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# Changes in Expression of Dopamine, Its Receptor, and Transporter in Nucleus Accumbens of Heroin-Addicted Rats with Brain-Derived Neurotrophic Factor (BDNF) Overexpression

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Data Collection B  
Statistical Analysis C  
Data Interpretation D  
Manuscript Preparation E  
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**Background:** The aim of this study was to explore how changes in the expression of BDNF in MLDS change the effect of BDNF on dopamine (DA) neurons, which may have therapeutic implications for heroin addiction.

**Material/Methods:** We established a rat model of heroin addiction and observed changes in the expression of BDNF, DA, dopamine receptor (DRD), dopamine transporter (DAT), and other relevant pathways in NAC. We also assessed the effect of BDNF overexpression in the NAC, behavioral changes of heroin-conditioned place preference (CPP), and naloxone withdrawal in rats with high levels of BDNF. We established 5 adult male rat groups: heroin addiction, lentivirus transfection, blank virus, sham operation, and control. The PCR gene chip was used to study gene expression changes. BDNF lentivirus transfection was used for BDNF overexpression. A heroin CPP model and a naloxone withdrawal model of rats were established.


**Results:** Expression changes were found in 20 of the 84 DA-associated genes in the NAC of heroin-addicted rats. Weight loss and withdrawal symptoms in the lentivirus group for naloxone withdrawal was less than in the blank virus and the sham operation group. These 2 latter groups also showed significant behavioral changes, but such changes were not observed in the BDNF lentivirus group before or after training. DRD3 and DAT increased in the NAC of the lentivirus group.

**Conclusions:** BDNF and DA in the NAC are involved in heroin addiction. BDNF overexpression in NAC reduces withdrawal symptoms and craving behavior for medicine induced by environmental cues for heroin-addicted rats. BDNF participates in the regulation of the dopamine system by acting on DRD3 and DAT.

**MeSH Keywords:** **Dopamine Plasma Membrane Transport Proteins • Heroin • Nucleus Accumbens • Receptors, Dopamine**

**Abbreviations:** **Adcy1** – adenylyltransferase1; **Adcy5** – adenylyltransferase5; **BDNF** – brain-derived neurotrophic factor; **CPP** – conditioned place preference; **DA** – dopamine; **DAT** – dopamine transporter; **Dusp1** – dual-specificity phosphatase1; **DRD** – dopamine receptor; **Fos** – FBJ osteosarcoma oncogene; **ITPR1** – inositol triphosphate receptor1; **Mapk1** – mitogen-activated protein kinase1; **NAC** – nucleus accumbens; **PLCB1** – 1-phosphatidylinositol 4, 5-bisphosphate phosphodiesterase beta-1; **PPP1R1B** – protein phosphatase 1 regulatory subunit 1B; **TH** – tyrosine hydroxylase

**Full-text PDF:** <http://www.medscimonit.com/abstract/index/idArt/904670>

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## Background

Drug addiction is seriously harmful to human health and social development, being a chronic relapsing mental disease [1], mainly involving compulsive and reckless drug-seeking behavior, the loss of limited drug intake capacity, and withdrawal symptoms after drug discontinuance [2,3].

The nucleus accumbens (NAc) is an important component of the brain reward system, and it is also an effective target for many addictive drugs. At present, it is believed that opioid addiction can lead to functional and structural adaptive changes in NAc neurons [4], which may lead to memory and craving for drug reward [5]. NAc mainly consists of dopaminergic (DA) neurons, which generates DA using tyrosine as a raw material. The chemical reaction on the DA generated by the action of tyrosine hydroxylase and DA decarboxylase is released through the cytoplasm. DA regulates various neural mechanisms, such as cognitive memory, natural reward, drug reward, and emotional activity. DA is the most important neurotransmitter of the reward system of NAc involved in the regulation of drugs [6].

The physiological role of DA *in vivo* involves the dopamine receptor and the dopamine transporter (DAT). Five kinds of dopamine receptors have been cloned so far, which can be divided into 2 categories: D1 and D2. D1 receptors include D1R and D5R, and D2 receptors include D2R, D3R, and D4R [7]. Different receptor subtypes mediate different transduction processes and intracellular signals, causing significant differences in intracerebral distribution.

DAT is a membrane protein located in central dopaminergic nerve endings, with a physiological role in there-uptake DA in synaptic space. Its physiological effect in the cytoplasm of neurons involves reuse, regulation of timing, extent and scope of DR activation, and regulation of the DA energy system signal transmission [8]. DAT determines the duration of the action of DA and maintains the steady state of DA in the neurons [9].

Brain-derived neurotrophic factor (BDNF) is a member of the family of neurotrophic factors, which is widely expressed in NAc, and it plays an important role in the survival, growth, differentiation, repair, and regeneration after nerve injury of DA neurons [10]. The high-affinity binding of TrkB (a family of tyrosine kinases) and BDNF *in vivo* can lead to downstream pathway activation, and eventually transmits BDNF signals to the cell nucleus, causing lasting biological effects of drug addiction [11]. The effect of BDNF on the role of opioid addiction has been reported inconsistently. Studies have shown that after morphine addiction, BDNF mRNA and protein levels in different brain regions were significantly increased [12]; but in some other reports, the decrease of BDNF-positive cells in VTA of a morphine addiction model indicated that BDNF functions

had decreased [13]. The emergence of these contradictory results suggests that BDNF plays an important role in opioid addiction, but its exact mechanism has not been clarified.

Studies have found that BDNF is a negative regulator of opioid addiction in DA neurons [14], and injection of BDNF in the nucleus accumbens can produce abnormal reward circuit excitement, similar to that induced by opioid-class drugs [15]. Hence, in this study, we propose that changing the expression of BDNF in NAc intervenes in the effect of BDNF on the DA neurons, which may have therapeutic implications for heroin addiction.

