(4): 1506–16
14. Ikemoto S: Dopamine reward circuitry: two projection systems from the ventral midbrain to the nucleus accumbens-olfactory tubercle complex. Brain Res Rev, 2007; 56(1): 27–78
15. McLaughlin PJ, Zagon IS: Duration of opioid receptor blockade determines biotherapeutic response. Biochem Pharmacol, 2015; 97(3): 236–46
16. Strang J, McDonald R, Alqurshi A et al: Naloxone without the needle – systematic review of candidate routes for non-injectable naloxone for opioid overdose reversal. Drug Alcohol Depend, 2016; 163: 16–23
17. Katz RJ, Gormezano G: A rapid and inexpensive technique for assessing the reinforcing effects of opiate drugs. Pharmacol Biochem Behav, 1979; 11(2): 231–33
18. Haigis MC, Guarente LP: Mammalian sirtuins – emerging roles in physiology, aging, and calorie restriction. Genes Dev, 2006; 20(21): 2913–21
19. Peck B, Chen CY, Ho KK et al: SIRT inhibitors induce cell death and p53 acetylation through targeting both SIRT1 and SIRT2. Mol Cancer Ther, 2010; 9(4): 844–55
20. Kelley AE: Memory and addiction: Shared neural circuitry and molecular mechanisms. Neuron, 2004; 44(1): 161–79
21. Edbauer D, Neilson JR, Foster KA et al: Regulation of synaptic structure and function by FMRP-associated microRNAs miR-125b and miR-132. Neuron, 2010; 65(3): 373–84

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

22. Giraldez AJ, Cinalli RM, Glasner ME et al: MicroRNAs regulate brain morphogenesis in zebrafish. Science (New York, NY), 2005; 308(5723): 833–38
23. Kasanetz F, Lafourcade M, Derochegamonet V et al: Prefrontal synaptic markers of cocaine addiction-like behavior in rats. Mol Psychiatry, 2013; 18(6): 729–37
24. Meffert MK, Chang JM, Wiltgen BJ et al: NF-kappa B functions in synaptic signaling and behavior. Nat Neurosci, 2003; 6(10): 1072–78
25. Boccia M, Freudenthal R, Blake M et al: Activation of hippocampal nuclear factor-kappa B by retrieval is required for memory reconsolidation. J Neurosci, 2007; 27(49): 13436–45
26. Kaltschmidt B, Kaltschmidt C: NF-kappaB in the nervous system. Cold Spring Harb Perspect Biol, 2009; 1(3): a001271
27. Russo SJ, Wilkinson MB, Mazei-Robison MS et al: Nuclear factor kappa B signaling regulates neuronal morphology and cocaine reward. J Neurosci, 2009; 29(11): 3529–37
28. Yang J, Yu J, Jia X et al: Inhibition of nuclear factor-κB impairs reconsolidation of morphine reward memory in rats. Behav Brain Res, 2011; 216(2): 592–96
29. Hawasli AH, Bibb JA: Alternative roles for Cdk5 in learning and synaptic plasticity. Biotechnol J, 2007; 2(8): 941–48
30. Salas E, Alonso E, Polanco MJ et al: Differential regulation of CDK5 and c-Fos expression by morphine in the brain of Lewis and Fischer 344 rat strains. Neuroscience, 2013; 230(5): 151–56
31. Chen ZG, Liu X, Wang W et al: Dissociative role for dorsal hippocampus in mediating heroin self-administration and relapse through CDK5 and RhoB signaling revealed by proteomic analysis. Addict Biol, 2017; 22(6): 1731–42
32. Xiong S, Salazar G, Patrushev N, Alexander RW: FoxO1 mediates an autofeedback loop regulating SIRT1 expression. J Biol Chem, 2011; 286(7): 5289–99
33. Wang C, Chen L, Hou X et al: Interactions between E2F1 and SirT1 regulate apoptotic response to DNA damage. Nat Cell Biol, 2006; 8(9): 1025–31
34. Christoffel DJ, Golden SA, Russo SJ: Structural and synaptic plasticity in stress-related disorders. Rev Neurosci, 2011; 22(5): 535–49
35. Duman RS, Aghajanian GK: Synaptic dysfunction in depression: Potential therapeutic targets. Science (New York, NY), 2012; 338(6103): 68–72
36. Xu W, Schlüter OM, Steiner P et al: Molecular dissociation of the role of PSD-95 in regulating synaptic strength and LTD. Neuron, 2008; 57(2): 248–62
37. Wheeler TC, Chin LS, Li Y et al: Regulation of synaptophysin degradation by mammalian homologues of seven in absentia. J Biol Chem, 2002; 277(12): 10273–2

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]