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Research report

Learning induced epigenetic modifications in the ventral striatum are necessary for long-term memory

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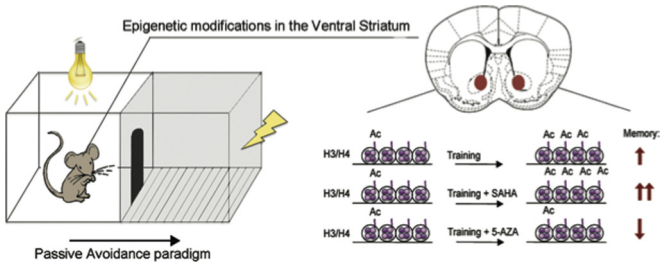
HIGHLIGHTS

- Passive avoidance training increases histone acetylation in the ventral striatum.
- The histone deacetylase inhibitor, SAHA, in the ventral striatum improves memory.
- The DNA methyl transferase inhibitor, 5-AZA, in the ventral striatum impair memory.
- SAHA, and 5-AZA have opposite effects on training induced acetylation in the striatum.
- Histone acetylation in the ventral striatum is necessary to store aversive memories.

GRAPHICAL ABSTRACT



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ABSTRACT

Epigenetic modifications such as histone acetylation in cortical or allocortical regions have been shown to be necessary for the formation of long-term memories. Here we investigated whether similar changes were occurring also in the ventral striatum and whether they are necessary for the consolidation of aversive memory. To this purpose we performed immediate post-training focal administrations of the histone deacetylase inhibitor suberoylanilide hydroxamic acid (SAHA, 5, 10 or 15 μg/side) or the DNA methyltransferase (DNMT) inhibitor, 5-aza-2′-deoxycytidine (5-AZA, 0.0625 or 0.125 μg/side) in the ventral striatum of mice trained in one-trial inhibitory avoidance task. Intra-ventral striatal SAHA administrations, immediately after training, improved memory retention. Opposite effects were found with 5-AZA. We also found that training in the one-trial inhibitory avoidance is accompanied by increased acetylation of specific residues that can be further increased by intra-VS SAHA administrations. Intra-VS 5-AZA administrations on the other hand reduced training-induced histones acetylation at the same residues. These findings imply the occurrence of histone acetylation in the ventral striatum in order to store aversive memory. Moreover, they suggest that the effects induced by the DNMT inhibitor 5-AZA may at least

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partially due to blockade of H3 and H4 acetylation. These results suggest that the contemporary activation of similar molecular mechanisms might be needed in different brain regions to enable the formation of long-term memories.

1. Introduction

Formation of long-term memories (LTM) is believed to involve a dynamic process by which a labile memory is progressively converted into a more stabile and potentially permanent trace. This process begins with neurotransmitter receptor activation that induces short-term changes in synaptic efficacy based on receptor phosphorylation and trafficking [1]. Subsequently, alterations in gene expression and protein synthesis occur that are the basis for long-term structural modifications [2]. However, given the timescale of protein turnover there has been an extensive search for gene regulatory mechanisms that can be triggered by transient environmental stimuli, but persist over the extended timescale associated with the enduring, potentially lifelong, learning induced neural adaptations. Histone modifications, particularly acetylation and DNA methylation, are major epigenetic modifications most intensively studied as key regulators of transcription in pathological events such as oncogenic processes and possess all the biochemical properties necessary for the long-term maintenance of information. Histone acetylation is regulated by the enzymes histone deacetylase (HDAC) and histone acetyltransferase (HAT), and relaxes chromatin structure making it accessible to transcriptional machinery [3]. Conversely, DNA methylation, catalyzed by DNA methyl transferases (DNMT), has generally been associated with transcriptional repression [4]. Interestingly these marks are dynamically regulated and despite there is not a general consensus in the literature on which epigenetic mechanism initiate the communication to modulate gene transcription an interplay between histone acetylation and DNA methylation is supported by experimental evidence [5,6].

