Epigenetic basis of alcoholism

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Introduction

Alcohol is one of the most widely addictive drugs within society, the continued abuse of which can result in tolerance and dependence. It is a psychiatric disorder with heterogeneous etiology. Incomplete phenotypic concordance between monozygotic twins suggests that environmental and epigenetic factors are influential in susceptibility to alcoholic disease (AD). It is reinforced by several studies that ethanol can induce some epigenetic alterations, particularly histone acetylation and methylation as well as DNA hipomethylation. This provides new insights into the actions of ethanol at nucleosome level in relation to gene expression and pathophysiological consequences.

Brain histone acetylation and deacetylation induced by ethanol

Table 1: Effects of ethanol in different nuclei of the amygdala

Treatment	HDAC	HAT (CBP)	H3 ac.	H4 ac.	Chromatin structure	Gene expression	p-CREB	NPY	Effect
Acute ethanol	↓	↑	↑	↑	Relaxed	Increased	↑	↑	Anxiolytic
Chronic ethanol	-	-	-	-	Normal	Normal	-	-	Normal anxiety levels
Withdrawal	↑	↓	↓	↓	Condensed	Decreased	↓	↓	Anxiogenic

HDAC: histone deacetylase
HAT: histone acetyltransferase
CBP: CREB binding protein
CREB: cAMP response element-binding
H3 ac : acetylated histone 3
H4 ac.: acetylated histone 4
NPY: Neuropetide Y

Figure 1

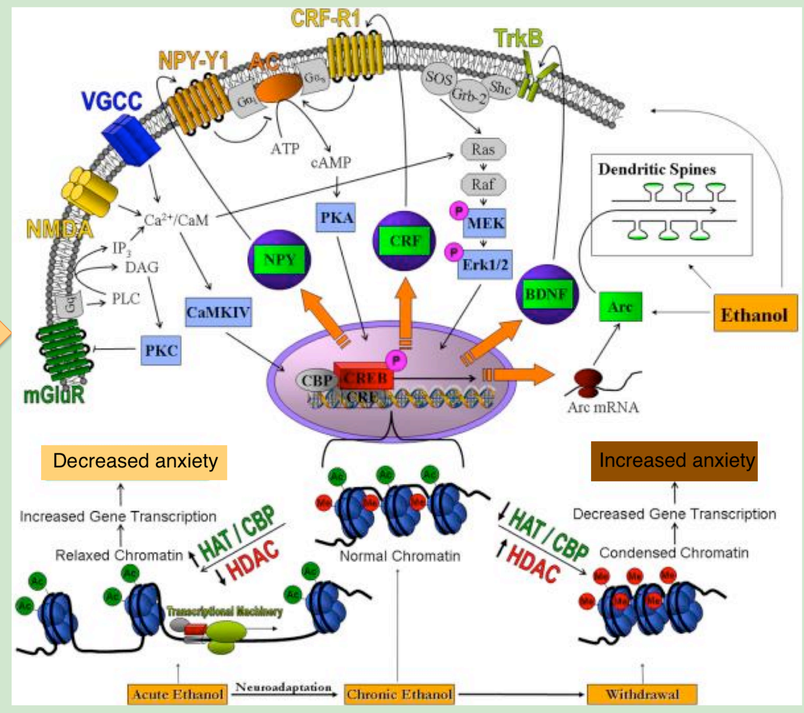
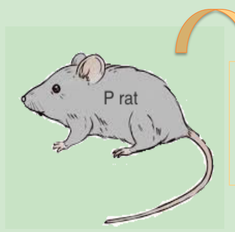


Figure 1: Acute ethanol exposure reduced HDAC activity and increased HAT activity by increasing CBP levels. Opposite effects would be observed in ethanol withdrawal. Figure modified from ref. 1



↓ pCREB
↓ NPY
↑ HDAC2
↓ H3K9 ac.

ANXIOGENIC EFFECTS → CONSUME ALCOHOL → ↓ HDAC, Pka ACTIVATION → ↑ p-CREB → ANXIOLYTIC EFFECTS

Figure 2: "P rats " are rats with preference for alcohol. They showed the protein levels described in Table 1 and had anxiety states that prompt them to consume alcohol in order to restore these protein levels and revert the anxiety states to anxiolytic states.