## Material and Methods

### Materials

We used lentiviral overexpression plasmid pLVX-mCMV-ZsGreen (Biowit), 293T cell lines (Biowit), BDNF first antibodies (Abcam), DAT first antibodies (Abcam), DRD1 first antibodies (Abcam), DRD2 first antibodies (Abcam), DRD3 first antibodies (Abcam), heroin (Guizhou Province Public Security Bureau, with purity of 92.09%), naloxone (Chongqing Yaoyou pharmaceutical), RNeasy Mini Kit (Qiagen), RT2 First-Strand Kit (Qiagen), and RT2 SYBR Green Mastermix (Qiagen).

### Experimental animals

Adult male Sprague-Dawley (SD) rats, weighing 180–220 g, provided by the Experimental Animal Center of Guizhou Medical University, were randomly divided into 4 groups: a heroin addiction group, a BDNF lentivirus group, a blank virus group, a sham operation group, and a saline control group. Rats were fed in cages and were allowed to freely drink and eat. The Experimental Animal Ethics Committee of Guizhou Medical University approved the experimental program.

### Heroin-addicted rats model

Heroin-addicted rats were given progressively increasing injections of the heroin solution by weight, with the dose of 3 mg/kg in the first day, 2 times/d (8 AM, 3 PM). The daily increasing dose was 3 mg/kg, with continuous subcutaneous injection of heroin for 9 days. On the 9<sup>th</sup> day, the amount of heroin injection given was 27 mg/kg. No treatment was given to the rats in the normal control group. Rats in the heroin addiction group and normal control group were decapitated after anesthesia on the 10<sup>th</sup> day, and NAc was removed and frozen at –80°C using liquid nitrogen for preservation.

### PCR Gene Chip Production (RT<sup>2</sup>Profiler™ PCR Array)

RT<sup>2</sup>Profiler™ PCR Array (Biosciences, SA) was used to analyze and screen the expression changes of 84 genes associated with the DA system and the relevant pathways in NAc brain areas that are important in drug addiction. The main steps were: the Mini Kit RNeasy (Qiagen) was used to extract total RNA and detect purity, followed by electrophoresis of the RNA sample. After using the RT<sup>2</sup> First-Strand Kit (Qiagen) for synthesis of cDNA, the RT<sup>2</sup> SYBR Green Mastermix (Qiagen) was used for the detection of genes.  $\Delta\Delta C_t$  method was used to calculate the  $\Delta\Delta C_t$  of each gene in the 2 groups, which can be expressed as  $\Delta\Delta C_t = \Delta C_t$  (heroin addiction group) -  $\Delta C_t$  (normal control group), and  $2^{-\Delta\Delta C_t}$  was used for calculation of the corresponding gene expression differences in the heroin addiction group and the normal control group. Compared with normal control rats, gene expression more than 2 times or less than 0.5 times were regarded as differentially expressed in the heroin addiction group [16].

### pLVX-rBDNF-mCMV-ZsGreen Plasmid construction

The plasmid pLVX-mCMV-ZsGreen was digested by EcoRI+BamHI double enzyme, and the large fragment and the target gene were recycled and then ligated to the corresponding gene (connection response was in 22°C, reaction for 2 h). The ligation products were transformed into JM109-competent bacteria, 100  $\mu$ L of which were inoculated in the LB plate containing 100  $\mu$ g/ml Ampicillin, and inversely cultured overnight in a culture box with constant temperature of 37°C. Two single colonies were selected to be inoculated into 5 ml LB culture liquid with 100  $\mu$ g/ml Ampicillin and allowed to grow with overnight shaking at 250 rpm and a constant temperature of 37°C. The plasmids were extracted with a mini Plasmid Extraction Kit, followed by plasmid EcoRI+BamHI double-enzyme digestion and 1% agarose gel electrophoresis. Sequencing analysis was conducted to identify the correct insertion of the DNA fragment.

### Transfection of 293T cells and viral titer determination

Subculture cells: 293T cells were digested with 0.25% trypsin, and the cell density was adjusted by DMEM culture medium containing 10% serum, then  $6-8 \times 10^6$  cells were inoculated in 10cm cell culture dishes at 37°C, and cultured in a 5% CO<sub>2</sub> incubator, which can be used for transfection until 80-90% cell density. The recombinant plasmid pLVX-rBDNF-mCMV-ZsGreen carrying the target gene pLVX-rBDNF-mCMV-ZsGreen and the system plasmid were co-transfected to 293T cells. The viral supernatant was collected with 1000 rpm centrifugation for 5 min, followed by virus particles precipitation and virus suspension with 1 mL PBS, and then aliquoted into tubes of 10  $\mu$ L/EP and stored at -70°C. Trypsin-digested 293T cells were inoculated into 6-well plates according to  $2 \times 10^5$ /

holes. The above were cultured in a 37°C and 5% CO<sub>2</sub> incubator until 50% fusion of cells. The lentivirus liquid was serially diluted ( $10^{-1}$ ~ $10^{-5}$ ) with the final volume of 1 mL. The cell culture fluid was discarded, and the above concentration of the virus (including liquid solution) was added to the 6-well plates.

Virus titer determination: after 72 h, positive cells were counted under fluorescence microscope for calculating virus titer (virus titer=GFP positive cells rate×total number of cells/lentivirus liquid volume×lentivirus dilution ratio) (TU/ml). To avoid error, only 1~30% of holes of the GFP positive cell rates were selected, and the average values of each group were calculated.

