LETTER TO THE EDITOR

Methamphetamine Induces Dynamic Changes of Histone Deacetylases in Different Phases of Behavioral Sensitization

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In recent years, abuse of amphetamine-type stimulants (ATS) has become a severe problem to the whole world, and methamphetamine (METH) has accounted for the majority percent of global ATS seizures. It has been confirmed that the changes of histone acetylation involve in the regulation of METH addiction [1]; however, the effects of METH on histone deacetylase (HDAC) are poorly understood. Behavioral sensitization is an important animal model to reflect the neurological changes in drug addiction [2]. It has been confirmed that the prefrontal cortex (PFC) is related with the circuitry of sensitization [3]. The development of sensitization may involve excitatory amino acids projections originating in the PFC [3], and the expression of sensitization to systemic cocaine was blocked after the PFC lesion [4]. This study aimed to investigate the activity and expression changes of HDACs in the PFC of rats with METH-induced behavioral sensitization.

The model of behavioral sensitization was established by 7 days' METH (5 mg/kg, s.c., once daily) administration followed by 7 days' withdrawal, and challenged by single METH (1 mg/kg, s.c.) on the 15th day (Figure 1A). When challenged by METH, rats in METH-METH group showed significantly higher locomotor activity than the NS-METH group ($F_{2,12} = 4.859$, $P < 0.01$, Figure 1B), indicating the enhanced locomotor response of sensitization.

This study found that the expressions of both HDAC1 and HDAC2, two isoforms of Class I HDACs, decreased after acute METH treatment ($P < 0.05$, Figure 2A), while the total HDACs activity did not change. Kennedy et al. [5] reported that HDAC1 knockdown inhibited the development of cocaine-induced behavioral sensitization, which suggested that HDAC1 played an important role in the induction of behavioral sensitization, and our data further proved the hypothesis.

Long-term treatment with METH produced histone hyperacetylation [1], but it is not clear that the relationship between HDACs

Figure 1 METH-induced behavioral sensitization. **(A)** Scheme of the behavioral training. **(B)** Total locomotion of METH-induced behavioral sensitization in 60 min. Animals were injected once daily with METH (5 mg/kg, s.c.) in METH-METH group or saline (1 mL/kg, s.c.) in NS-NS and NS-METH group for 7 days and were challenged with METH (1 mg/kg, s.c.) for NS-METH and METH-METH group or saline (1 mL/kg, s.c.) for NS-NS group after 7 days' withdrawal, the locomotor activity in the 60 min phase following challenge was recorded. * $P < 0.05$ versus NS-NS, *** $P < 0.001$ versus NS-NS, ** $P < 0.01$ versus NS-METH. NS: 0.9% saline, METH: methamphetamine, one-way ANOVA followed by a post hoc *t*-test.

Figure 2 The mRNA expressions and total activity of HDACs in rat PFC during different phases of METH-induced behavioral sensitization. **(A)** The mRNA expressions and total activity of HDACs after acute METH (5 mg/kg, s.c.) or saline (NS, 1 mL/kg, s.c.) administration. **(B)** The mRNA expressions and total activity of HDACs after 7 days' METH (5 mg/kg, s.c.) or saline (1 mL/kg, s.c.) administration in the development phase. **(C)** The mRNA expressions and total activity of HDACs after 7 days' METH (5 mg/kg, s.c.) or saline (1 mL/kg, s.c.) administration followed by 7 days' withdrawal. **(D)** The mRNA expressions and total activity of HDACs after 7 days' METH (5 mg/kg, s.c.) or saline (1 mL/kg, s.c.) administration followed by 7 days' withdrawal, and challenged on the 15th day with METH (1 mg/kg, s.c.) or saline (1 mL/kg, s.c.). NS-NS: saline (1 mL/kg, s.c.) for 7 days, and challenged by saline (1 mL/kg, s.c.) on the 15th day. NS-METH: saline (1 mL/kg, s.c.) for 7 days, and challenged by METH (1 mg/kg, s.c.) on the 15th day. METH-METH: METH (5 mg/kg, s.c.) for 7 days, and challenged by METH (1 mg/kg, s.c.) on the 15th day. Mean $\pm$ SEM, $n = 4-6$, * $P < 0.05$ versus NS or NS-NS, ** $P < 0.01$ versus NS. NS: 0.9% saline, METH: methamphetamine, t -test or one-way ANOVA followed by a post hoc t -test.

