

# Histone 3 lysine 9 acetylation of *BRG1* in the medial prefrontal cortex is associated with heroin self-administration in rats

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**Abstract.** Heroin addiction is a chronic relapsing brain disorder with negative social consequences. Histone acetylation serves a role in drug-induced behavior and neuroplasticity impairment. Brahma/SWI2-related gene-1 (*BRG1*) participates in cerebellar development, embryogenesis and transcriptional regulation of neuronal genes concurrent with histone modifications. However, little is known about the relationship between histone H3 lysine 9 acetylation (H3K9ac) and *BRG1* in response to heroin. The present study aimed to assess the contribution of histone 3 lysine 9 acetylation of *BRG1* to heroin self-administration. The present study established a Sprague-Dawley rat model of heroin self-administration under a fixed-ratio-1 paradigm. Chromatin immunoprecipitation followed by reverse transcription-quantitative PCR (RT-qPCR) was used to detect the accumulation of H3K9ac on *BRG1* in the medial prefrontal cortex (mPFC) and nucleus accumbens (NAc) following heroin self-administration. The relative expression levels of *BRG1* were analyzed by RT-qPCR. H3K9ac at the promoter region of *BRG1* was significantly elevated ( $P=0.002$ ), and the expression of *BRG1* in the mPFC increased 1.47-fold in the heroin self-administration group compared with the control group. No significant difference in H3K9ac at the *BRG1* locus was observed in the NAc ( $P=0.323$ ), with the expression of *BRG1* decreasing 1.38-fold in the heroin self-administering rats compared with the control group. H3K9ac is associated with transcriptional activation, and the increased *BRG1*

expression suggested an essential and novel role for *BRG1* and its H3K9ac-mediated regulation in the mPFC after heroin self-administration; and this may function through epigenetically modulating the activation of neuroplasticity-associated genes. This association may provide a novel therapeutic target for the treatment of heroin addiction.

## Introduction

Abuse of opioids, particularly heroin, causes substantial medical and economic harm. The United Nations Office on Drugs and Crime indicated that, globally, opioids accounted for 66% (110,000) of the 167,000 cases of drug-associated mortality in 2017 (1). Among users of opioids, there were 29.2 million past-year users of opiates (heroin and opium), accounting for 0.6% of the global population aged 15-64 in 2017. In China, a national survey on drug use showed that there were 889,000 past-year users of heroin, accounting for 37% of the global number of drug abuse in 2018 (2). Heroin addiction is a relapsing brain disorder characterized by continuous, compulsive drug-taking, uncontrollable drug-seeking behavior and high recurrence rates (3-5). Heroin addiction interferes with central nervous system plasticity and adaptability by triggering the brain reward centers, which are prompted to initiate and maintain the drug-taking habit (6). In the context of addictive disorders, the mesocorticolimbic circuitry underlying drug addiction comprises of the ventral tegmental area (VTA) and the regions of the brain that are innervated by projections from the VTA, including the nucleus accumbens (NAc), the prefrontal cortex (PFC), the hippocampus and the prelimbic areas (7,8). Acting as the main component of the brain reward circuit, the PFC and NAc code for various aspects of reward-related behavior (9,10). The PFC exerts a strong influence on relapse to opiate seeking behavior during periods of drug abstinence, especially the ventral and medial parts of the PFC (11,12), with the medial PFC (mPFC) contributing to drug-, stress- and cue-induced drug seeking (13-16). The NAc serves an important role in drug self-administration through mediating Pavlovian influences on instrumental seeking behavior (17).

Neuronal activation leads to epigenetic changes in gene expression, such as those modulated by histone modifications (18). In particular, histone acetylation serves a crucial role

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in drug addiction, memory formation and transcriptional regulation by relaxing the chromatin structure, thus allowing better access to transcriptional activators (19,20). A previous study has reported that histone H3 ac (H3ac) may serve an essential role in the long-term neural and behavioral response to addictive drugs (21). For example, hyperacetylation of H3 lysine 27 (H3K27ac) increases chromatin accessibility at hyperacetylated regions in a rat model of heroin self-administration and in humans with heroin addiction behavior (22). Furthermore, one study revealed that methamphetamine injection decreases the level of H3K9ac in nuclear extracts (23). Another study reported that long-term administration of cocaine can significantly upregulate H3ac in the NAc of rats (24). Additionally, class IIa histone deacetylases (HDACs), such as HDAC5 in the NAc, were previously demonstrated to reduce the cocaine reward behavior (25,26). Our previous study has revealed that the HDAC inhibitor sodium butyrate increases the reinstatement of heroin seeking (27). Another HDAC inhibitor, valproic acid is essential for the reduction of behavioral and reinforcement-related effects of alcohol (28).

