The role of epigenetic regulation in Alcohol Use Disorders and co-occuring Alcohol Use and mood disorders

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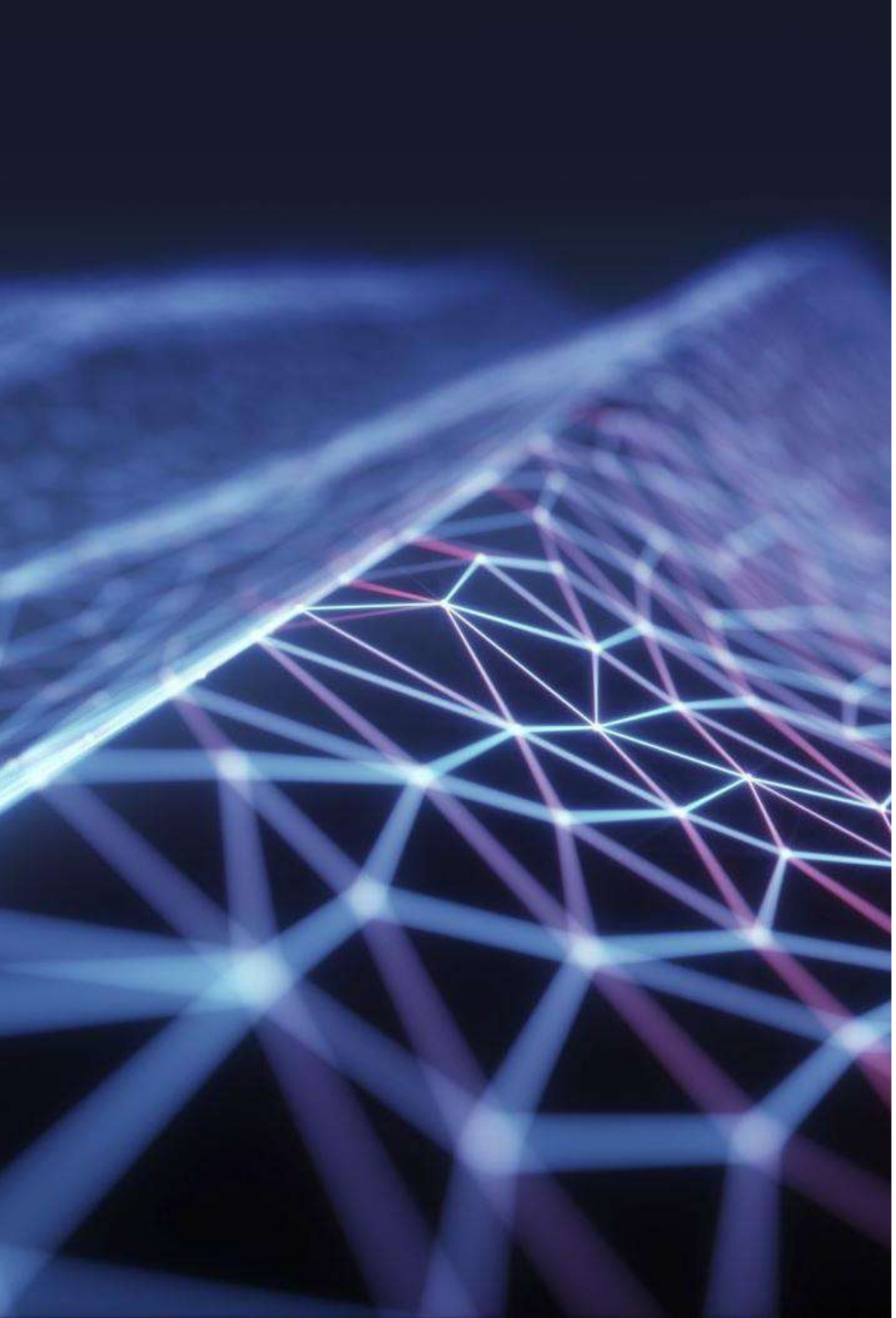
- 20000-30000 students
- 3500 medical students



Contents

- Definition of drug addiction
- Epigenetic
- Epidemiology of alcohol use disorders
- Animal models
- Comorbidity between Alcohol use and anxiety disorders
- Preclinical data: role of epigenetic modifications on alcohol and anxiety related behaviors