### Microinjection of intracranial lentivirus in NAc region of rats

Rats were injected and anesthetized by 3% intraperitoneal sodium, and fixed on the brain stereo-positioning instrument. Rat scalps were disinfected by alcohol and iodophor, and a surgical knife was used for cutting off the skin, then we performed blunt separation of muscle and periosteum, with exposure to the surgical field of vision. The anterior midline, lambdoid suture, coronal suture, and other landmark positions were found. The related brain regions of rats with coordinates (bregma was regarded as 0, NAc: the anterior fontanelle 1.2 mm, lateral to the midline open 2 mm, subdural 7 mm) were determined through the brain stereo-positioning instrument. Holes were drilled in the skull by use of a dental drill. Rats in the BDNF lentivirus group were slowly injected with BDNF lentivirus using micro-needles; rats in the sham operation group were only cut using a scalpel, without drilling or microinjection; and rats in BDNF blank virus groups were slowly injected with the blank virus as a control. After suturing the scalp, rats were allowed to recover for 7 days. NAc brain tissue was taken from the rats after anesthesia and transfection with a slow virus vector, and then the expression level of BDNF was determined by Western blot technique to finally identify the effect of the transfection of the virus.

### Establishment of naloxone withdrawal model

Rats in the BDNF lentivirus group, blank virus group, and sham operation group were progressively given increased injections of heroin solution by weight injection, with the dose of 3 mg/kg in the first day and given 2 times/d (8 AM, 3 PM). The daily increasing dose was 3 mg/kg, with continuous subcutaneous injection of heroin for 9 days. On the 9<sup>th</sup> day, the amount of heroin injected was 27 mg/kg. Rats in the saline group were injected with the same dose of normal saline every day.

On the 10<sup>th</sup> day, rats in each group were injected with 5 mg/kg naloxone. The rats in each group were observed for 30 min and the numbers of withdrawal symptoms were recorded. The

withdrawal symptoms included tooth flutter, jumping, twisting, "wet-dog" shaking, and standing. Weight measurements were performed both before and after observation, and then the rats were anesthetized by intraperitoneal injection of 3% sodium and the brain tissue was taken. By referring to the rat brain stereotaxic atlas [17], NAc brain regions were located and removed, frozen using liquid nitrogen, and stored at  $-80^{\circ}\text{C}$ .

### CPP model building

Rats in the CPP experiment box were allowed to adapt for 3 days in the black box and white box, 15 min each time, once a day. On the third day, the residence time of rats within 15 min in the white box and black box were recorded, and rats with natural preference for the black box were selected. Black boxes were used as saline water tanks and the white boxes were used for training rats.

Rats were trained in a white box for 40 min after injection, with continuous subcutaneous injection of heroin for 9 days. In the afternoon, the rats were injected with the same dose of normal saline injection, and trained in a black box for 40 min after injection by the same method. In the control group, rats were treated with normal saline instead of heroin and the training method was the same as above.

On the 10<sup>th</sup> day, the rats were moved into the CPP box, the central partition was removed for free activities in the box for 15 min, the residence time of rats in the medicine chest was recorded and compared with the residence time before training. After the test, the rats were anesthetized by intraperitoneal injection of 3% sodium and the brain tissue was taken, frozen in liquid nitrogen, and stored at  $-80^{\circ}\text{C}$ .

### Western blot detection

For preparation of SDS-PAGE gel, the extracted samples of the total protein were denatured, separated using SDS-PAGE gel, and transferred to a PVDF membrane using standard methods. For detection, the primary antibody was diluted 1: 1000 and the membrane was incubated overnight in the primary antibody working fluid at  $4^{\circ}\text{C}$ . After washing in TBST, the membrane was incubated in the secondary antibody working liquid for 60 min. Chemical luminescence method was used for detection and collection of images.

### Statistical analysis

Data obtained were statistically analyzed by use of the SPSS 11.5 software package. Single-factor analysis of variance was used for comparison between groups, and  $P < 0.05$  indicated that the difference was significant.

## Results

### Gene chips

Gene expression changes for 20 genes associated with DA in the NAc of heroin-addicted rats are summarized in Table 1 (Figures 1, 2).

Two signal pathways most evidently showed gene expression change: the cAMP/PKA pathway (TH, DRD, DAT, BDNF, ADCY1, ADCY5, DUSP1, FOS, MAPK1, PPP1R1B), and the PLC pathway (TH, DRD, DAT, BDNF, ITPR1, PLCB1). The common genes in the 2 pathways were TH, DRD, DAT, and BDNF (Figures 3), among which the expressions of DRD1, DRD2, DRD3, and DAT were higher than in the normal group. TH and BDNF showed decreased expression, while DRD4 and DRD5 showed no significant difference (Figures 3, 4).

### pLVX-rBDNF-mCMV-ZsGreen plasmid enzyme digestion results

The results of enzyme digestion showed a clear band at 750 bp, indicating positive clones (Figure 3).

### pLVX-rBDNF-mCMV-ZsGreen Plasmid sequencing results

The pLVX-rBDNF-mCMV-ZsGreen plasmids were sequenced and the results were as follows:

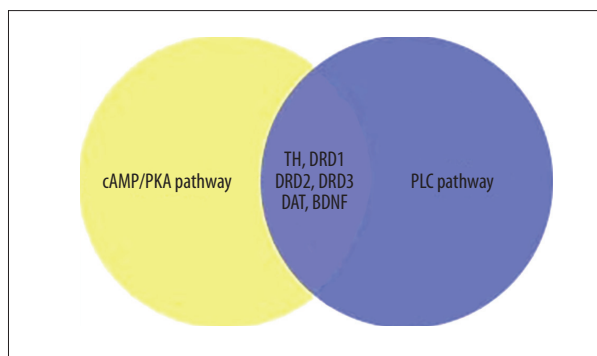
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GCAGGAAGAAGCGGAGACAGCGCAAGAGCGCCATGAAAGA
AGCAAACGTCACCGACAAGGCAACTTGGCCTACCGAGCTGTGCGG
ACCCATGGGACTCTGGAGAGCGTGAATGGGCCAGGGCAGGTTCCGA
GAGGTCTGACGACGACGTCCTGGCTGACACTTTTGAGCACGTGAT
CGAAGAGCTGCTGGATGAGGACCAGAAGGTTCCGCCAACGAAGAA
AACATAAGGACGCGGACTTGACACTCCCGGGTATGCTCAGCAGT
CAAGTGCTTTGGAGCCTCCTCTGCTTTCTGCTGGAGGAATACA
AAAATTACCTGGATGCCGAAACATGTCTATGAGGGTTCGGCGCCACT
CCGACCCCGCCCGCTGGGAGAGCTGAGCGTGTGTGACAGTATTAGC
GAGTGGGTACAGCGGAGATAAAAAGACTGCAGTGGACATGTCCGG
TGGGACGGTACAGTCTGGAGAAAGTCCCGGTATCAAAGGCCA
ACTGAAGCAATATTTCTACGAGACCAAGTGAATCCCATGGGTTACAC
GAAGGAAGGCTGAGGGGCATAGACAAAAGGCACTGGAACCTCGAA
TGCCGAACACTCCAATCGTATGTTCCGGCCCTTACTATGGATAGCAAAA
AGAGAATTGGCTGGCGGTTACATAAGGATAGACACTTCTGTGTATGTA
CACTGACCATAAAAAGGGGAAGACTCGAGCACCACCACCACCACCAC
TGAGATCCGCTGCTAACAAAGCCGAAAGGAAGCTGAGTTGGCTG
CTGCCACCGCTGAGCAATAACTAGCATAACCCTTGGGGCCTCTAAAC
GGGTCTTGAGGGGTTTTTGTCTGAAAGGAGGAACTATATCCGGAT
TGGCGAA
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The sequencing results showed that the plasmid was constructed successfully.

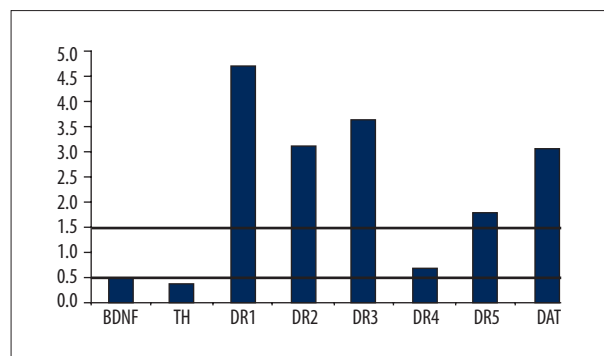
**Table 1.** Expression of DA related genes in NAc of heroin-addicted rats.

| Gene name | Description                                     | Folds up or down regulation |
|-----------|---|-----------------------------|
| Adcy1     | Adenylatecyclase 1                              | 2.29↑                       |
| Adcy5     | Adenylatecyclase 5                              | 2.70↑                       |
| DRD1      | Dopamine receptor D1A                           | 4.71↑                       |
| DRD2      | Dopamine receptor D2                            | 3.12↑                       |
| DRD3      | Dopamine receptor D3                            | 3.66↑                       |
| Dusp1     | Dual specificity phosphatase 1                  | 3.10↑                       |
| Fos       | FBJ osteosarcoma oncogene                       | 2.03↑                       |
| Alox12    | Arachidonate 12-lipoxygenase                    | 2.22↑                       |
| Mapk1     | Mitogen activated protein kinase 1              | 3.16↑                       |
| Nr4a1     | Nuclear receptor subfamily 4, group A, member 1 | 4.28↑                       |
| Nr4a3     | Nuclear receptor subfamily 4, group A, member 3 | 4.01↑                       |
| Pde10a    | Phosphodiesterase 10A                           | 10.35↑                      |
| Pde4a     | Phosphodiesterase 4A                            | 2.61↑                       |
| Pdyn      | Prodynorphin                                    | 3.16↑                       |
| DAT       | Dopamine transporter                            | 3.08↑                       |
| Slc18a2   | Solute carrier family 18, member 2              | 0.12↓                       |
| Tdo2      | Tryptophan 2,3-dioxygenase                      | 0.41↓                       |
| TH        | Tyrosine hydroxylase                            | 0.38↓                       |
| Tph2      | Tryptophan hydroxylase 2                        | 2.67↑                       |
| BDNF      | brain derived neurotrophic factor               | 0.50↓                       |

↑ Increase in gene expression; ↓ decrease in gene expression.



**Figure 1.** Venn diagram for DA-associated gene expression in NAc of heroin-addicted rats.



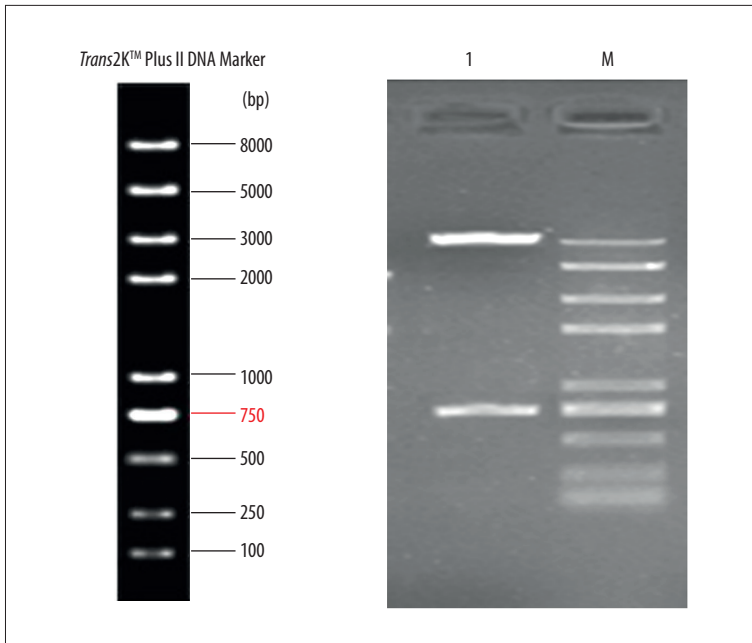
**Figure 2.** Expression of TH, DRD, and DAT in NAc brain area of heroin-addicted rats.