Brahma/SWI2-related gene-1 (*BRG1*) encodes the ATPase subunit of the switch/sucrose non-fermentable (SWI/SNF) chromatin remodeling complex (CRC), which contributes to chromatin remodeling and transcriptional regulation of target genes by using energy from ATP hydrolysis (29). Emerging evidence suggests that *BRG1* is required to regulate transcription of neuronal genes concurrent with histone modifications. Rodent models of drug self-administration provide a way to simulate the process of heroin addiction in humans and to systematically evaluate brain plasticity and adaptability following heroin self-administration.

The present study aimed to elucidate the possible relationship between heroin self-administration and H3K9ac associated with *BRG1* in the specific brain subregions involved in the reward circuitry, thus gaining insight into the potential function of the epigenetic regulation of addictive behaviors.

## Materials and methods

**Heroin preparation.** Heroin was provided by the National Institute of Forensic Science and dissolved in physiological saline at a final concentration of 0.2 mg/ml, as previously described (30). Heroin was administered at a dose of 50  $\mu$ g/kg per infusion based on a previous report (31).

**Animals.** Male Sprague-Dawley (SD) rats (weight, 250–300 g; age, 7–8 weeks) were provided by the Experimental Animal Center of Zhejiang Province. A total of 20 SD rats were housed in cages controlled for temperature (22–25°C) and humidity (40–60%) under a standard 12-h light/dark cycle (lights on between 7:00 p.m. and 7:00 a.m.) with free access to water and food. Rats were randomly divided into two groups (n=10 rats/group): i) Heroin addiction group, which received self-administered heroin for 14 days, and ii) Control group, which received saline injections for 14 days.

**Self-administration apparatus.** Plexiglas custom-made operant boxes (working area 29x29x29 cm) were enclosed inside a sound-attenuating plywood chamber. Two nose-poke response devices were located 5 cm from the floor in each

operant box, an active nose-poke hole and an inactive one. A yellow LED cue light was placed inside each nose-poke hole, and a white LED (10 cm in diameter) was placed on the wall above the holes. The drug solution was delivered at a constant flow rate (1.1 ml/min) through Tygon tubing, which was attached to an infusion pump (PHM-100; Med Associates, Inc.). The tubing was protected by a leash spring and hung from the ceiling with a plastic fluid swivel (PHM-115; Med Associates, Inc.). The leash spring was fitted to a connector on the animal's jacket. The experimental procedure was controlled by an IBM-compatible PC, using input/output interfaces and computer software (Super State Version 1.0; Ningbo Institute of Microcirculation and Henbane) (32).

**Surgery.** Chronically indwelling intravenous catheters were implanted in the rats after sodium pentobarbital anesthesia (50 mg/kg, intramuscular; Sigma-Aldrich; Merck KGaA). A silicon catheter (length, 3.5 cm; inner diameter, 0.5 mm; outer diameter, 0.94 mm) was placed into the right external jugular vein, passing through the back of the body to the right atrium.

Catheters were irrigated daily with 0.2 ml of saline containing sterile benzylpenicillin sodium (60,000 units) and heparin (5 units) in a syringe to prevent bacterial infection and blockage. All rats were allowed to recover for at least 1 week after surgery. All animal procedures were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

**Heroin self-administration.** Four-hour training sessions of heroin self-administration were performed daily for 14 consecutive days under a fixed-ratio-1 paradigm after rats had recovered from surgery. Rats were placed in the operant chambers, and their connectors were attached to the infusion lines. The trial began with illumination of the yellow light. Each response in the active hole was immediately reinforced with an infusion of heroin. Responding in the inactive nose-poke resulted in no heroin delivery. Rats received a single heroin infusion at a dose of 50  $\mu$ g/kg, paired with 2 sec illumination of the white light and in combination with the noise of the infusion pump, followed by a 20-sec refractory period with no injection. During the training cycle, there were no heroin infusions on the right side of the operant box (inactive nose-poke). After each refractory period, the yellow light was turned on again, and another trial began. Rats were put back into their individual home cages after the training sessions ended. Rats in the control group received the same treatment schedule as above but with an injection of saline instead of heroin.

At 24 h after the last behavioral session, all rats were anesthetized with sodium pentobarbital (50 mg/kg, intramuscular) and rapidly decapitated. Brain tissues were dissected from the bregma [2.2 to 0.8 mm according to coordinates from a rat brain atlas of Paxinos and Watson (33)] using a rat brain matrix, and were sectioned (1.4 $\pm$ 0.02 mm). The NAc and mPFC were harvested using a biopsy punch (2 mm in diameter).

**Chromatin immunoprecipitation (ChIP) assay.** Brain tissues were minced into small pieces and homogenized in 1 ml 10X PBS. Next, they were centrifuged at 800  $\times$  g (4°C, 5 min) and resuspended in 1 ml cold PBS according to the manufacturer's instructions (Magna ChIP™ A kit; Merck KGaA), and

crosslinked with 0.1% formaldehyde for 8 min at room temperature. Crosslinking was stopped with 100  $\mu$ l glycine (10X), then precipitates were collected by centrifugation (800 x g, 4°C, 5 min). Precipitates were washed with 1 ml of cold PBS [containing 5  $\mu$ l Protease Inhibitor Cocktail II (Magna ChIP™ A kit; Merck KGaA)], resuspended with 500  $\mu$ l cell lysis buffer, centrifuged at 800 x g (4°C, 5 min) and resuspended with 300  $\mu$ l nuclear lysis buffer containing Protease Inhibitor Cocktail II (Magna ChIP™ A kit; Merck KGaA).

