Behavioral Sensitization Induced by Methamphetamine Causes Differential Alterations in Gene Expression and Histone Acetylation of the Prefrontal Cortex in Rats

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Abstract

Background: Methamphetamine (METH) is one of the most widely abused illicit substances around the world; unfortunately, its addiction mechanism remains unclear. Increasing evidence indicates that the change in gene expression and the involvement of chromatin modifications might be related to the lasting effects of METH on the brain. In this study, we took advantage of METH-induced behavioral sensitization as an animal model that reflects some aspects of drug addiction and examined the transcription and histone acetylation changes in gene expression in the prefrontal cortex (PFC) of adult rats.

Methods: We conducted mRNA microarray and chromatin immunoprecipitation (ChIP) coupled to DNA microarray (ChIP-chip) analysis to test and screen transcriptional changes and histone acetylation modifications. Functional enrichment analysis, including Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG), was performed to analyze the differentially expressed genes. We then further identified alterations in ANP32A (acidic leucine-rich nuclear phosphoprotein-32A) and POU3F2 (POU domain, class 3, transcription factor 2) by qPCR and ChIP-PCR assays.

Results: In the rat model of METH-induced behavioral sensitization, METH challenge caused 275 differentially expressed genes and a number of hyperacetylations (821 genes with H3 acetylation and 10 genes with H4 acetylation). Based on mRNA microarray and GO and KEGG enrichment analysis, 24 genes may be involved in METH-induced behavioral sensitization, and 7 of them were confirmed by qPCR. We further tested the alterations in ANP32A and POU3F2 transcription and histone acetylation at the different periods of METH-induced behavioral sensitization. The results showed that H4 hyperacetylation contributed to the increased mRNA of ANP32A and that H3/H4 hyperacetylation contributed to the increased mRNA of POU3F2 induced by METH challenge-induced behavioral sensitization but not by acute METH exposure.

Conclusions: The present results revealed alterations in transcription and histone acetylation in the rat PFC by METH exposure and provided evidence that modifications of histone acetylation contributed to the alteration of gene expression caused by METH-induced behavioral sensitization.

Background

Methamphetamine (METH) has been one of the most widely abused illicit substances around the world for many years. Abusers suffer cognitive, behavioral and emotional regulation dysfunctions with long-term use and have a high relapse potential with METH. The neurobiological mechanism of METH addiction is attributed to adaptations in signal cascades and neural plasticity in the central nervous system. The enduring impact of these drugs, even after long periods of abstinence, has suggested persistent molecular events caused by transcriptional, epigenetic and translational effects[1] within addiction-related brain regions[2], such as the nucleus accumbens (NAc), prefrontal cortex (PFC), and hippocampus. Notably, a growing body of evidence has shown the important role of epigenetic

regulation in the effects of psychostimulants[3] (e.g., cocaine[4, 5]) and found modifications of histones present in chromatin[6, 7], DNA methylation[8, 9], and DNA hydroxymethylation involved in the development of addiction. With respect to METH, however, only a few studies have investigated the epigenetic effects of these drugs, mainly focusing on the NAc and striatum[10-14].

In animals, repeated exposure to METH produces behavioral sensitization manifested as a progressive enhancement of locomotor responses that can be elicited by a challenge dose of the drug after long-term withdrawal. Behavioral sensitization has been suggested to be a useful animal model of drug abuse that may reflect the properties of craving[15]. Alterations to the associated brain regions, such the PFC, are suggested to mediate the etiology and maintenance of METH-induced behavioral sensitization[16]. Repeated exposure to drugs such as cocaine or METH was also reported to produce long-lasting changes in synaptic plasticity in the ventral tegmental area (VTA), NAc, and PFC[17-19]. A study in nonhuman primates has shown that changes in the PFC following METH administration could persist for as long as 3.5 years[20].

In the present study, we focused our attention on whether the changes in gene expression in METH-induced behavioral sensitization are related to histone acetylation in the PFC. Given the importance of the PFC in the development of METH abuse, we screened the alterations in gene expression as well as histone acetylation in the PFC following METH-induced behavioral sensitization and further investigated the contribution of histone acetylation to the expression of candidate genes in the different phases of the behavior model. We found that alterations in histone acetylation might, in part, influence METH-induced changes in gene expression in the PFC.