and METH-induced behavioral sensitization. Our study showed that total HDACs activity was downregulated in the development phase of behavioral sensitization ($P < 0.05$, Figure 2B), which provided direct evidence that HDACs involved in the elevated histone acetylation. In consistent with the decreased HDACs activity, the mRNA of HDAC2 and HDAC4 was downregulated ($P < 0.05$, Figure 2B). Single 20 mg/kg METH increased the protein level of HDAC2 in the nucleus accumbens (NAc) [6], while the HDAC2 mRNA decreased in PFC after acute and 7 days' administration in this study. The difference between NAc and PFC further proved that each subregion of brain played a unique role in addiction.

After withdrawal of METH, the total HDACs activity was upregulated ($P < 0.05$, Figure 2C); however, the mRNA of HDAC2 was recovered back to normal level and HDAC4/5, two isoforms of Class II HDACs, was decreased ($P < 0.05$, Figure 2C). The opposite changes in the mRNA expression level of HDACs and the activity of HDACs suggested that HDAC 4/5 mRNA showed an adaptive response of the increased activity of HDACs to maintain the homeostasis of the histone hyperacetylation. Similarly, the total

HDACs activity was increased to about 1.84-fold of the NS-NS group ($F_{2,8} = 5.672$, $P < 0.01$, Figure 2D), while HDAC5 still decreased after challenge (vs. NS-NS, $F_{2,6} = 4.086$, $P < 0.05$, vs. NS-METH, $F_{2,6} = 6.565$, $P < 0.01$, Figure 2D), which were different to the trend of HDAC1/2. Therefore, HDAC4/5 exerted different, even opposite effect on drug addiction compared with Class I HDACs. The difference was confirmed in several previous references. Cocaine-induced conditioned place preference could be inhibited by over-expression of HDAC4 or HDAC5 in the NAc [7]. On the contrary, cocaine addiction was attenuated by inhibition of HDAC1 or HDAC2 activity [5]. Therefore, the different changes of HDAC4/5 and HDAC1/2 might reflect their opposite effect on drug addiction. HDAC5 was the only isoform that did not change after acute METH treatment, but kept decreasing after drug withdrawal as well as low dose METH challenge. It can be concluded that the change of HDAC5 mRNA was a reaction to the METH-induced behavioral sensitization instead of the instantaneous change to acute METH treatment. Several studies have demonstrated the role of HDAC5 in drug addiction. Overexpression of

HDAC5 attenuated the behavioral adaption to chronic cocaine treatment, but not acute treatment [7]. Knockout of HDAC5 promoted the hypersensitive responses, which was completely reversed by re-expression of HDAC5 in NAc [7]. Our present study further suggested the special role of HDAC5 in METH-induced behavioral sensitization.

Administration of HDAC inhibitors (HDACis) increased histone acetylation in the central nervous system and was supposed to modulate drug addiction [8,9]. However, different HDACis were reported to have different, even opposite effects on drug addiction. Valproic acid (VPA), the Class I and Class II HDACi, inhibited the development of behavioral sensitization induced by METH [8]; however, sodium butyrate (SB), another kind of Class I and Class II HDACi, led to the enhancement of behavioral sensitization [9]. The mechanism for the different effects of HDACis has not been well understood, but one study suggested that drug addiction may induce the expression of different transcription factors, which was modified by different HDACis [10]. Our present study found that METH induced different changes on the mRNA levels of Class I and Class II HDACs in the behavioral sensitization session, and again inferred that Class I and Class II HDACs might exert different effects. Different HDACis, such as VPA and SB, might have

different binding affinities with Class I and Class II HDACs, therefore produced opposite effects on the development of behavioral sensitization.

In summary, the present study found that HDAC activity in PFC was dynamically regulated in METH-induced behavioral sensitization. From acute to challenge, HDACs' mRNA showed a shift from Class I HDACs to Class II HDACs. Class I HDACs appeared to contribute to the decreased HDAC activity in the development phase, while Class II HDACs exerted the adaptive changes to the increased HDAC activity after withdrawal of METH. Therefore, our finding further demonstrated that HDAC played an important modulated role in METH addiction.

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Conflict of Interest

The authors declare no conflict of interest.

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