Nuclear lysates were sonicated using a Bioruptor UCD-200 (Diagenode SA) with optimal conditions determined to shear crosslinked DNA to 100-500 bp. Briefly, samples were sonicated at 60 KHz for 30 cycles for 30 sec on and 30 sec off at 4°C and briefly spun down (800 x g, 4°C, 10 sec) for every 5 cycles. To evaluate the quality of sonicated chromatin, a 20  $\mu$ l aliquot was removed, reverse-crosslinked and purified with the Magna ChIP A kit for agarose gel electrophoresis (2% agarose gels were prepared, ethidium bromide was added to the gel matrix to enable fluorescent visualization of the DNA bands, and electrophoresis was performed at 150 mA for 30 min at room temperature).

Chromatin extracts were diluted 10-fold with ChIP buffer H from the Auto Histone ChIP-seq kit (Diagenode, SA) to a final volume of 200  $\mu$ l. A 2  $\mu$ l aliquot of pre-immunoprecipitated lysates (1%) was saved as 'input' for normalization.

The Diagenode SX-8G IP-Star® Automated System (Diagenode SA) was used for immunoprecipitation. Immunoprecipitations were performed with 1.5  $\mu$ l (2  $\mu$ g) rabbit anti-H3K9ac (cat. no. C15410004; Diagenode SA) and 2  $\mu$ l (2  $\mu$ g) normal rabbit immunoglobulin G (IgG; cat. no. C15410206; Diagenode SA), which was used as a negative IP control. Then 10  $\mu$ l of protein A coated with magnetic beads were incubated at 4°C with the anti-H3K9ac or the IgG for 2 h before immunoprecipitation of chromatin extracts for 13 h, and washing for 5 min at 4°C. The immunoprecipitated material and the input were de-crosslinked at 65°C for 4 h under high-salt conditions (4  $\mu$ l; 0.585 g/ml NaCl). Immunoprecipitated DNA was extracted with Auto IPure kit (Diagenode, SA) and resuspended in 25  $\mu$ l Buffer C.

**Quantitative PCR (qPCR).** Two different regions of the *BRG1* gene, namely the promoter (*BRG1*<sup>+</sup>) and the last exon (*BRG1*<sup>-</sup>), were amplified with primers shown in Table I. Primers were selected with Primer Premier 5.0 software (Premier Biosoft International; Fig. 1). All oligomers were synthesized by Sangon Biotech Co., Ltd.

The level of specific histone modification at *BRG1* was determined by qPCR with LightCycler® 480 (Roche Applied Science). qPCR was performed with 5  $\mu$ l immunoprecipitated or input DNA, 1  $\mu$ l primer pairs (10  $\mu$ M each), 12.5  $\mu$ l SYBR Green Master mix (Roche Corporation) and 6.5  $\mu$ l DNase/RNase-free water in a final volume of 25  $\mu$ l. Amplification reactions were run in triplicate for every sample; the thermocycling conditions were: Pre-incubation at 95°C for 5 min, followed by 40 cycles of 95°C for 30 sec, 60°C for 30 sec, 72°C for 30 sec for primer extension, and then a melting curve period (90°C for 10 sec, 60°C for 30 sec and 90°C for 10 sec), followed by final cooling step of 40°C for 5 min. The differential quantitation cycle ( $\Delta$ Cq) value (34) was used to quantify the relative amount of the target regions, and

melting curves were generated to evaluate the specificity of the amplification products.

The efficiency of ChIP and DNA recovery of a particular genomic locus was normalized to the input DNA calculated from qPCR data and reported as a percentage of starting material: % (ChIP/total input) =  $2^{[(Cq(1\% \text{ input}) - \log(1\% \text{ log2}) - Cq(\text{ChIP}))]} \times 100$ . Fold enrichment over IgG was calculated by the recovery of immunoprecipitated genomic loci.

**Reverse transcription-qPCR (RT-qPCR).** Total RNA was extracted from 15-20 mg of brain tissues by using TRIzol® Reagent (Invitrogen; Thermo Fisher Scientific, Inc.) and trichloromethane (Jiangsu Tongsheng Chemical Reagent Co., Ltd.). RT was performed with the miScript II RT kit (Qiagen GmbH), and cDNA was synthesized from 1  $\mu$ g total RNA. The nucleotide sequences of the qPCR primers are shown in Table I. *GAPDH* was used for normalization. All reactions were performed in a LightCycler® 480 (Roche Applied Science). The reaction mixture contained 5  $\mu$ l SYBR Green premix, 2  $\mu$ l primer pairs (10  $\mu$ M each), 1.5  $\mu$ l cDNA, and 1.5  $\mu$ l water. The thermocycling conditions were: 95°C for 10 min, 45 cycles of 95°C for 10 sec, 60°C for 10 sec and 72°C for 10 sec. The  $2^{-\Delta\Delta Cq}$  method was used to analyze the RT-qPCR data and to report the relative change in *BRG1* mRNA expression (33).

