Journal of Physiology - Paris 108 (2014) 278–285

Contents lists available at ScienceDirect

Journal of Physiology - Paris

journal homepage: www.elsevier.com/locate/jphysparis

Review Paper

Epigenetic mechanisms and memory strength: A comparative study

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ARTICLE INFO

Article history:
Available online 28 June 2014

Keywords:
Long-term memory
Memory persistence
Chromatin modifications
Histone acetylation
Gene expression

ABSTRACT

Memory consolidation requires *de novo* mRNA and protein synthesis. Transcriptional activation is controlled by transcription factors, their cofactors and repressors. Cofactors and repressors regulate gene expression by interacting with basal transcription machinery, remodeling chromatin structure and/or chemically modifying histones. Acetylation is the most studied epigenetic mechanism of histones modifications related to gene expression. This process is regulated by histone acetylases (HATs) and histone deacetylases (HDACs). More than 5 years ago, we began a line of research about the role of histone acetylation during memory consolidation. Here we review our work, presenting evidence about the critical role of this epigenetic mechanism during consolidation of context-signal memory in the crab *Neohelice granulata*, as well as during consolidation of novel object recognition memory in the mouse *Mus musculus*. Our evidence demonstrates that histone acetylation is a key mechanism in memory consolidation, functioning as a distinctive molecular feature of strong memories. Furthermore, we found that the strength of a memory can be characterized by its persistence or its resistance to extinction. Besides, we found that the role of this epigenetic mechanism regulating gene expression only in the formation of strongest memories is evolutionarily conserved.

Contents

1. Introduction

Regulation of gene expression is a key process for long-term memory (LTM) storage. During LTM consolidation, the expression of a set of genes leads to proteins synthesis, an important process for the regulation of synaptic function that underlies memory. Macromolecules synthesis induces changes in the morphology of synapses involved and/or genesis of new synapses in the memory trace (Montarolo et al., 1986; Glanzman et al., 1990). Some transcription factors (TFs), such as cyclic AMP responsive element binding protein (CREB) (Kaang et al., 1993; Yin and Tully, 1996), zinc finger inducible factor (ZIF/268) (Tischmeyer and Grimm, 1999; Davis et al., 2003), CCAAT enhancer binding protein (C/EBP) (Alberini et al., 1995; Taubenfeld et al., 2001) and the nuclear factor κappa B (NF-κB) (Romano et al., 2006), have been involved in memory consolidation. Among them, CREB and NF-κB are considered key synapse-nucleus signaling molecules in the induction of gene expression during LTM formation (Alberini, 2009). These two TFs are rapidly activated after learning, regulating the transcription of early and late genes during memory consolidation.

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http://dx.doi.org/10.1016/j.jphysparis.2014.06.003
0928-4257/

The magnitude and the extent of the gene expression pattern induced by learning could be regulated by epigenetic mechanisms (Barret and Wood, 2008). The genome of all cells is packaged into a structure called chromatin, comprising deoxyribonucleic acid (DNA) and proteins that are associated to it at different levels, performing the compaction of chromatin structure and generating its different degrees of packing. Epigenetic marks are known as those modifications in chromatin structure which affect transcription of genes. These marks may be post-translational modifications (PTMs) of nucleosomal histones, such as acetylation, phosphorylation, ubiquitination and methylation, as well as changes at the methylation patterns of DNA cytosine residues. Other epigenetic mechanisms include histone variants incorporation to nucleosomes, nucleosome remodeling, and changes in the position of the chromosome in relation to pores in the nuclear envelope (Raisner and Madhani, 2006, Kundu and Peterson, 2009; Draker and Cheung, 2009). All these epigenetic processes occur in an interdependent and coordinated manner, in order to regulate the organization of the various functional genomic microdomains (Mehler, 2008, for a review).

Chromatin-modifying enzymes that carry out acetylation and deacetylation of histones are the histone acetyl transferases (HATs) and deacetylases (HDACs), respectively (Sterner and Berger, 2000). Histone acetylation is generally associated with transcriptional activation, and histone deacetylation with transcriptional repression. The involvement of epigenetic mechanisms such as histones acetylation, phosphorylation and methylation has been described in neuronal plasticity processes in invertebrates and long-term memory consolidation in vertebrates (Guan et al., 2002; Alarcon et al., 2004; Korzus et al., 2004; Levenson et al., 2004; Wood et al., 2005, 2006a; Gupta et al., 2010; Gupta-Agarwal et al., 2012). For example, histone H3 acetylation in the hippocampus has been associated with the formation of conditioned fear memory in rodents (Levenson et al., 2004; Bredy and Barad, 2008; Lubin et al., 2008). The CREB binding protein (CBP) is one of the most studied HAT and it was demonstrated as a chromatin structure regulator during memory consolidation in vertebrate models (Alarcon et al., 2004; Korzus et al., 2004; Oliveira et al., 2007; Vecsey et al., 2007). Some studies showed that genetic disruption of CBP and other HATs activity interferes with memory formation (Alarcon et al., 2004; Korzus et al., 2004; Oliveira et al., 2007; Maurice et al., 2007). Furthermore, it has been demonstrated that inhibition of HDACs activity facilitates memory in rodent models (Yeh et al., 2004; Levenson et al., 2004; Vecsey et al., 2007; Fischer et al., 2007; Stefanko et al., 2009), and it also reverses memory deficits induced by genetic engineering into the *cbp* gene (Alarcon et al., 2004; Korzus et al., 2004; Guan et al., 2009). In contrast, inhibition of HAT activity with drugs has proven challenging, as most inhibitors generated to date cannot be used *in vivo* due to their cell impermeability and/or metabolic instability (Dal Piaz et al., 2010). Some evidence has shown that pharmacological inhibition of p300/CBP impaired memory enhancement by estradiol (Zhao et al., 2012), impaired memory formation (Federman et al., 2013), and enhanced memory extinction (Marek et al., 2011).