Café Table With Absinthe, Vincent van GoghVan Gogh Museum

Alcohol use disorders are defined by continued alcohol use despite negative psychological, biological behavioral and social consequences (DSM5, ICD 11)

Diagnosis and Statistical Manual (DSM-5) Criteria for a Substance Use Disorder

According to DSM-5, a substance use disorder may be an appropriate diagnosis when at least two of the following characteristics occur within a 12 months period and cause significant impairment or distress:

- The quantity of the substance used, or the amount of time spent using is often greater than intended;
- Efforts to control use of the substance, recovering from its effects, or attempting to obtain the substance;
- A strong desire, craving, or urge to use the substance is present;

Loss of control/Craving

- Substance use interferes with major role obligations at work, school, or home;
- Use of the substance continues despite harmful social or interpersonal effects caused or made worse by substance use;
- Participation in social, work, or leisure activities is avoided or reduced due to substance use;
- Substance use occurs in situations where substance use may be physically hazardous;
- Continued substance use occurs even when the substance is causing physical or psychological problems or making these problems worse;

Compulsivity

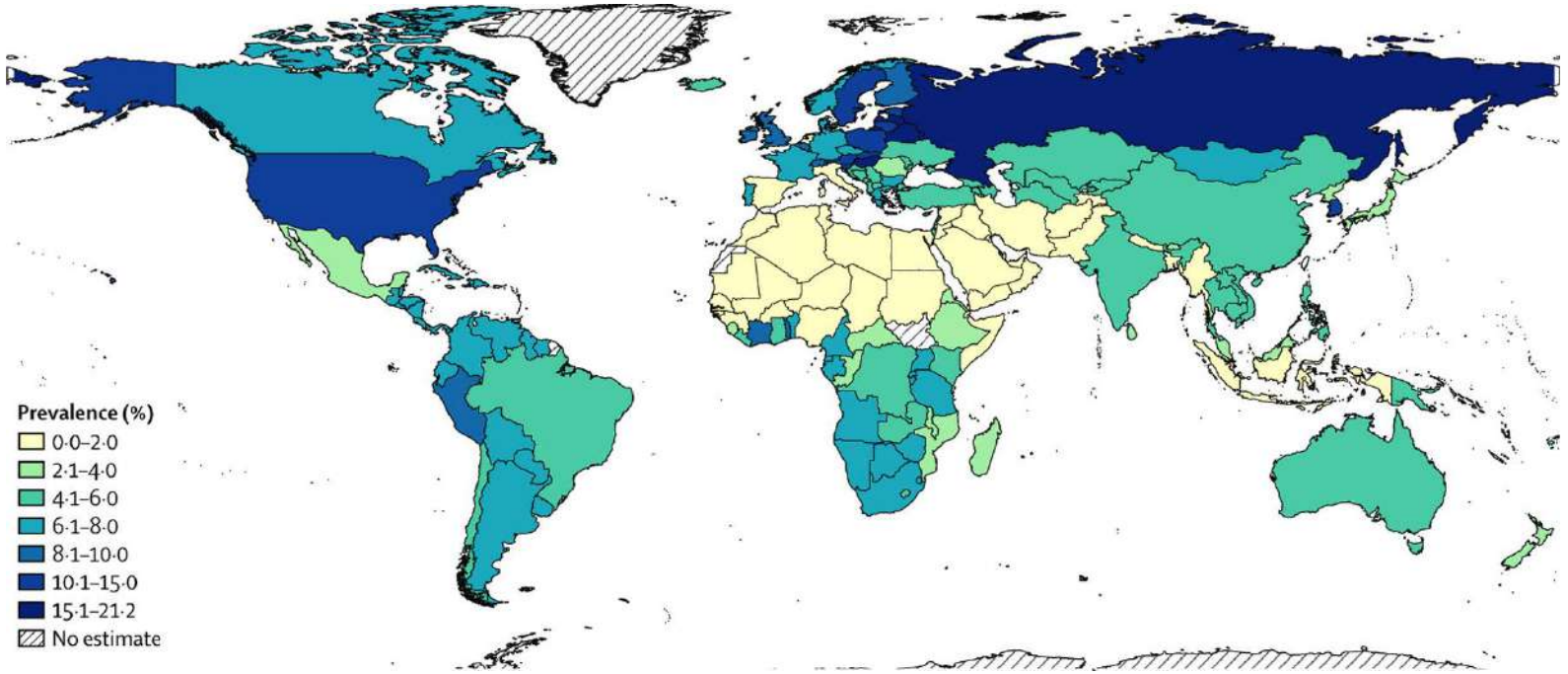
- Tolerance for the substance develops, including a need for increasing quantities of the substance to achieve int or a noticeable decrease in effects when using same amount of the substance;

Tolerance

- After heavy or sustained use of a substance, reduction in or abstinence from the substance results in withdrawal symptoms or precipitates resumption of use of the substance or similar substances to relieve or avoid withdrawal symptoms.