**Statistical analysis.** One-way ANOVA for repeated measures was used to compare the number of infusions or active and inactive nose-poke responses in the two groups during the training sessions. Student's t-test was used to evaluate the statistical differences in the mPFC and NAc between the heroin self-administration and control group. Statistical analysis was performed using SPSS 16.0 (SPSS, Inc.) and GraphPad Prism 5.0 (GraphPad Software, Inc.). P<0.05 was considered to indicate a statistically significant difference.

## Results

**Establishment of a rat model of heroin self-administration.** The number of nose-pokes (active or inactive responses) in rats are presented as mean  $\pm$  SEM during 14 consecutive days of training (Fig. 2A). The nose pokes in the active holes and the number of infusions reached a stable level within 14 days of self-administration training (Fig. 2A and B). Notably, the number of inactive pokes remained stable at baseline during the training program. The criterion of stable heroin self-administration was defined as <10% variation in the number of active pokes that rats touched on the last 3 days (27). A total of 18 rats were included in the final analysis (n=9 rats/group); the remaining 2 rats were excluded from further analysis as they did not meet the criteria for stable model.

**Quality control of ChIP assay.** To evaluate chromatin quality after sonication, the length of sheared DNA was analyzed by agarose gel electrophoresis. The results showed that sheared DNA fragments were 100-500 bp long (Fig. 3A). H3K9ac of *BRG1* was evaluated by qPCR. Amplification curves for one qPCR product are presented in Fig. 3B. The curves demonstrated good reproducibility. Moreover, the qPCR results of the H3K9ac antibody-immunoprecipitated DNA over rabbit IgG (negative control) showed that the IgG

Table I. Primers for ChIP and RT-qPCR.

Reaction	Gene	Primer sequence (5'→3')	Length (bp)	T <sub>m</sub> (°C)
ChIP	<i>BRG1</i> <sup>+</sup>	F: ACAGAGCCTTGCAGAGCA	18	60
		R: GAGGAAAGTGAAGCCGAGA	19	
	<i>BRG1</i> <sup>-</sup>	F: CTTAGCAGTGATGGGTAGCA	20	60
		R: CAGACCAGGGCCAACAGA	18	
RT-qPCR	<i>BRG1</i>	F: CCTGATGACCCACGATAACAACC	22	60
		R: GGAGGCATGTTTCAGAGCCACC	21	
	<i>GAPDH</i>	F: ACCCAGAAGACTGTGGATGG	20	60
		R: TTCTAGACGGCAGGTCAGGT	20	

*BRG1*, Brahma/SWI2-related gene-1; *BRG1*<sup>+</sup>, promoter region of *BRG1*; *BRG1*<sup>-</sup>, specific region in last exon of *BRG1*; ChIP, chromatin immunoprecipitation; F, forward; R, reverse; RT-qPCR, reverse transcription-quantitative PCR.