Transfection of 293T cells with pLVX-rBDNF-mCMV-ZsGreen plasmid

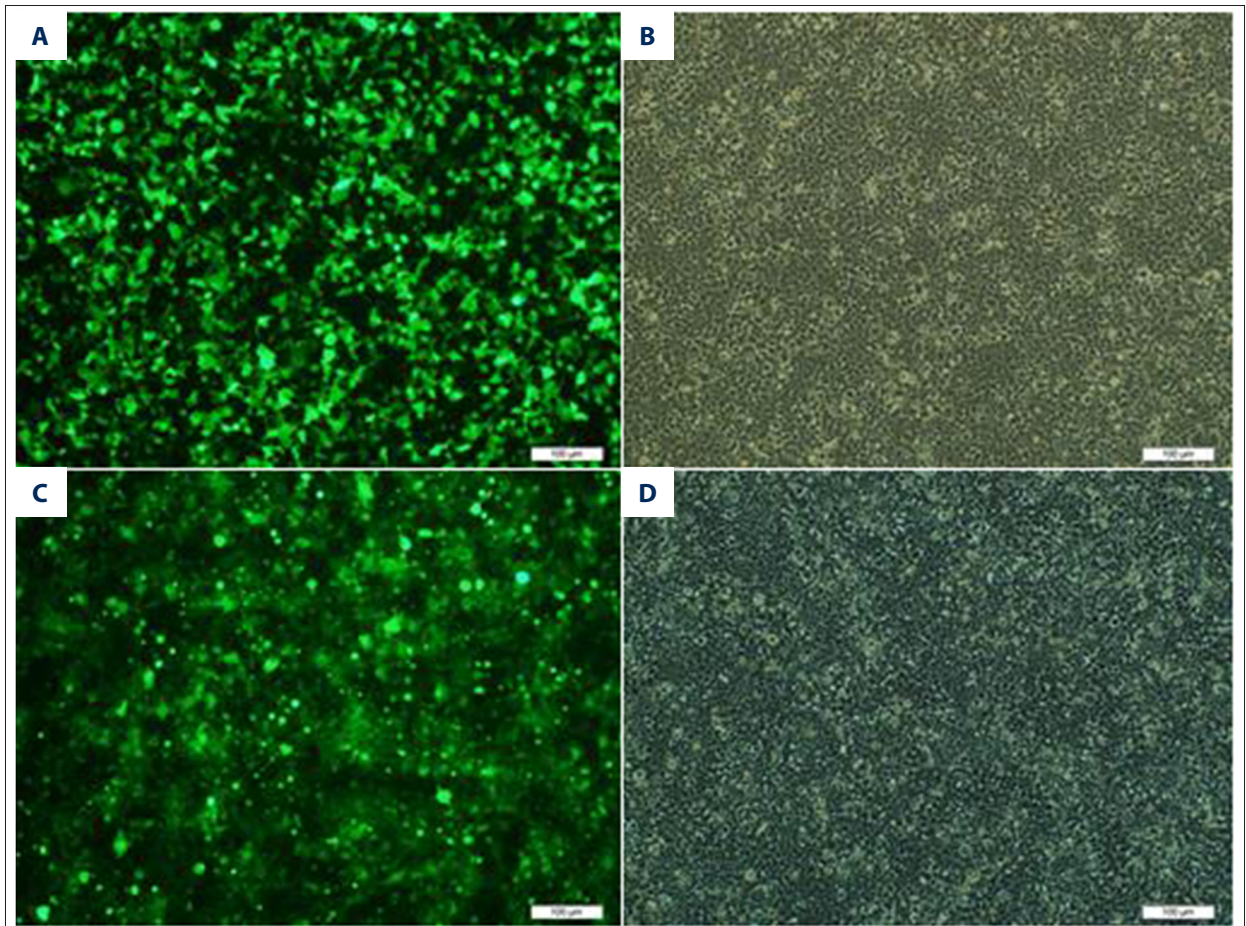
The recombinant plasmid pLVX-rBDNF-mCMV-ZsGreen with the target gene were used for transfecting the 293 T cells, and the fluorescence was observed under the microscope within 24 h before and after transfection (Figure 4).

### Effect of BDNF over expression

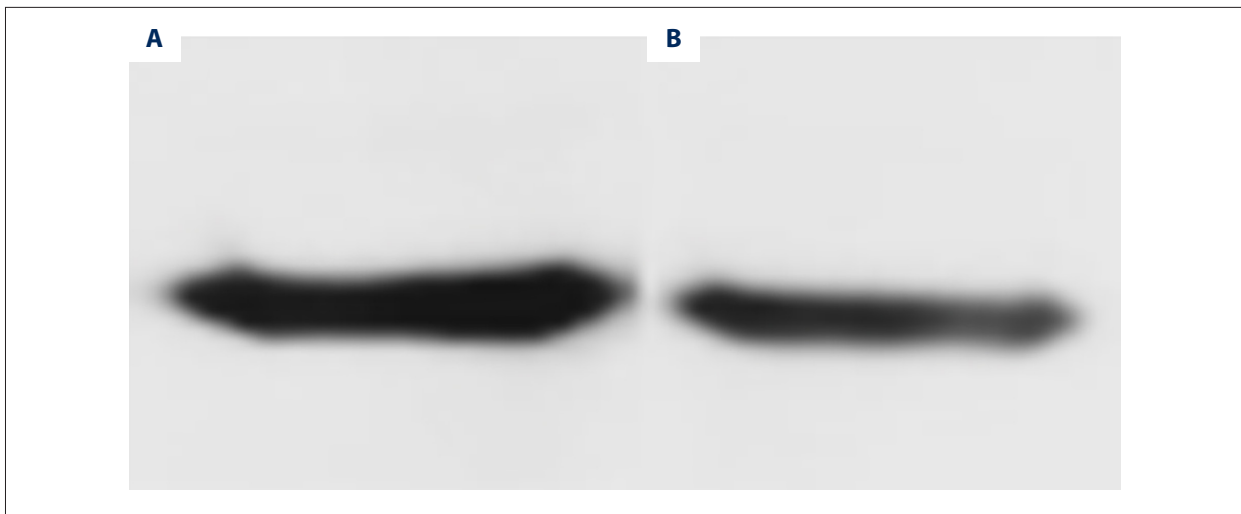
BDNF was transfected into rats in the lentivirus group and normal rats. After anesthesia, the NAc brain areas were taken and BDNF was detected by Western blot method. Compared with the normal control group, rats in the BDNF lentivirus group showed a higher BDNF expression in the NAc region,