Dependence

Prevalence of alcohol use disorders in 2016



- Higher prevalence in high income countries
- Higher prevalence in individuals with low socioeconomic status

Gender discrepancies (northern Europe)
Addiction 5 times more common among men
8.7% males, 1.5% females

Carvalho et al., Lancet 2019

Prevalence of Alcohol Use in Sweden

- In recent years, nearly 6% of the Swedish population are estimated to be either dependent on or abuse alcohol.

- On average, nearly 19% of the drivers killed in traffic accidents during the past decade had blood alcohol levels exceeding the legal limit.

CAN: the Swedish council for information on alcohol and other drugs 2017

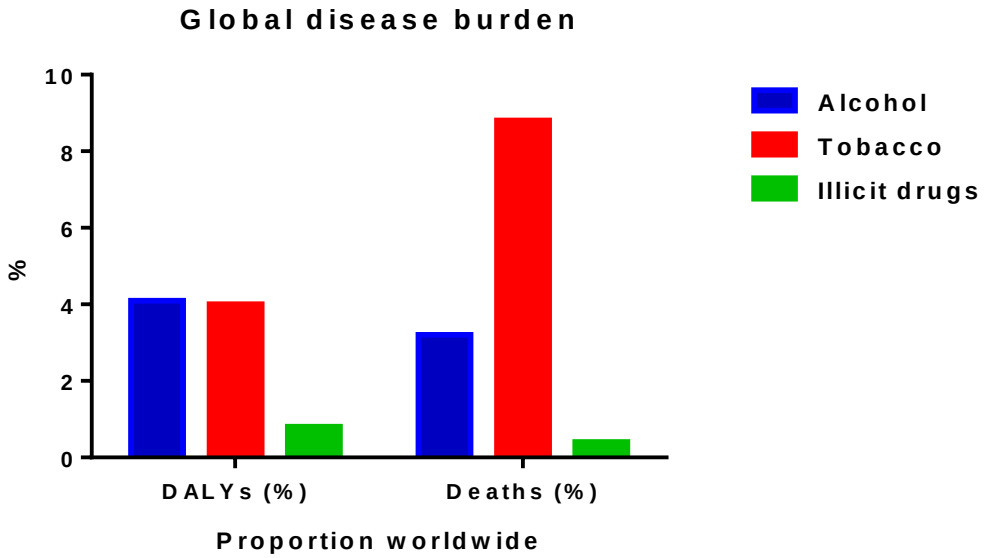
Alcohol use disorders

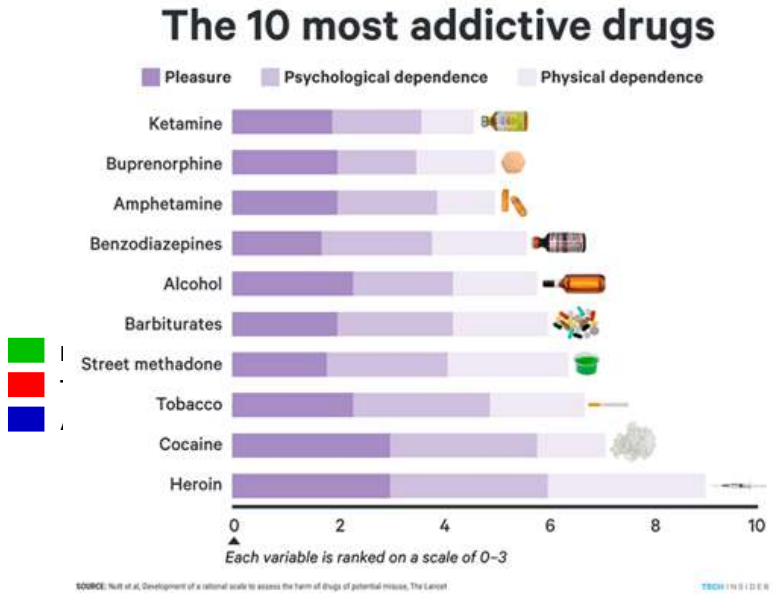
- Major cause of health problems and death globally
  - 3.3M net deaths 2012
  - Responsible for 5.1% of the global burden of disease

Global burden of disease = impact of a health problem as measured by financial cost, mortality. It is often quantified as the number of year lost due to disease.