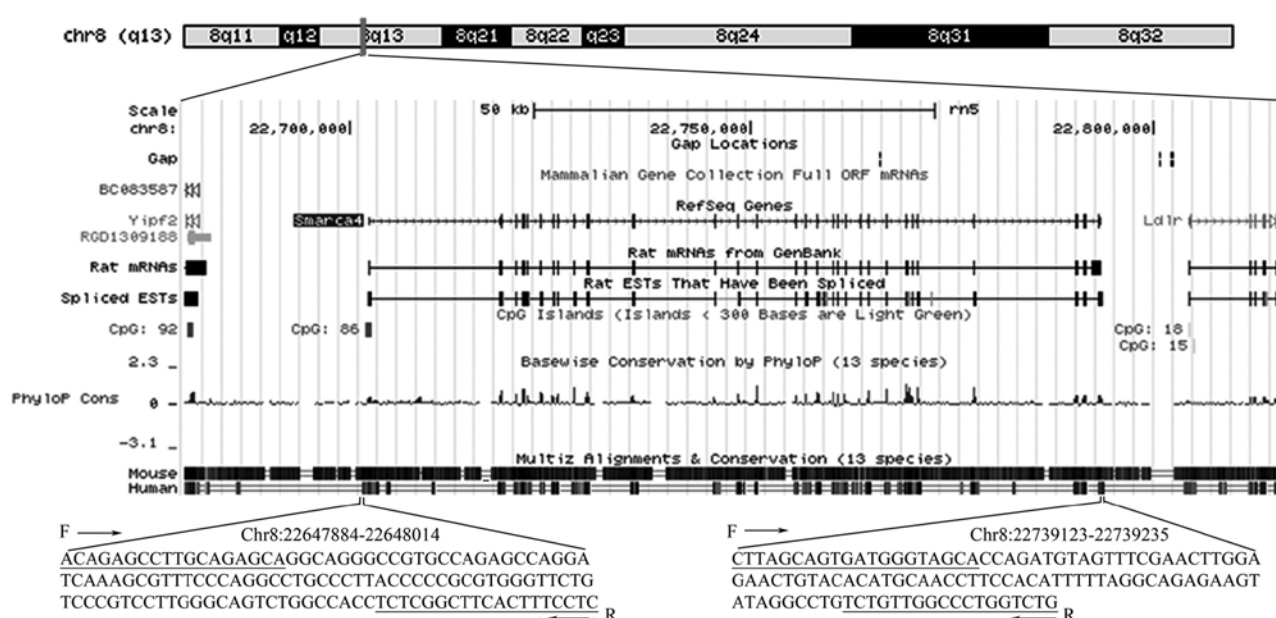


Figure 1. Location and sequence of Brahma/SWI2-related gene-1. Chromatin immunoprecipitation was performed on the fragment (hg19, chr8:22647884-22648014) in the promoter region and the fragment (hg19, chr8:22739123-22739235) in the last exon of the *SMARCA4/BRG1* gene. F, forward; R, reverse.

antibody was not amplified by qPCR, which indicated that the experimental system was reliable (data not shown). To avoid errors introduced by differences in the amount loaded, *BRG1*<sup>-</sup> was considered as the internal negative reference for qPCR; the results demonstrated that 0.08-0.16% of the *BRG1*<sup>-</sup> with H3K9ac was recovered in the mPFC and NAc (Fig. 4B). Highly sensitive and specific ChIP enabled >10-fold enrichment; this value depended not only on the antibody but also on the target. In the present, study the fold enrichment over H3K9ac was calculated by the recovery of the target *BRG1*<sup>+</sup>/*BRG1*<sup>-</sup>. It was revealed the fold enrichment  $\geq 30$ -fold, which indicated that the signal-to-noise ratio was acceptable (Fig. 4A). Therefore, the sensitivity and specificity of the H3K9ac antibody was satisfactory.

**H3K9ac recovery rate.** Recovery is a measure of the association of an antibody with a specific locus. As the target

and negative regions, the promoter region and the final exon region of *BRG1* were tested. Results showed the quantity of *BRG1*<sup>+</sup> combined with H3K9ac differed among the four groups (Fig. 4B). H3K9ac increased in response to heroin self-administration in the mPFC; H3K9ac enrichment was significantly higher in the heroin self-administration group compared with the control group (recovery % input,  $7.79 \pm 0.81$  vs.  $10.09 \pm 0.81$ , respectively;  $P=0.002$ ; Fig. 4B). However, no significant differences were identified between the two groups in the NAc (recovery % input,  $4.15 \pm 0.74$  vs.  $3.88 \pm 0.95$ , respectively; Fig. 4B;  $P=0.323$ ). Additionally, there were significant differences within treatment groups between the mPFC and the NAc ( $P<0.05$ ).

**Expression of *BRG1*.** *BRG1* mRNA expression was elevated after heroin self-administration, corresponding to 1.47-fold of the saline control ( $P<0.05$ ) in the mPFC. Whereas *BRG1*

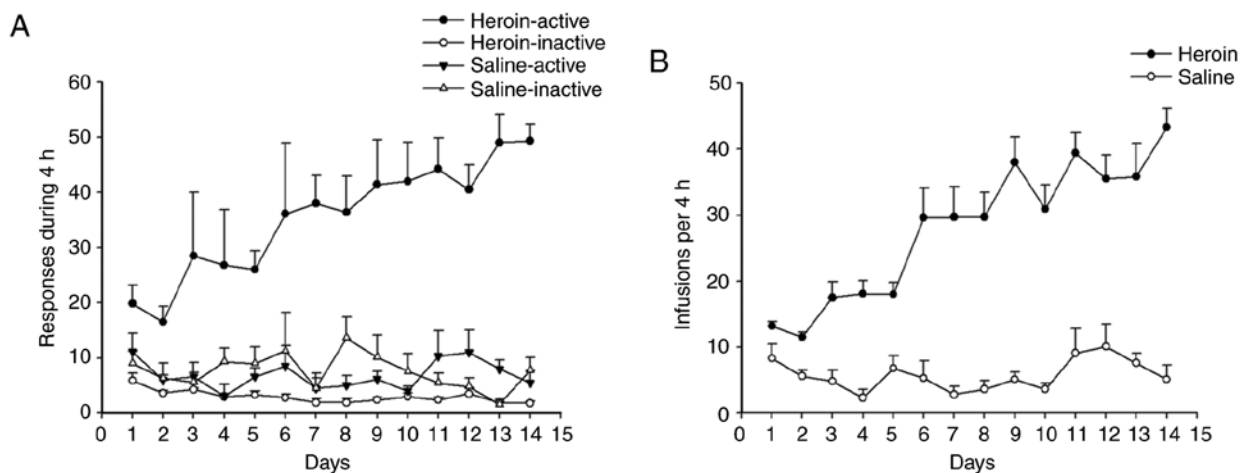


Figure 2. Establishment of a rat model of heroin self-administration. (A) Number of responses (nose pokes) in the active and inactive holes during 4 h for each of the 14 days of training between the heroin self-administration group and the saline control group. (B) Number of infusions in the two groups during the training session.