The PTMs of histones and chromatin remodeling have been implicated in a wide variety of functions in the nervous system (Bhaumik et al., 2007; Blasco, 2007; Feng et al., 2007; Hsieh and Gage, 2004; Kondo, 2006; McCarthy et al., 2009; Mikkelsen et al., 2007; Ooi and Wood, 2007; Shi et al., 2007; Taniura et al., 2007; Tsankova et al., 2007). The involvement of epigenetic mechanisms in memory formation has been postulated as a continuous supply of gene expression. Their regulation is specifically required for maintaining neuronal long-term changes induced by learning, providing potentially stable marks in the genome (Tsankova et al., 2004; Kumar et al., 2005; Hsieh and Gage, 2005; Barret and Wood, 2008; Levenson and Sweatt, 2006; Colvis et al., 2005; Borrelli et al., 2008). Through these control mechanisms, generation of stable changes in gene expression pattern during memory consolidation could be an important mechanism for its stability (Alberini, 2009). The existence of an *epigenetic code* involved in memory formation has already been proposed, by means of which specific patterns of histones PTMs and DNA methylation contribute to encode the salience of extra and intracellular signals and its contingence (Wood et al., 2006b; Roth and Sweatt, 2009). This *epigenetic code* hypothesis for memory stems from the original idea of a *histone code* proposed by Allis (Jenuwein and Allis, 2001), but it also includes DNA methylation (Roth and Sweatt, 2009; Day and Sweatt, 2011). In this context, *epigenetics* comprises the covalent modifications of chromatin that influence in gene expression, which are induced by neuronal activity and are necessary for cognition. In the last decade, an increasing amount of evidence has begun to shed light on the role of such processes in the encoding, storage and retrieval of acquired information during learning (Peleg et al., 2010; Lesburguères et al., 2011; Gräff et al., 2012). Here we review our work in both invertebrates and vertebrates on the critical role of the histone acetylation in long-term memory.

2. Histone acetylation in context-signal memory: a case in invertebrates

We began our study in the grapsid crab *Neohelice granulata*. In the last 20 years, a considerable research effort has been focused on the study of the context-signal memory (CSM) in this model. In the CSM, repeated presentation of a visual danger stimulus (an opaque screen that moves above the animal) provokes the fading of the initial escape response, which is actively replaced by a freezing response (Lozada et al., 1990) (Fig. 1a). Fifteen or more spaced danger stimulus presentations (trials) induce an association between the iterated stimulus and contextual features (container, room light, etc). A LTM is formed, which lasts at least for a week and entails *de novo* protein and mRNA synthesis (Pedreira et al., 1996), activation of cAMP-dependent protein kinase (PKA) (Locatelli et al., 2002), activation of extracellular signal-regulated kinase (ERK) (Feld et al. 2005), and activation of the NF-kB transcription factor (Freudenthal and Romano, 2000; Merlo et al., 2002). Memory retention of the learning acquired during training is defined as a significantly lower mean response level at testing session of the trained group versus a control group that was not stimulated with the VDS during the training session (Fig. 1b). The memory retention at testing session is similarly evident in animals trained either with the standard (15 trials) or the strong (30 trials) protocols (Freudenthal and Romano, 2000). In contrast, weak protocol of five trials is unable to induce LTM formation (Romano et al., 1996) (Fig. 1b).

Using this invertebrate model, our group has focused the study on histone acetylation during memory consolidation and its relation with memory strength. For this purpose, we trained the animals with two different protocols, standard and strong trainings, using 15 and 30 trials, respectively. We found an increase in the level of histone H3 acetylation in the brain during consolidation only after a strong training protocol (Fig. 2). We also found that the memory induced by a strong training of 30 trials, in contrast to standard training memory, was resistant to extinction (Fig. 3, Federman et al., 2012). Memory extinction is the temporary inhibition of the response acquired during training, and the resistance to extinction is considered as indicative of memory strength (Tully and Quinn 1985; De Oliveira Alvares et al., 2013). Thus, our result showed that the strong training induced in fact a stronger LTM (sLTM).

Furthermore, when we trained the animals with a weak training protocol of 5 trials, pharmacological blockade of the action of