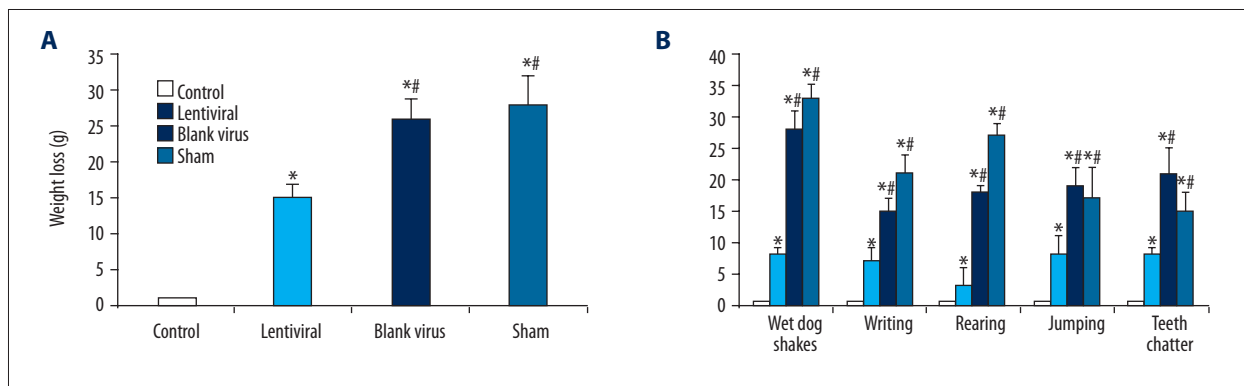
**Figure 3.** pLVX-rBDNF-mCMV-ZsGreen plasmid enzyme digestion results. Band M – *Trans2K* plus marker; Band1 – pLVX-rBDNF-mCMV-ZsGreen plasmid.



**Figure 4.** Cell fluorescence expression of pLVX-rBDNF-mCMV-ZsGreen transfected in 293T cells. (A) Green fluorescence after transfection, (B) White fluorescence after transfection, (C) Green fluorescence after transfection at 48 h, (D) White fluorescence after transfection at 48 h.



**Figure 5.** BDNF protein expression. (A) BDNF lentivirus, (B) Normal control.



**Figure 6.** Comparison of withdrawal symptoms and weight loss in each group. \* Compared with control group,  $P < 0.05$ ; # compared with lentivirus group,  $P < 0.05$ .

confirming the successful construction of the BDNF overexpression model (Figure 5).

### Observation of withdrawal symptoms

Naloxone withdrawal rats showed statistically significant reduced body weight compared with the control group. The weight loss of rats in the BDNF virus group was less than that in the blank virus group and sham operation group (Figure 6A).

The rats in the blank virus group and the sham operation group showed obvious jumping, standing, “wet-dog” shaking, torsion, and tooth fibrillation. The withdrawal symptoms of rats in the BDNF lentivirus group were mild, and these symptoms were not observed in the control group (Figure 6B).

### CPP test

After CPP training for 9 days, rats were put into the CPP box. The central partition was removed for free activities for 15 min

in the box, and the residence time of rats in the box was recorded. In the control group, the residence time of rats in the white box was not significantly different ( $P > 0.05$ ) from that before training. In the blank virus group and sham operation group, the residence time of rats in the white box was significantly longer than that before training ( $P < 0.05$ ). In the BDNF lentivirus group, the residence times of rats in the white box before and after training were not significantly different (Table 2, Figure 7). CPP results showed that in the blank virus group and sham operation group, rats showed preference for the white box, and in BDNF the lentivirus group, the residence time in the white box of rats was not significantly different from that before training.

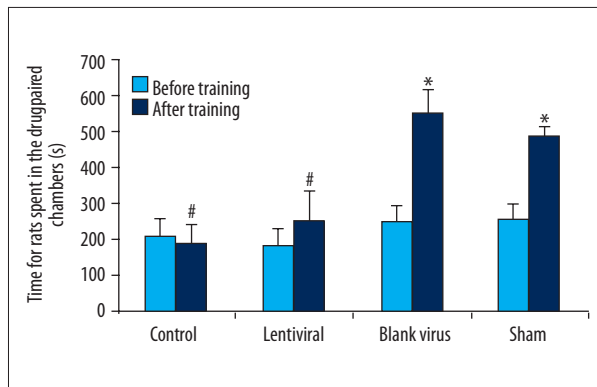
### Western blot analysis

Western blot results showed that in the CPP test and naloxone precipitated withdrawal experiment, in the virus control group and sham operation group, DRD1, DRD2, DRD3, and DAT levels in NAc decreased, and in the BDNF lentivirus group, DRD3 and DAT levels in NAc increased (Figures 8, 9).