Materials And Methods

Subjects

Adult male Sprague-Dawley rats (Grade SPF, Beijing Animal Center, Beijing, China) weighing 160-180 g were used in this study. Animals were maintained under a 12/12-hour light/dark cycle (lights on at 6:30 and off at 18:30), with ambient temperature set at 20°C to 22°C. Animals were housed in 470×300×200 mm cages and given access to rat maintenance food and water ad libitum. During housing, animals were monitored twice daily, and no abnormal events regarding health status were detected. All experiments on the animals were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals (8th edition) and the guidelines of the National Research Council ([2006] 398, China). All sections of this report adhere to the ARRIVE Guidelines for reporting animal research. A completed ARRIVE guidelines checklist is included in [Checklist S1](#).

Reagents

METH-HCl was provided by the Beijing Public Security Bureau Forensic Medical Examination Center (China). The drug was dissolved in saline (0.9% NaCl).

Behavior experiment

The locomotor activity was measured in chambers (40 cm×40 cm×65 cm) using Digbehv spontaneous activity monitors[21] (Shanghai Jiliang Software Technology Co. Ltd., Shanghai, China). The total distance of horizontal locomotor activity was recorded with a video camera placed above the chamber and analyzed with the Digbehv software SuperState v3.0 system (AniLab Software & Instruments Co., Ningbo, China).

The behavioral sensitization procedures were conducted according to the method we have reported[14]. Behavioral sensitization consists of three phases (development, withdrawal and challenge phase) (Figure 1A). The rats were administrated with METH (5 mg/kg, s.c.) or normal saline (NS) (1 ml/kg, s.c.) once daily in the acute phase or for 7 days in the development phase. On days 8-14, the rats were housed in home cages, and nothing was injected in the withdrawal phase. On day 15, METH (1 mg/kg, s.c.) was used to challenge the rats that were administered NS or METH in the development phase, and then the locomotor activity was recorded for 60 min in the challenge phase. The experiment was conducted using 40 rats (n=8 for each group, 5 groups). In order to reduce animal use and experimental error, a randomized METH and saline administration schedule was created using the standard=RAND() function in Microsoft Excel.

RNA isolation and cDNA synthesis

Twenty-four hours after the behavioral experiment, rats were anesthetized by pentobarbital (70 mg/kg, i.p.) and then killed by decapitation. PFCs were dissected (+5.16 mm to 3.00 mm from Bregma) according to the stereotactic atlas of Paxinos and Watson. Frozen PFCs were thawed in TRIzol (Invitrogen, Cat. No. 15596018) and then processed according to the manufacturer's protocol. Total RNA was reverse-transcribed using the ReverTra Ace qPCR RT Kit (Qiagen, Cat. No. 205313).

Real-time PCR

Real-time PCR was run using a SYBR Green PCR kit (Qiagen, Cat. No. 204145) and quantified using the $2^{-\Delta\Delta Ct}$ method. Real-time PCR was then run using an ABI 7300 Real-Time PCR System (Applied Biosystems, Foster City, CA), amplified using SYBR Green (TOYOPO, Cat. No. QPS-201) and quantified using the $2^{-\Delta\Delta Ct}$ method. The primers for the testing genes are shown in Table S1. Each sample was assayed in duplicate and normalized using GAPDH levels.

ChIP assay

PFCs were crosslinked with 1% formaldehyde at 37°C for 10 min, and then glycine was added to a final concentration of 0.125 M for 5 min to stop the crosslinking. Samples were spun at 800 *g* at 4°C for 10 min to pellet tissues. The PFCs were sonicated on wet ice with 300 μ l of nuclear lysis buffer using a Sonicator (Sonics Vibra-cell™, Sonics & Materials Inc., Newtown, Connecticut, USA). The sonication conditions were as follows: almp 70%, pulse 10 sec, pause 59 sec for 9 cycles. The supernatant was

collected in accordance with a chromatin immunoprecipitation kit (Millipore, Cat. No. 17408). The lysate was precipitated with antibodies against acetyl-histone H3 (Millipore, Cat. No. 06599) and acetyl-histone H4 (Millipore, Cat. No. 06866). Sequences for ChIP-PCR are as follows: POU3F2: forward primer 5'-CAGTACCGCTGAATAACGC-3' and reverse primer 5'-CTGAGCCTGCTTACGATGA-3'; ANP32A: forward primer 5'-CCATCGGAGCATGTTTCGT-3' and reverse primer 5'-AGGGTGAGATCGCCGTCTT-3'.