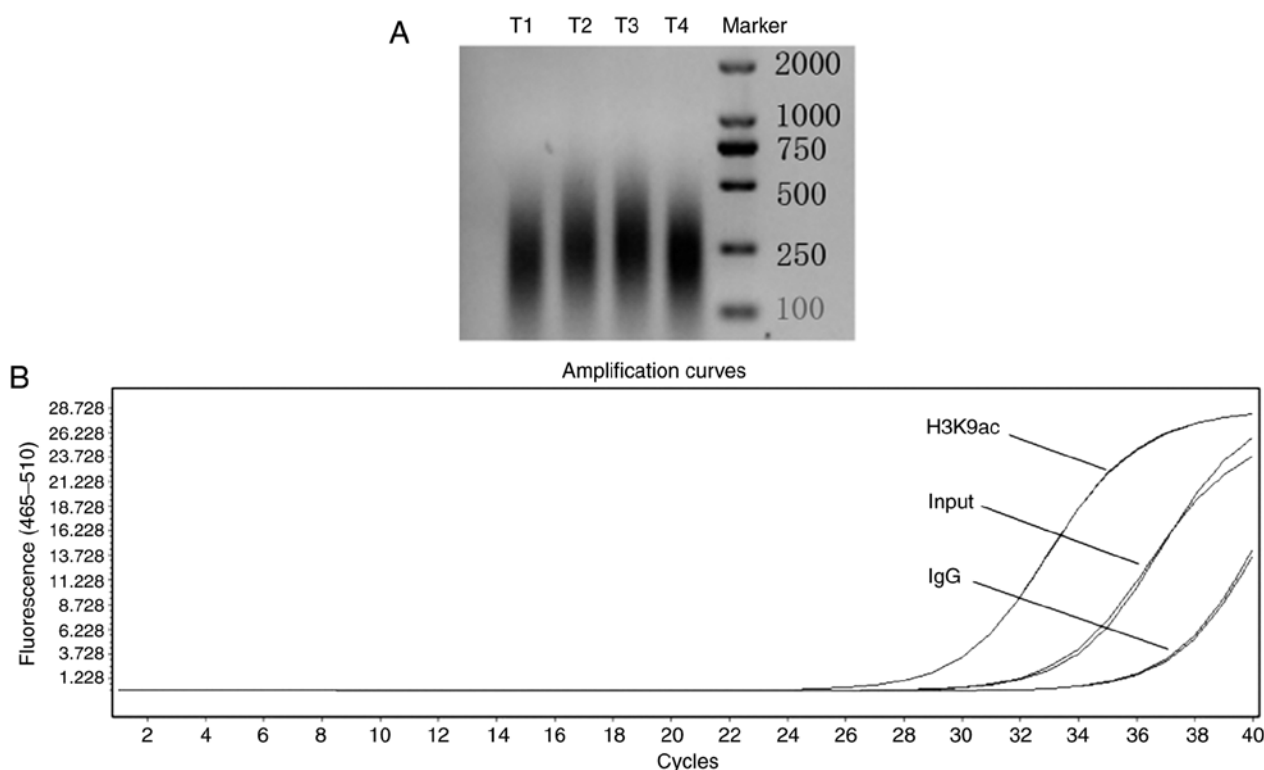


Figure 3. DNA fragmentation analysis and amplification curves. (A) Analysis of sonicated DNA by agarose gel electrophoresis. T1/T2, self-administered delivery of heroin; T3/T4, saline control. T1/ T3, mPFC; T2/ T4, NAc. (B) Amplification curves of quantitative PCR in a sample (mPFC-Heroin) with H3K9ac and IgG antibodies (negative control). H3K9ac, histone H3 lysine 9 acetylation; IgG, immunoglobulin G.

mRNA expression was reduced in the NAc, corresponding to 0.72-fold of the saline control ( $P>0.05$ ; Fig. 5). The relative change in the mRNA expression of *BRG1* following heroin self-administration between the mPFC and NAc was 2.04-fold (data not shown).

## Discussion

Heroin addiction is a debilitating psychiatric disorder characterized by compulsive heroin craving behavior and

relapse, despite prolonged periods of abstinence (35), posing a significant threat to human health. Heroin addicts often exhibit long-lasting and complicated cognitive impairment and neural deficits (36). Neurobiological adaptations or drug-induced synaptic neuroplasticity, such as epigenetic alterations in the brain reward circuitry, are thought to contribute to this chronic disease (37).