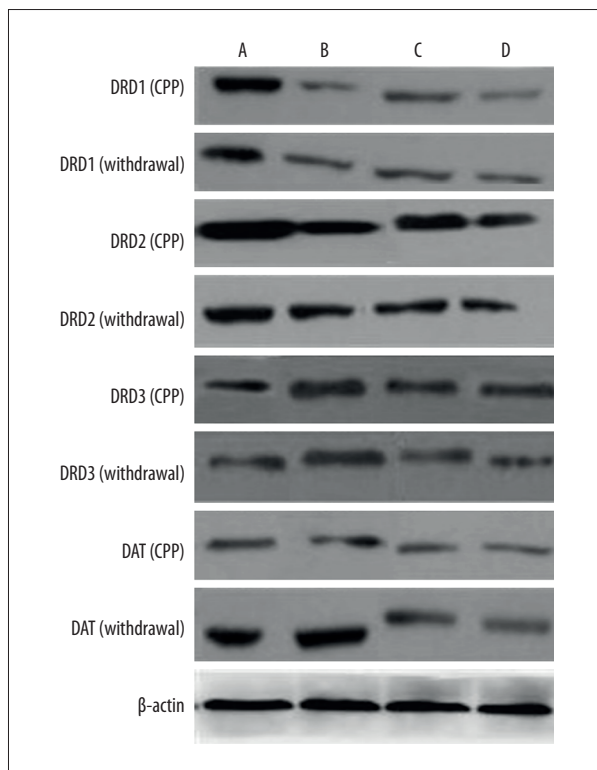
**Table 2.** Residence time of rats in the white box before and after training (Time scale: 0–900 seconds).

|                 | Control group       | Blank virus group   | Sham operation group | BDNF lentivirus group |
|-----------------|---------------------|---------------------|----------------------|-----------------------|
| Before training | 212±48              | 258±36              | 262±41               | 231±43                |
| After training  | 195±50 <sup>#</sup> | 555±61 <sup>*</sup> | 492±25 <sup>*</sup>  | 255±80 <sup>#</sup>   |

\* Compared with that before training,  $P < 0.05$ ; # compared with that before training,  $P > 0.05$ .



**Figure 7.** Residence time of rats in the white box before and after training (Time scale: 0–900 s). \* Compared with that before training,  $P < 0.05$ ; # compared with that before training,  $P > 0.05$ .



**Figure 8.** Protein expression of DRD1, DRD2, DRD3, and DAT in each group as determined by Western blot method. A – Control group, B – BDNF lentivirus group, C – Blank virus group, D – Sham operation group.