Microarray experiments

The samples extracted from a total of 8 rat PFCs per group were processed for the mRNA microarray or ChIP on chip DNA microarray. mRNA microarray analysis was conducted using the Rat Whole Genome One Array that examines 19,803 unique genes using 24,358 probes (Phalanx Biotech Group, Hsinchu, Taiwan). The fluorescently amplified RNA targets were prepared from 2.5 µg of total RNA samples using the OneArray® Amino Allyl aRNA Amplification Kit (Phalanx Biotech Group) and labeled with Cy5 dye (Amersham Pharmacia, Piscataway, NJ, USA) and then hybridized to the Rat Whole Genome OneArray® (ROA 1.1) (Phalanx Biotech Group) according to the Phalanx hybridization protocol. The slides were scanned using an Axon 4000 scanner (Molecular Devices, Sunnyvale, CA, USA). The Cy5 fluorescence intensity of each spot was analyzed using GenePix pro software (Molecular Devices). The signal intensity of each spot was loaded into the Rosetta Resolver System® (Rosetta Biosoftware) for data analysis.

For ChIP on chip, DNA microarray analysis was processed using the Rat 3×720K RefSeq Promoter Array that examines 15,287 unique gene promoters overlapping 1070-4280 bp (Nimblegen). The input DNA was amplified using the WGA2 kit (Sigma, Cat. No. WGA2-50R) and purified by the QIAquick PCR purification kit (Qiagen). The product was labeled with Cy3 and Cy5 dyes and hybridized using a Roche NimbleGen hybridization kit. After drying, the slides were scanned by a Roche NimbleGen MS200, and the data were extracted by NimbleGen2.6 software. For each probe on the array, the log₂ ratios of the Cy5-labeled ChIP DNA/Cy3-labeled input DNA were calculated using Signal MAP software. Peak detection was performed using NimbleScan software.

Differential gene expression analysis and functional enrichment

Functional enrichment analysis, including Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG), was performed to identify which differentially expressed genes were significantly enriched in GO terms and metabolic pathways at the thresholds of corrected *P*-value [also named FDR (false discovery rate)] ≤0.05 and log₂ fold change (log₂ FC). GO functional enrichment and KEGG pathway analysis were carried out with the KOBAS program (<http://kobas.cbi.pku.edu.cn/home.do>[22]).

Statistics

All data are presented as the mean ± SEM. The challenge data were assessed using one-way analysis of variance (ANOVA) followed by a post hoc test. For other data, a t-test was used. Values of *P*<0.05 were considered statistically significant.

Results

METH induced behavioral sensitization

The model of behavioral sensitization was established by 7 days of METH (5.0 mg/kg, s.c.) administration followed by 7 days of withdrawal and challenged by single METH (1.0 mg/kg, s.c.) on day 15. One-way ANOVA showed the difference between groups ($F_{(2, 21)} = 14.58$, $P < 0.001$). Rats in the METH sensitization group (METH-METH group) showed significantly higher locomotor activity than either the saline control (NS-NS) or METH acute treatment group (NS-METH) (Tukey's post hoc test, $P < 0.001$, vs NS-NS group; $P < 0.01$, vs NS-METH group, Figure 1B), indicating the enhanced locomotor response of sensitization. Compared with the NS-NS group, rats in the NS-METH group showed longer distances ($P < 0.05$, Figure 1B).

Changes of mRNA in the PFC of METH-induced behavioral sensitization rat

An mRNA microarray was performed to determine a genome-wide profile of METH-induced behavioral sensitization. The cut-off value was set at a fold change of ≤ 0.5 or ≥ 2.0 ($P < 0.01$). Figure 2 shows the clustering and a heat map of the mRNA expression among the NS-NS group, NS-METH group, and METH-METH group. A large number of genes were changed by METH exposure ($P < 0.01$) (Figure 2B). Among these, 275 genes were differentially expressed in the METH-METH group compared with the NS-METH group (97 genes were upregulated and 178 genes were downregulated), and 232 genes were changed in the NS-METH group compared with the NS-NS group (122 genes were upregulated and 110 genes were downregulated) (Table S2).