H3K9ac is a chromatin mark that is increased significantly following nerve injury and is closely related to gene transcription (38,39). Previous studies have shown that *BRG1*

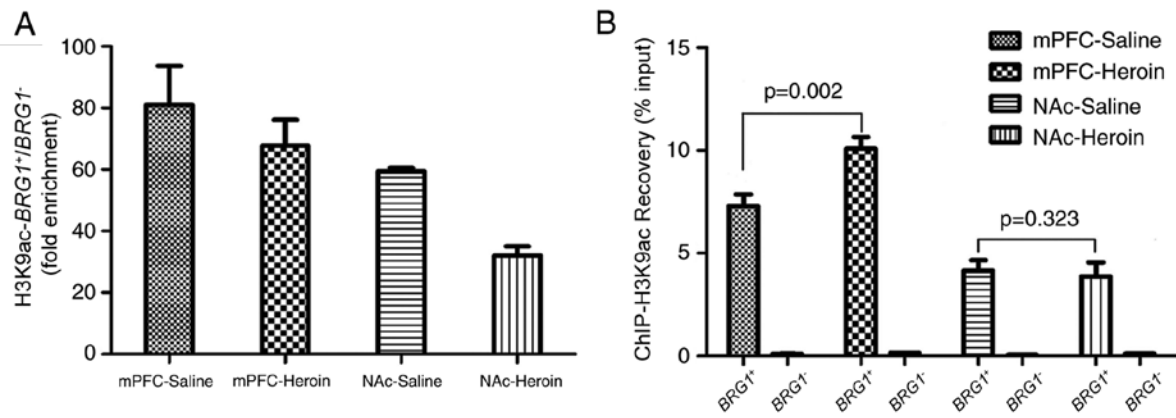


Figure 4. Specificity of the H3K9ac antibody and quantity of *BRG1* bound to H3K9ac. (A) Fold enrichment over IgG of H3K9ac-associated *BRG1* following ChIP and quantitative PCR (B) Recovery rate of H3K9ac-associated DNA input. The ChIP assay was used to recover the total percentage input of *BRG1* acetylated at H3K9 within the mPFC and NAc of the heroin self-administration and saline control groups. *BRG1*, brahma/SWI2-related gene-1; ChIP, chromatin immunoprecipitation; H3K9ac, histone H3 lysine 9 acetylation; mPFC, medial prefrontal cortex; NAc, nucleus accumbens; *BRG1*+, promoter region of *BRG1*; *BRG1*-, last exon of *BRG1*.

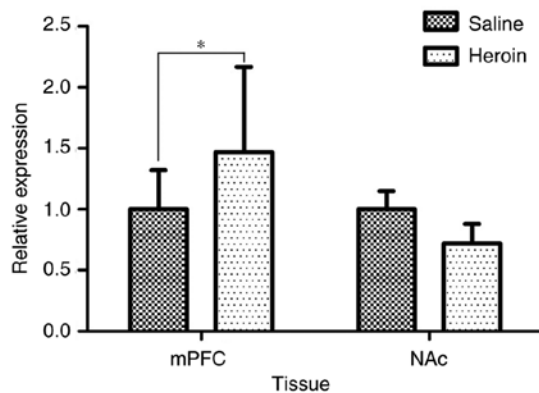


Figure 5. Expression of *BRG1* mRNA in the mPFC increased in heroin-self administration rats. The mRNA expression levels of *BRG1* in the heroin self-administration group or saline control group were determined by reverse transcription-quantitative PCR in the mPFC and NAc of rats. mRNA expression levels were normalized against *GAPDH*; data are presented as mean  $\pm$  SEM; \* $P < 0.05$ . *BRG1*, brahma/SWI2-related gene-1; mPFC, medial prefrontal cortex; NAc, nucleus accumbens.

expression is enriched in neurons (40), and *BRG1* contributes to transcription factor-DNA interactions (39). For instance, *BRG1* forms a complex with SLIT-ROBO Rho GTPase Activating Protein 3, which is related to GTPase activator activity, subsequently disrupting the *BRG1*-related SWI/SNF CRC and affecting transcription of neuron-specific target genes in cortical neurons or Neuro2a cells, which are essential during neural development (41). Furthermore, *BRG1* combined with calcium-responsive transactivator regulates promoter activation downstream of calcium-dependent signaling in resting neurons (42) and our previous study demonstrated that H4K5ac of *BRG1* in the VTA may be related to heroin administration, but not addiction (43).

The present study assessed a possible relationship between H3K9ac and the *BRG1* locus after heroin self-administration. The data demonstrated that in contrast to a saline control, there was a significant increase in the level of H3K9 acetylation at the *BRG1* promoter in the mPFC after heroin self-administration, and an increased expression of *BRG1* mRNA. However, heroin

self-administration did not induce any significant alteration in H3K9ac at the *BRG1* locus in the NAc and the mRNA expression of *BRG1* was decreased.