In the last decade a growing body of evidence has been accumulating supporting an active role of epigenetic processes in learning driven regulation of gene transcription in post-mitotic brain cells [7–22]. For example, mice carrying a mutation to the CREB binding protein (CBP), acting also as a HAT, show reduced histone 3 (H3) acetylation paralleled by impaired memory for objects and reduced fear conditioning [7]. Inhibition of HDAC, on the other hand, improves memory in fear conditioning [8], and object recognition [11,16]. Interestingly, in most of these studies while H3 acetylation is placed in a causal relationship with memory improvement, the role for histone 4 (H4) acetylation seems more controversial [8,10,12,19]. Although less evidence is available, DNA methylation has also been shown important in modulating synaptic plasticity and memory formation [10,19,23]. The effects of DNA methylation in memory formation, however, seem opposite to what expected. In fact, though it is generally associated with transcriptional repression, experimental findings demonstrate learning induced increase of DNMTs levels after fear conditioning training and coherently memory impairing effects of DNMTs inhibition [10,19].

An important issue in the investigation of memory consolidation is the neuroanatomical site at which these processes occur. The ventral striatum (VS) is a structure located in the basal forebrain that receives excitatory inputs from prefrontal cortex, hippocampus, thalamus, and amygdala, as well as a major dopaminergic innervation from the ventral tegmental area [24–26]. Because of the rich dopaminergic innervation, this structure has been generally associated with modulation of locomotor activity and learning controlled by appetitive stimuli [27,28]. In more recent years, a role of this brain region in learning and memory, has also been envisaged by many studies focusing on appetitive learning [29–32]. Nevertheless, the VS has also been implicated in learning controlled by aversive stimuli. This is demonstrated, for example, by the effects induced by temporary inactivation of this structure in cue and contextual fear condition [33,34] or on one-trial inhibitory avoidance [35–38]. Interestingly recent studies demonstrated the occurrence of epigenetic modifications, such as altered histones acetylation, and DNA methylation, within VS after cocaine self administration [39], acquisition and extinction of cocaine induced place preference [14,18,21,22,40], supporting the view that consolidation of appetitive learning might depend upon changes in gene expression profiles within this structure [18,41–43]. Available evidence on the involvement of epigenetic modifications in the memorization of information acquired in tasks controlled by aversive stimuli generally focused on the hippocampal formation [8,10], the cerebral cortex [44] and the amygdala [19]. Therefore in this study we investigated the occurrence of one-trial inhibitory avoidance induced epigenetic modifications in the VS and their possible causal role in memory stabilization through histone acetylation. Moreover, in light of the described interplay between histone acetylation and DNA methylation we verified also whether the effects DNMT inhibition on learning could be partially explained by histone acetylation changes.

2. Materials and methods

2.1. Subjects

The subjects were adult CD1 male outbred mice obtained from Charles River Laboratories (Calco, Italy). They were about 7 weeks old at the time of arrival and their weight was between 35 and 40 g. Immediately after the arrival mice were housed in groups of five in standard breeding cages (26 cm × 20 cm × 14 cm) and adapted to vivarium conditions for at least 1 week before surgery. They were maintained at a constant temperature (22 ± 1 °C) on a 12-h/12-h light-dark cycle (lights on at 07:30 h), with food and water available ad libitum. The behavioral tests were performed during the first part of the light period (between 09:30 and 12:30 h).

Every possible effort was made to minimize animal suffering, and all animal procedures were in strict accordance with European community and Italian national laws and regulations of the use of animals in research as well as NIH guidelines on animal care.

2.2. Surgery

Immediately prior to surgery animals were anaesthetised with chloral hydrate (500 mg/kg, i.p.; Fluka) and placed in a stereotaxic apparatus (David Kopf Instruments, USA) with a mouse adapter and lateral ear bars. The head skin was cut longitudinally and bilateral guide cannulae (7 mm in length, 0.5 mm in diameter) were fixed on the calvarium with acrylic cement (Riccardo Ilic, Italy). Ventral striatum coordinates were: anterior to bregma, +1.6 mm; lateral to midline, ±1 mm; ventral from the dura, −2.3 mm, according to the mouse atlas [45]. Mice were left in their home cages for at least 1 week before all behavioral tests in groups of four in standard breeding cages (26 cm × 20 cm × 14 cm).