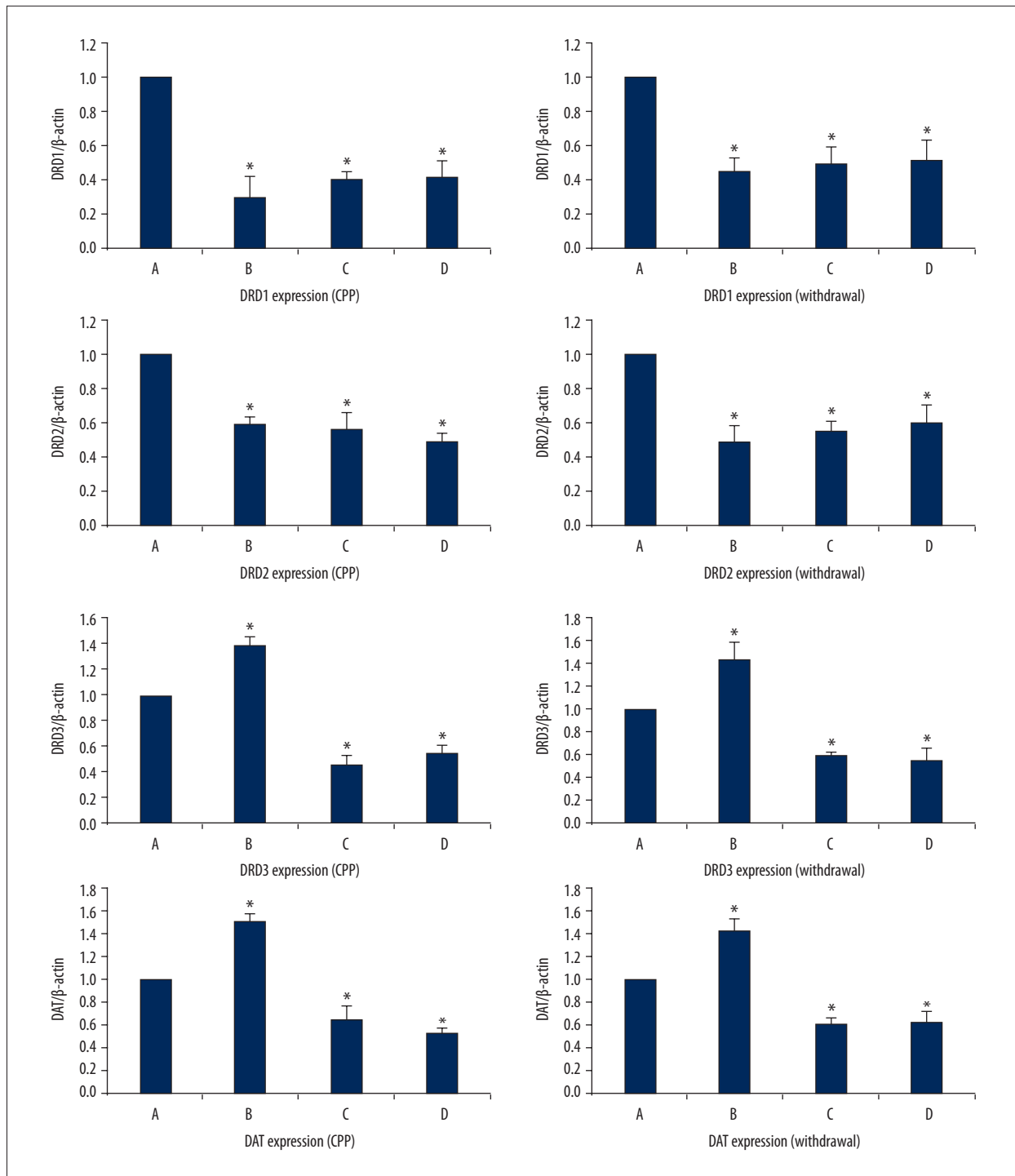
## Discussion

In this study, we used PCR gene chip technology to detect the 84 genes associated with DA in the NAc brain region of heroin-addicted rats. We determined 20 genes showing expression changes. There were 2 signalling pathways that were most evidently showing gene expression changes, namely: the cAMP/PKA pathway (TH, DRD, DAT, BDNF, ADCY1, ADCY5, DUSP1, FOS, MAPK1, PPP1R1B), and the PLC pathway (TH, DRD, DAT, BDNF, ITPR1, PLCB1). The common genes in the 2 pathways were TH, DRD, DAT, and BDNF. Therefore, we showed that the DA system and BDNF are involved in the regulation of the heroin addiction process.

The reward effect, sensitization, and craving of drug addiction were all related to the DA system. The common feature of addictive drugs is that these drugs can cause the increased release of DA from DA neuron endings in NAc, as well as cause reward effect of drugs on DRD, resulting in “heart addiction”. In addition, opioids not only promote the release of DA, but also inhibit the heavy absorption of transmitter DA for neurons [18], so that DA transmitters in synaptic gaps increase, thus enhancing the function of the DA system, resulting in a reward effect. This rewarding effect is the basis of the compulsive drug-seeking behavior of addicts [19,20]. DA transmitter can only play a role mediated through DAT, and DRD regulates DA nerve transmission. Moreover, DRD can control the DA homeostasis in neurons through the heavy intake of DA [8,21]. Therefore, the present research mainly focused on DRD and DAT. Inhibiting the increase of DA release or blocking reward effect may become a therapeutic tool for drug addiction.

It has been reported that BDNF has an important regulatory role for DA [22], and can also interact with DRD and DAT [23–25]. BDNF injection for NAc can produce a similar abnormal reward circuit excitement induced by opioids [15]. Therefore, we put forward the hypothesis that by changing the expression of BDNF in NAc, interrupting the effect of BDNF on DA neurons may have therapeutic implications for heroin addiction. In this study, BDNF overexpression was induced by BDNF lentivirus transfection, then the heroin CPP model and naloxone withdrawal model were established, and the effect of CPP and naloxone withdrawal on the high level of BDNF was observed. CPP results showed that, in the blank virus group and sham operation group, rats showed preference for the CPP





**Figure 9.** Quantitation of the relative expression of DRD1, DRD2, DRD3, and DAT in each group by Western blot. A – Control group, B – BDNF lentivirus group, C – Blank virus group, D – Sham operation group. \* Compared with the control group,  $P < 0.05$ .

box, and in BDNF the lentivirus group, the residence time in the CPP box of rats had no statistical significance compared to that before training. This indicates that BDNF intervention can reduce the drug craving of rats induced by environmental factor stimulation.

DRD1, DRD2, DRD3, and NAC levels in the DAT brain regions of the blank virus and sham operation group were decreased, while in the BDNF lentivirus group, DRD3 and DAT levels in the NAc area were increased. Reports showed that DRD3 is regulated by BDNF regulation. If the role of BDNF receptor was

blocked, it reduces the DRD3 expression [24], so we speculated that BDNF mainly regulates the body by acting on DRD3. Hence, the overexpression of BDNF could activate the DRD3 function [26], thus leading to a reward effect after combination with DA. BDNF and DRD3 can also enhance the function of DAT on DA re-uptake, so that DA levels in VTA and NAc are relatively stable [27,28]. DRD in the blank virus group and sham operation group were observed at low levels, which could not be combined with DA to produce enough reward and led to the appearance of the drug craving, as well as CPP behavior induced by environmental cues.

Naloxone precipitating in withdrawal can cause the body to change the secretion of neurotransmitter and increase the release of DA and its metabolites, which is speculated to participate in mediating withdrawal symptoms [29–31]. It is also reported that drug withdrawal can enhance the inhibitory effect of GABA neurons on the VTA area, thus the postsynaptic potential of DA neurons was inhibited [32]. In addition, drug addiction also caused the binding force of DRD1, DRD2, and DRD3 to decrease [33,34]. Due to the inhibition of DA neuron function and DRD binding force reduction, DA cannot combine with DRD to play a physiological function, causing a

sharp withdrawal reaction, which can drive addicts to drug-seeking and relapse [35]. In the naloxone withdrawal test, we observed that the DRD and DAT levels in the rats were lower than those in the control group, but the level of DAT and DRD3 of the NAc region in the BDNF lentivirus group was higher than that in the control and the sham operation group. The reasons for the mild withdrawal symptoms of rats with BDNF intervention were as follows: (1) BDNF can increase DA [36]; (2) BDNF increased the expression of DRD3 [26], which could combine with DA to produce partial physiological function; and (3) BDNF itself can produce a reward effect similar to that induced by opioid drugs [15].

## Conclusions

Gene chip results showed that BDNF, DA, DRD1, DRD2, DRD3, and DAT in the NAc brain region are involved in the process of heroin addiction. BDNF overexpression in NAc can induce up-regulation of DRD3 and DAT, which is helpful to reduce the withdrawal symptoms and craving behavior induced by environmental cues in heroin-addicted rats.

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