These results suggested an important and novel role for *BRG1* in heroin-mediated neural adaptation. This is consistent with the recent publication from Martin *et al* (44), which demonstrated that *BRG1* expression in the PFC increased after heroin self-administration, but remained unchanged in the NAc. In addition, genes regulated by *BRG1*, such as the sex determining region Y-box 10 (*Sox10*), which is crucial for neural crest and peripheral nervous system development (45,46), have been seen to change during heroin self-administration in the PFC. Upon further investigation, it was demonstrated that the overexpression of *Sox10* or *BRG1* decreased the motivation to obtain heroin infusions (44). These results demonstrated the crucial role of *BRG1* in regulating motivation for heroin. A study on cocaine demonstrated that *BRG1* expression increased in the NAc, and *BRG1* can be incorporated into the transcriptional complex with SMAD family members, which are signal transducers that mediate multiple signaling pathways and modulate gene expression and behavioral changes following cocaine exposure (47). Interestingly, the overexpression of *BRG1* can exacerbate the cocaine-reinstatement behavior. In conclusion, *BRG1* contributes to addiction phenotypes through association with other genes, modulators and epigenetic mechanisms that regulate histone lysine acetylation. The current study provided evidence that the direct relationship of *BRG1*-H3K9ac might be implicated in heroin self-administration. Notably, the relationship between H3K9ac and *BRG1* has been previously reported in human cancer (48). A previous study showed that *BRG1* remodels chromatin to facilitate the action of HDAC2 by binding to the transcription start site of the telomerase reverse transcriptase (*hTERT*) promoter, which catalyzes the *de novo* synthesis of telomeric repeats using a telomere-specific RNA template, leading to H3K9 deacetylation and suppression of *hTERT* transcription. Thus, *BRG1* protein regulates H3K9ac through HDAC and possibly by regulating *hTERT* expression. Overall, these data suggested that the *BRG1* gene is regulated by H3K9ac at its promoter and increased H3K9ac might



correlate with increased BRG1 expression, which in turn may downregulate neuron-addictive behavior through promoting the accessibility of HDACs.

In the present study, there were significant differences in H3K9ac at the *BRG1* promoter between the heroin self-administration group and the control group in the mPFC, but not in the NAc, which suggested region-specific regulation. The NAc, which serves as a critical node within the brain's reward circuitry, directs attention and behavior toward multiple stimuli, including drug abuse and natural rewards such as food and sex, in addition to other secondary reinforcers (49). In addition, it integrates mnemonic and emotional signals from cortical and limbic structures to mediate goal-directed behaviors (49,50). The PFC has long been suspected to be involved in cognitive control; the ability to orchestrate thought and action in accordance with internal states or intentions (51), and other aspects of the addiction phenotype (52). Activity in the dorsal mPFC is thought to initiate drug-seeking behavior and is crucial for cue-induced nicotine or morphine relapse (15,53), whilst lesions in the mPFC may lead to insensitivity to changes in compulsive behavior (17) and marked inability to make choices (52). Results from the present study demonstrated the specificity of the H3K9ac-*BRG1* association in response to heroin self-administration for the mPFC, which may inform further research in specific brain regions. Nevertheless, this study has several limitations. Firstly, the influence of heroin on H3K9ac was only examined in the mPFC and NAc; other tissues related with addiction, including the VTA and hippocampus, should be studied in the future. Secondly, the mechanisms by which BRG1 affects heroin self-administration through association with H3K9ac remain largely elusive. Further investigations are required to reveal how the increased expression of BRG1 and its association with H3K9ac affects heroin self-administration behavior.

In conclusion, the present study demonstrated an elevated acetylation of H3K9 in the *BRG1* promoter in the mPFC after heroin self-administration, indicating a potential novel role for *BRG1* and its relationship with H3K9ac in a heroin-mediated addicted phenotype and neuroadaptations. Specifically, BRG1 may be an essential component of transcriptional complexes mediating gene expression after heroin self-administration. This may provide a novel biomarker for detecting and monitoring heroin addiction, or even a novel therapeutic target for addiction treatment.

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#### Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

#### Authors' contributions

WZ and HL designed the study. QH and JL contributed equally, reviewed the publication and drafted this manuscript. QH, JL and ZL contributed to data interpretation and revised the manuscript critically. DZ, WX and ZX performed laboratory work. ML and HZ participated in data acquisition and data analysis. All authors contributed to finalize the manuscript and approved the final version of the manuscript. All authors agree to be accountable for all aspects of the research.

#### Ethics approval and consent to participate

All procedures involving animals were approved by the Laboratory of Behavioral Neuroscience, Animal Care and Use Committee in Ningbo Institute of Microcirculation and Henbane (Ningbo, China) and conformed to guidelines for the Care and Use of Laboratory Animals of the Ministry of Health, China.

#### Patient consent for publication

Not applicable.

#### Competing interests

The authors declare that they have no competing interests.

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