Histone acetylation in hepatocytes

- **Acute ethanol treatment**: increased acetylation of histone H3 at Lys 9 mediated by ROS, with an induction of ADH1 mRNA expression. **Acetate treatment also increased H3acK9**. Yet this acetylation could not simply be explained by acetate metabolism to acetyl-CoA (figure 3).
- **Chronic ethanol treatment**: did NOT change significantly H3acK9 global levels → "acute" and "chronic" exposure generate different patterns of global histone acetylation.
- **Ethanol metabolism**→ increased NADH/NAD⁺ ratio resulting in an inhibition of an HDAC, sirtuin 1 (Sirt 1) and promoting gene activation (figure 4).

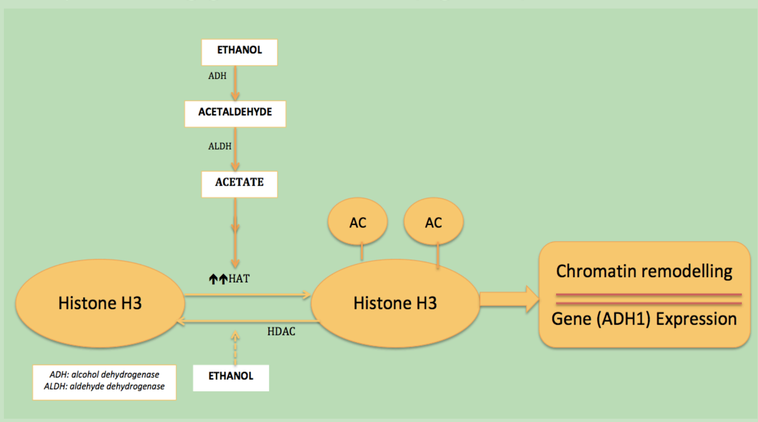


Figure 3: Ethanol increased accumulation of acetylated histones with the ADH 1 gene, suggesting that the increase of H3-Lys9 acetylation might be involved in the ethanol-induced ADH I gene expression. Figure modified from ref. 2

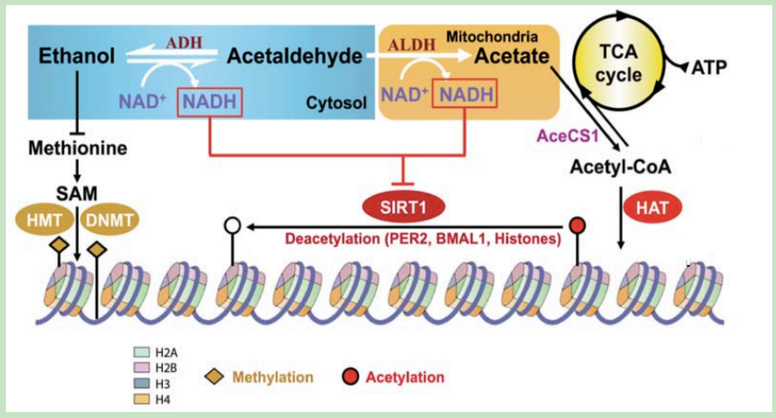


Figure 4: Ethanol metabolism increase NADH/NAD+ ratio dealing with the inhibition of SIRT1 and interfering with normal histone acetylation patterns. Figure modified from ref. 3.

Histone methylation in hepatocytes induced by ethanol

↓ H3me2K9
↑ H3me2K4
} promoter zone ADH1 & GSTYc2

↑ H3me2K9
↓ H3me2K4
} promoter zone Lsdh & CYP2CII

H3me2K4 : gene expression
H3me2K9: gene repression
ADH1: alcohol dehydrogenase
GSTYc2: glutathione-S Yc2-transferase
Lsdh: L-serine dehydratase
CYP2CII: cytochrome P450 2CII

Figure 5

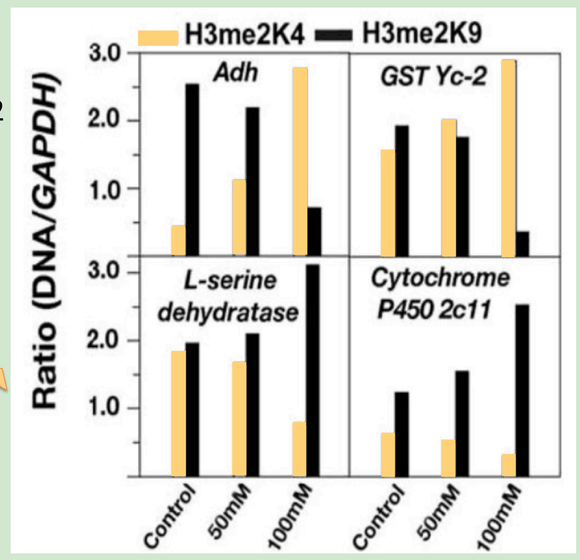


Figure 5: Graphs plotted from the results obtained using chromatin immunoprecipitation of hepatocytes treated with ethanol during 24 h and employing antibodies noted in the panel. Immunoprecipitated DNA contained promoter DNA sequence of two up-regulated (Adh-1 and GST-Yc2) and two down-regulated (CYP2C11, Lsdh) genes and a house keeping gene GAPDH (internal control). The DNA was amplified by semi quantitative PCR and further validated by real-time PCR analyses. Figure modified from ref. 4