Ontological and pathway analysis of differentially expressed mRNAs in the PFC of METH-induced sensitization rats

In order to investigate whether the clustering of differentially expressed genes induced by METH exposure correlates with functional groupings, we performed GO analysis and categorized the differentially expressed genes. Figure 3A and Table S3 show the top 10 ranked GO terms and the number of genes in a category. Compared with METH acute treatment (NS-METH), the biological process in the METH-induced behavioral sensitization (METH-METH) group was markedly constructed of biological regulation, cellular process and organism process. For molecular function, binding and protein binding were enriched in the METH-METH group.

To assess the functional features of METH-induced behavioral sensitization-mediated gene sets, we further performed KEGG pathway enrichment analysis. The top 10 significantly ranked pathways are shown in Figure 3B and Table S4. Many signal transduction pathways were enriched in the METH-induced behavioral sensitization group, including cell adhesion, PI3K-AKT signaling pathway, olfactory transduction, and cell cycle (METH-METH group vs. NS-METH, $P < 0.0001$). For the METH acute treatment (NS-METH) group, the genes categorized by KEGG analysis were enriched in the pathways including the cell adhesion, the pathway in cancer, and dopaminergic pathway.

Changes in histone acetylation in the PFC of METH-induced sensitization rats

ChIP coupled with DNA microarray analysis revealed that METH resulted in increased histone acetylation modification (H3 or H4) on a large number of gene promoters (Figure 4), in accordance with mRNA activation. Compared with the NS-METH group, the METH-METH group induced much more acetylation modifications of H3 than H4 on the promoters; specifically, 821 genes presented H3 hyperacetylation and 10 genes showed H4 hyperacetylation (Table S5). The NS-METH group itself also induced hyperacetylation modifications on the gene promoters, including 947 genes at H3 and 4902 genes at H4. There was very little H3/H4 hyperacetylation that overlapped between the METH-METH and NS-METH groups.

Real-time PCR confirmation of the candidate genes

Based on the mRNA microarray, GO and KEGG enrichment analyses, the genes listed in Table S6 were likely to associate with METH-induced behavioral sensitization. Of these genes, *Avp*, *Bcl2l1*, *Egr1*, *E2f3*, *Ln timer*, *Shoc2*, *Stx2* and *Zfp36* clustered into the top 10 GO and KEGG classifications, and *Anp32a*, *Eml2*, *Exog*, *Hira*, *Metrn*, *Pou3f2*, *Stk32*, *Syt8*, and *Trim17* were included in the GO category. According to the GO category analysis, *Ln timer*, *Shoc2* and *Stx2* were changed by METH single injection, and the other genes above were affected by METH-induced behavioral sensitization (METH-METH) compared with METH acute treatment (NS-METH). We then used qPCR to confirm some genes of interest. The results showed that METH challenge in the behavioral sensitization model caused a significant increase in the mRNA levels of acidic nuclear phosphoprotein 32 family member A (*Anp32a*), calcium/calmodulin-dependent protein kinase II inhibitor 1 (*Camk2n1*), echinoderm microtubule associated protein like 2 (*Eml2*) and POU class 3 homeobox 2 (*Pou3f2*), and a decrease in syntaxin 2 (*Stx2*), tripartite motif-containing 17 (*Trim17*), and zinc finger protein 36 (*Zfp36*) ($P < 0.05$, one-way ANOVA followed by Turkey's post hoc test, Figures 5, 6).

Alterations in ANP32A and POU3F2 in the development, withdrawal and challenge periods of METH-induced sensitization

Then, we selected ANP32A and POU3F2 and further measured the expression of mRNA and histone modification of the two genes. In addition to the challenge of behavioral sensitization, the development and withdrawal phases were also involved to investigate the changes in ANP32A and POU3F2 throughout the whole process of METH-induced behavioral sensitization. After METH (5 mg/kg, s.c.) chronic treatment for 7 days (development phase), the expression of ANP32A mRNA and H4 acetylation was markedly increased compared with the NS group ($P < 0.05$, t-test, Figures 6A, 6B). However, ANP32A mRNA returned to the normal level, while H4 acetylation remained at the high level after 7 days of withdrawal ($P < 0.05$, t-test). Then, injection of METH (1 mg/kg, s.c.) was used for challenge on day 15. One-way ANOVA showed a significant difference in mRNA ($F_{(2, 18)} = 8.824$, $P < 0.01$) and H4 acetylation ($F_{(2, 26)} = 6.072$, $P < 0.01$) between groups, and Tukey's post hoc test showed that challenge induced the expression of ANP32A mRNA ($P < 0.01$, METH-METH vs. NS-NS) and H4 acetylation ($P < 0.01$, METH-METH vs. NS-

METH, $P < 0.05$, METH-METH vs. NS-NS) in the METH pretreatment group (METH-METH). The injection of METH alone also caused the elevation of ANP32A mRNA ($P < 0.05$, NS-METH vs. NS-NS) but did not affect histone acetylation.

