Epigenetics and Cognition: Histone acetylation in long term synaptic plasticity

**Preface:**

I was first interested in epigenetics in relation to memory, but the topic was very broad. Based on reference 1, there are multiple epigenetic mechanisms that influence memory formation including DNA methylation, post-transcriptional modification of histones, histone acetylation, histone methylation and chromatin remodeling. I decided to choose histone acetylation because I wanted to learn more about histone proteins, and histone acetylation was one of the most studied mechanisms in memory regulation. I specifically chose to research more about histone acetylation within the brain because I was interested in long term potentiation and memory.

I have learnt a little bit about histone acetylation from reference 1, which provides a simple and easy to understand background to histone acetylation. Histone acetylation is basically when an acetyl group is added to a lysine present on the N-terminus tail of the nucleosome. Histone acetylation is controlled by activities of HAT and HDAC. I was not sure what those were, so I had to do a quick search, where I found that HAT was short for histone acetyltransferases, and these enzymes catalyze the transfer of an acetyl group from acetyl CoA to the ε-amino on the histone lysine residue. HDAC was short for histone deacetylase which removes the acetyl group from the histone. Levels of gene expression can then be modified by targeting HAT or HDAC activities. (Reference 2)

Looking at reference 3, long term potentiation is a process of continuous strengthening of synapses that leads to long-lasting increase in synaptic transmission between neurons and is considered neural basis for long-term information storage in the brain—memory.

**Question:** What are the molecular pathways that lead to changes in histone acetylation specifically in memory formation?

* What methods were used to measure long term potentiation?
* What is the specific action of HAT and HDAC, what does it bind to?
* What are the experimental procedures?
* How did they determine that histone acetylation was involved in memory formation?
* How does histone acetylation promote transcription?

**Premise**

The main question draws out the first crucial question: how did researchers determine that histone acetylation was involved in memory formation? From reference 1, I found a citation that led me to a journal article on the regulation of histone acetylation during memory formation in the hippocampus. (reference 4) This seems relevant, as the investigation centered on whether mechanisms such as histone acetylation were activated during initial stages of memory formation in a fear-conditioning paradigm. The introduction of the paper brought up the activation of n-methyl-d-aspartate receptors and subsequent influx of calcium ions, leading to the activation of ERK (extracellular signal-regulated kinase). ERK then regulates gene expression by directly or indirectly regulating transcription factors.

This bit is not really important to my question, but it is interesting to know of. After I some searching, I found that ERK are protein kinases, which phosphorylate other proteins to modify them.

In reference 4, they used several ways to conclude that histone acetylation was involved in memory formation as follows:

1. Examining the structure of chromatin during long term memory formation in the hippocampus, specifically in the CA1 region after fear conditioning
2. Stimulation of a known memory-associated signaling pathway ERK through activation of protein kinase A or protein kinase C
3. Artificially elevating levels of histone acetylation in the hippocampus *in vivo*

The researchers used rats that were exposed to three consecutive electrical shocks and were then allowed to explore the training chamber. After that, the rodents were killed, and slices of their hippocampi were prepared. The researchers also performed other experiments concurrently such as latent inhibition to eliminate other reasons for the formation of memory. All above experiments carried out by the researchers indicated histone acetylation and was detected using antibodies (anti-Histone H3, anti-acetyl Histone H3, anti-acetyl Histone H4) in Western Blotting\*, and separating using gel electrophoresis. Long term potentiation was analyzed using data acquired before and after tetanic stimulation\*.

Results: Significant increases in the acetylation of histone H3 on Lys-14 in area CA1 in the hippocampus 1 hour after contextual fear conditioning.

Somewhat related findings from reference 5: histone deacetylases and histone acetyltransferases have new names, lysine deacetylases (KDAC) lysine acetyltransferases (KAT) respectively.

\*Western blotting is a laboratory technique used to detect specific protein molecules from among a mixture of proteins

\*Tetanic stimulation consists of high frequency sequencing of individual stimulations of a neuron

**Answering the Question**

Early studies discovered that the action of KDAC and KATS on histone acetylation is positively correlated to genome wide gene expression and memory formation. From reference 4, the use of KDAC inhibitors (tyrosine A, sodium butyrate) enhance long-term potentiation and memory formation in contextual fear conditioning. Because the use of KDAC inhibitors lead to memory enhancements, it seems that KDACs act as memory suppressor genes. There are several types of KDACs, and these have different functions. HDAC 1 inhibition is shown to be sufficient for memory enhancement. (reference 6) Studies have also shown that injections of sodium butyrate can facilitate long term memory for weaker stimuli and enhance persistence of long term potentiation. (reference 7) Hence, histone acetylation plays a role in memory formation.

Recognition of acetylated lysine during histone acetylation is more important than the charge itself. (reference 5) Acetylated histones are also able to serve as molecular tags.

Regulation of transcription necessary for synaptic plasticity and long-term memory is shown to be dependent on transcription factors cyclic AMP response element binding protein (CREB) as well as the NF-kB family. Several genes have been discovered to be targets, such as immediate-early genes. (reference 5) The effect of KDAC inhibitors is dependent on CREB and involve its interaction with CREB. (reference 8) It seems that CREB is a fundamental transcription factor for histone acetylation, and so I found this publication that focuses on CREB. CREB possesses intrinsic histone acetyltransferase activity.

The first study that linked CREB to long-lasting changes in neuronal functional plasticity was performed in the *Aplysia,* and a similar study was done as well in the *Drosophila.* CREB is mainly regulated through phosphorylation (though there are other CREB regulatory mechanisms) and binds to DNA sequences called cAMP response elements (CRE) which increase or decrease the transcription of the genes. Serine 133 is targeted by some kinases, including CaM dependent kinases, protein kinase A, etc. This kinase activity is initiated by increases in intracellular cAMP and Ca2+, which triggers phosphorylation of Serine 133. CREB then becomes a binding target of the KIX domain (CREB binding domain) in the CREB binding protein and p300, allowing induction of CRE-mediated transcription. (reference 9) (reference 10)

Many studies have been done to indicate the role of CREB in long term memory formation.

* CREB alpha/delta knockout mice showed impaired memory formation in contextual fear conditioning
* CREB shown to be involved in cued and contextual fear memory, various sensory-related memory

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