- During early brain development **ethanol up-regulate G9a**, a methyltransferase that increase H3me2K9 and H3me2K21. These histone dimethylation patterns are reflected in a transcriptional silence of genes encoding survival factors (e.g. p-CREB), producing a delay in neonatal development (**FASD**).

Hyperhomocysteinemia caused by ethanol

- Alcoholics suffer from a chronic deficiency of micro-nutrients:
  - **Folate**
  - **Vitamin B6**
  - **Vitamin B12**

  } **HYPERHOMOCYSTEINEMIA** ↓ **HYPOMETHYLATION**

- **Ethanol metabolism**: excessive reactive oxygen species formation (ROS) which can deplete glutathione (GSH)→ re-methylate reaction to produce S-adenosylmethionine (SAM) is reverted → **DNA HYPOMETHYLATION** (figure 6).

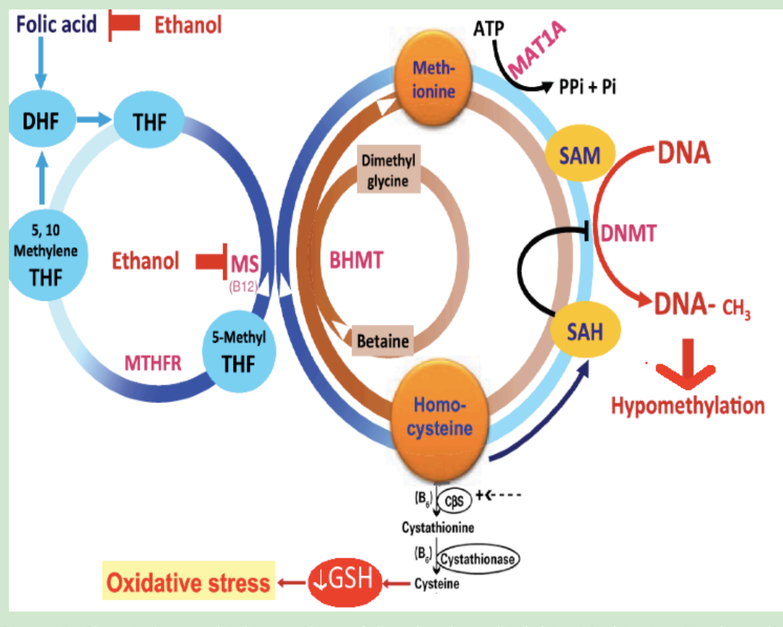


Figure 6: Chronic heavy drinking reduces folate levels and inhibits Methionine Synthase (MS), resulting in the reduction of methionine and SAM and a concurrent increase in homocysteine and S-adenosylhomocysteine (SAH). SAH, further inhibits DNA methyltransferases (DNMTs), ultimately resulting in global DNA hypomethylation . Figure modified from ref. 3

- Hypomethylation of the N2RB (N-methyl D-aspartate receptor subtype 2B) gene promoter region promoting CREB binding and increasing **NR2B gene transcription**.
- Acute ethanol administration to a pregnant mouse produces fetal DNA hypomethylation related with fetal abnormalities observed in fetal alcohol spectrum disorder (**FASD)**.

Conclusions

- In the amygdala, depending the ethanol-intake level, there is a regulation of both HAT(s) and HDAC(s) activities that could promote gene expression or repression within genes involved in the onset of different neurological effects.
- Ethanol produce different methylation and acetylation patterns in the promoter region of genes involved in ethanol metabolism, affecting in last instance to their regulation.
- Excessive ROS formation from ethanol metabolism and micronutrient deficiencies cause an hyperhomocysteinemia which could revert SAM formation resulting in a DNA hypomethylation as it has been observed in some genes.

It would be convenient to carry out an integrative study of different epigenetic mechanisms together to understand if there is a "crosstalk" between them, suggesting an "epigenetic code" that would be important to understand how gene regulation occurs. Taking all this knowledge together, it would be possible to start designing new therapeutic strategies in order to improve the pathophysiology of alcoholism.

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