We next measured the change in POU3F2 mRNA and histone modification. POU3F2 mRNA was significantly elevated by chronic METH treatment for 7 days and decreased after 7 days of withdrawal ($P < 0.05$, t-test), while H3 or H4 acetylation was not affected by the treatment (Figures 6C, 6D). In the challenge phase, one-way ANOVA showed that challenge with a low dose of METH caused a significant difference in mRNA ($F_{(2, 45)} = 4.01$, $P < 0.05$) and H3 and H4 hyperacetylation (H3: $F_{(2, 36)} = 4.17$, $P < 0.05$; H4: $F_{(2, 38)} = 3.47$, $P < 0.05$) between groups. Tukey's post hoc test showed that the behavioral sensitization group produced significant elevation in mRNA ($P < 0.05$, METH-METH vs. NS-NS), as well as H3 and H4 hyperacetylation (H3: $P < 0.05$, METH-METH vs. NS-NS, METH-METH vs. NS-METH; H4: $P < 0.05$, METH-METH vs. NS-NS). Notably, the single METH injection group (NS-METH) itself also increased the expression of POU3F2 mRNA ($P < 0.05$, vs. NS-NS) but not H3/H4 acetylation.

Discussion

Behavioral sensitization is an animal model that reflects some aspects of drug addiction, such as drug seeking and relapse, but the neurobiological mechanism is not yet fully elucidated. Drug-induced changes in gene expression and sustained changes in genes regulated by epigenetic mechanisms in addiction-associated regions are thought to contribute to long-lasting behavioral abnormalities and neuroadaptation. In order to further analyze the neurological mechanism underlying the development of METH addiction, the present study examined the transcriptional changes in gene expression and histone acetylation alterations in the PFC of adult rats that experienced the different phases of METH-induced behavioral sensitization. The mRNA microarray and ChIP microarray analysis revealed 275 differentially expressed genes and significant hyperacetylation, including 821 genes with H3 and 10 genes with H4, caused by the challenge of METH-induced behavioral sensitization in the PFC, suggesting that histone hyperacetylation is associated with increased expression of a set of genes caused by METH-induced behavioral sensitization.

Accumulating evidence has revealed that drug exposure causes modifications of global histone acetylation or specific changes in the gene-promoter regions in a complex manner. Wang et al. reported that chronic self- or experimenter-administered cocaine induced long-term increases in global H3 and H4 acetylation in the NAc shell[23], and cocaine abstinence caused the acetylation of histone H3K9 and histone H4K8 in the PFC[24]. ChIP sequencing provides access to precise patterns of histone acetylation. Renthal et al.[25] showed that chronic cocaine exposure caused a larger number of genes with H3 acetylation than H4 acetylation in the NAc. A set of genes, such as CBP in the NAc shell[26] or BDNF in the PFC[27], was shown to be activated by cocaine, which was associated with the hyperacetylation of H3 and/or H4. For METH exposure, Tracey et al. reported that a single METH injection induced time-dependent increases in acetylated H4K5 and H4K8 and decreases in acetylated H3K9, H3K18, and H4K16 in the NAc[13]. González et al. found that a single METH injection caused increased total H3 acetylation

and decreased H4 acetylation, while repeated METH injections induced decreased levels of total H3 and H4 acetylation in the mPFC[28, 29]. Our present results showed that METH-induced behavioral sensitization caused a large number of genes to have H3 hyperacetylation and few with H4 hyperacetylation, while more H4 than H3 hyperacetylation was induced by acute METH exposure in the PFC.

The present study found METH-induced behavioral sensitization led to a change in the mRNA transcription of the PFC, as manifested by the large number of differentially expressed genes categorized by GO and KEGG pathway enrichment, including cell adhesion, the PI3K-AKT signaling pathway, olfactory transduction, and the cell cycle, compared with METH acute treatment control. In fact, numerous studies have shown that exposure to drugs, such as cocaine and morphine, can induce cell adhesion[30] and intracellular signaling pathways, such as PI3K-AKT[31] activation and the prolactin signaling pathway[32], in mediating addiction effects. For the genes identified and confirmed in this study by qPCR, there are some potential impacts on behavior. We found that some genes for molecules involved in behavioral sensitization decreased (e.g., *Stx2*, *Zfp36* and *Trim17*) and some increased (e.g., *Anp32a*, *Camk2n1*, *Eml2* and *Pou3f2*) at the mRNA level induced by METH challenge. These genes include not only transcription factors but also genes for molecules involved in neuron differentiation, synaptic plasticity and intracellular signaling. *Stx2* was reported to play a role in CNS damage[33-35]. *Stx2* interacts with neurons and promotes structural changes in the cell membrane that may be indicative of the synaptic vesicle cycle[36]. *Stx2* induces the PI3K-involved signaling pathway to release glutamate, which contributes to CNS impairment[37]. *EML2* encodes echinoderm microtubule-associated protein-like 2 protein, which plays a role in the formation of the mitotic spindle and interphase microtubule network[38]. Some studies found that the *EML2* methylation status is different among individual cell types in brain tissue[39], suggesting that *EML2* is important in cell type differentiation in the brain[40].

We further identified *ANP32A* and *POU3F2* by qPCR and H3/H4 acetylation measurements. *ANP32A* is mostly localized in the nucleus and is also present on the endoplasmic reticulum as well as the cytoplasm[41]. To date, *ANP32A* has been found to play a role in a variety of cellular functions, including RNA transport, transcription, cell-mediated cytotoxicity, and tumor suppression[42-44]. In the CNS, *ANP32A* contributes to the survival and differentiation of neurons[45-47], as well as synaptic plasticity and memory, in aged mice or tau transgenic mice used as a model of Alzheimer's disease. In the present study, we found that *ANP32A* mRNA was increased by acute or chronic treatment with METH, as well as METH challenge-induced behavioral sensitization. The ChIP assay showed a robust induction of the acetylation of H4 at the promoter of *ANP32A* caused by chronic METH use or challenge-induced behavioral sensitization but not by acute METH treatment. The fact that H4 hyperacetylation was not affected by acute METH exposure but was potentially elevated in the development and challenge stages of METH-induced behavioral sensitization suggested that enhancement of H4 acetylation in *ANP32A* may have an important role in modulating the motivation for METH reinforcement.

POU3F2 (also known as *BRN2*), a class III POU protein[48], is prominently expressed in the neocortex and found to be upregulated in the progenitor cells of the subventricular zone, intermediate zone and outer

layer of the neocortex in the early stages of embryonic brain development[49, 50]. It has been reported that POU3F2 contributes to neural formation and cell fate determination and regulates cortical neural migration, neurogenesis and positioning of cortical neurons[49, 51]. Furthermore, POU3F2 has been suggested to regulate tyrosine hydroxylase and tryptophan hydroxylase 2, the rate-limiting enzymes of dopamine and 5-HT[52], both of which are important for the regulation of psychological and physiological functions. POU3F2^{Δ/Δ} mice in which all three homopolymeric amino acid repeats were deleted from the POU3F2 transactivation domain displayed cognitive impairment in object recognition and object location tests[53], suggesting that POU3F2 is involved in cognitive function. In the present study, we found that POU3F2 mRNA was upregulated by METH exposure, including acute or chronic METH treatment, and METH challenge-induced behavioral sensitization. However, when METH was withdrawn after chronic treatment, POU3F2 mRNA was downregulated instead. It is noted that histone acetylation at the promoter of POU3F2 showed a specific change during the course of METH treatment, which manifested as both H4 and H3 being hyperacetylated only in response to METH-induced behavioral sensitization but not acute, chronic METH treatment or withdrawal. The change in the patterns suggests that histone acetylation of POU3F2 is involved in the mechanism of neuroplasticity underlying the transition from recreational METH use to compulsive use. Moreover, it has been reported that POU3F2 is involved in schizophrenia[54, 55], the syndrome and pathology of which are very similar to METH addiction. Therefore, we speculated that POU3F2 could be one of the common genetic bases underlying schizophrenia and METH use.

Conclusion

We identified the changes in gene expression and the diverse alterations in histone acetylation in the PFC after the different phases of METH-induced behavioral sensitization. By further analyzing histone acetylation binding to the promoters of some genes (e.g., ANP32A and POU3F2), we revealed that histone modifications could participate in the transcriptional changes in gene expression caused by the specific manners of METH treatment, which further proved the epigenetic mechanisms of gene regulation underlying the lasting changes induced by drugs. Meanwhile, the present results indicated that METH-induced histone acetylation might be necessary but not sufficient to induce changes in gene expression. In the present study, the function of the genes of interest in the brain was not explored. The transcription and histone acetylation of the genes associated with functions in specific brain regions may offer new knowledge to elucidate the neurobiological mechanism underlying METH addiction and develop alternative pharmacological methods for treating psychiatric disorders, which should be extensively studied in the near future.

Declarations

Ethics approval and consent to participate

All animal experimental procedures were performed in accordance with the NIH Guide for the Care and Use of Laboratory Animals (8th edition) and the guidelines of the National Research Council ([2006] 398,

China).

Consent for publication

Not applicable.

Availability of data and materials

The datasets supporting the conclusions of this article are included within the article and its additional files.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

JL, RBS and FL conceived and designed the project. HL, JAC, GYL, FL and NW performed the experiments. HL, QZD and NW analyzed the data. HL and FL wrote the manuscript. JL revised the paper. All authors read and approved the final manuscript.

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Abbreviations

METH: methamphetamine; ChIP: chromatin immunoprecipitation; NAc: nucleus accumbens; PFC: prefrontal cortex; NS: normal saline; GO: Gene Ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes; ANP32A: Acidic leucine-rich nuclear phosphoprotein-32A; POU3F2: POU domain, class 3, transcription factor 2.

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Figures

Figure 1

METH-induced behavioral sensitization. (A) Scheme of the behavioral sensitization training. (B) Total or time-dependent locomotion of METH-induced behavioral sensitization in 60 min. Animals were injected once daily with METH (5 mg/kg, s.c.) followed by 7 days of withdrawal, and challenge was induced by single METH (1.0 mg/kg, s.c.) administration. Data are expressed as the means $\pm$ SEM, $n = 8$ per group.

***P<0.001 and *P<0.05 versus NS-NS. #P<0.05 versus NS-METH. One-way ANOVA followed by Tukey's post hoc test. NS: 0.9% saline. METH: methamphetamine.

Figure 2

Heat map and Venn diagram of genes by analysis of PFC samples from METH-induced behavioral sensitization. (A) The heat map represents the expression values of a panel of different groups in METH-

induced behavioral sensitization. (B) The Venn diagram depicts the overlap of genes identified in groups in METH-induced behavioral sensitization.

Figure 3

Functional enrichment of differentially expressed genes by GO (A) and KEGG (B) analysis. The horizontal axis represents the enrichment ratio.

Figure 4

Venn diagram of genes by analysis of PFC samples from METH-induced behavioral sensitization. The Venn diagrams show the overlapping genes marked by each of the increased histone H3/H4 acetylation modifications by different METH administration paradigms.

Figure 5

Confirmation of gene expression changes by real-time PCR in the PFC samples from METH-induced behavioral sensitization. METH-induced sensitization rats were challenged by METH single injection. Data are expressed as the means $\pm$ SEM, $n = 6-9$ per group. * $P < 0.05$, ** $P < 0.01$, one-way ANOVA followed by Tukey's post hoc test.

A ANP32A

B ANP32A

C POU3F2

D POU3F2

Figure 6

Validation of mRNA expression and H3/H4 histone acetylation modifications in ANP32A (A, B) or POU3F2 (C, D). The results of real-time PCR and ChIP-PCR revealed the changes in ANP32A and POU3F2 in the different phases of METH-induced behavioral sensitization. Data are expressed as the means $\pm$ SEM, $n = 9-10$ per group for testing ANP32A and 14 per group for testing POU3F2. * $P < 0.05$, ** $P < 0.01$, one-way ANOVA followed by Tukey's post hoc test for the challenge test and t-test for the development or withdrawal test.

Supplementary Files

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