Carvalho et al., Lancet 2019

Alcohol use is one of the dominant cause of disease burden from addictive disorders

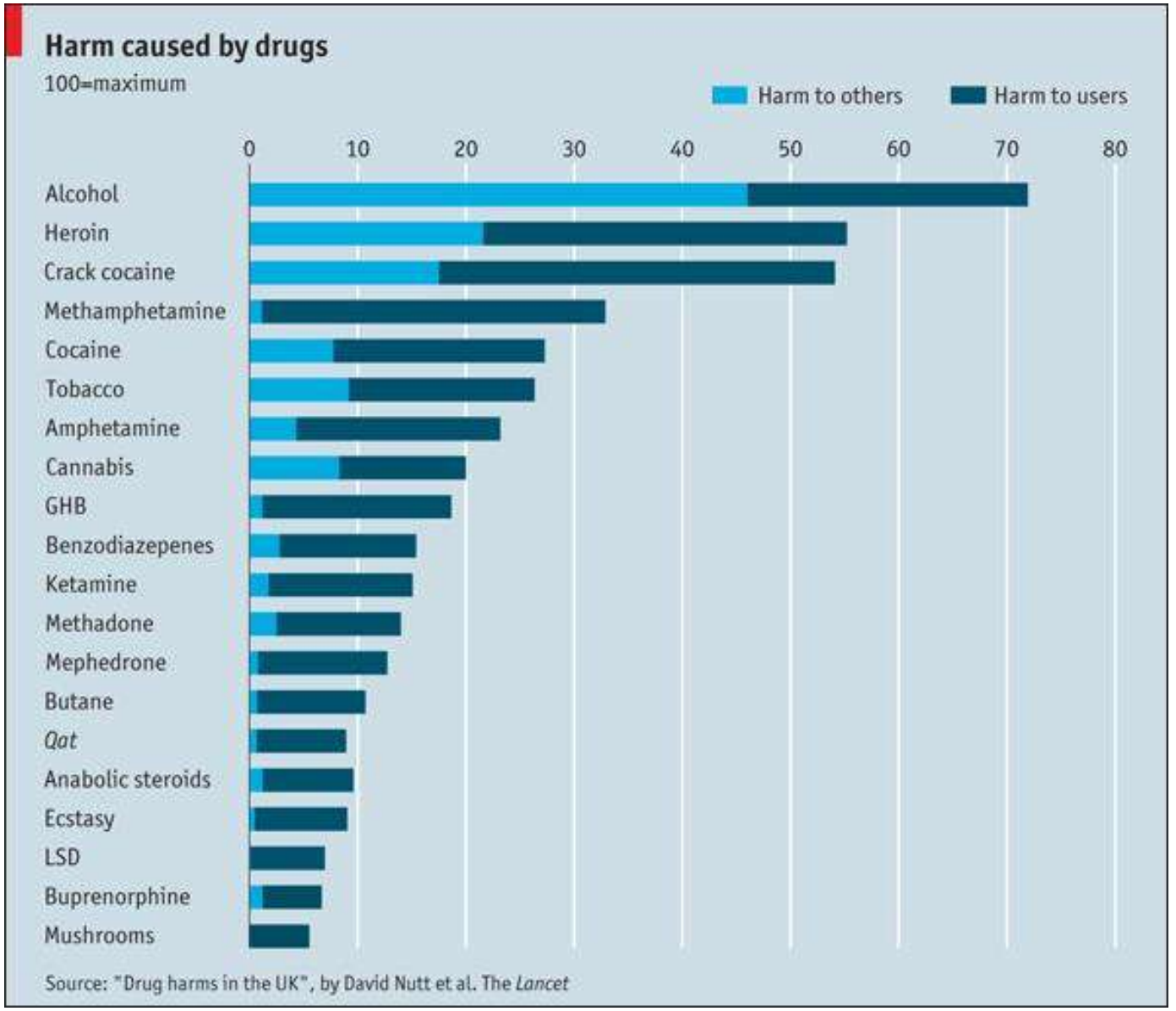




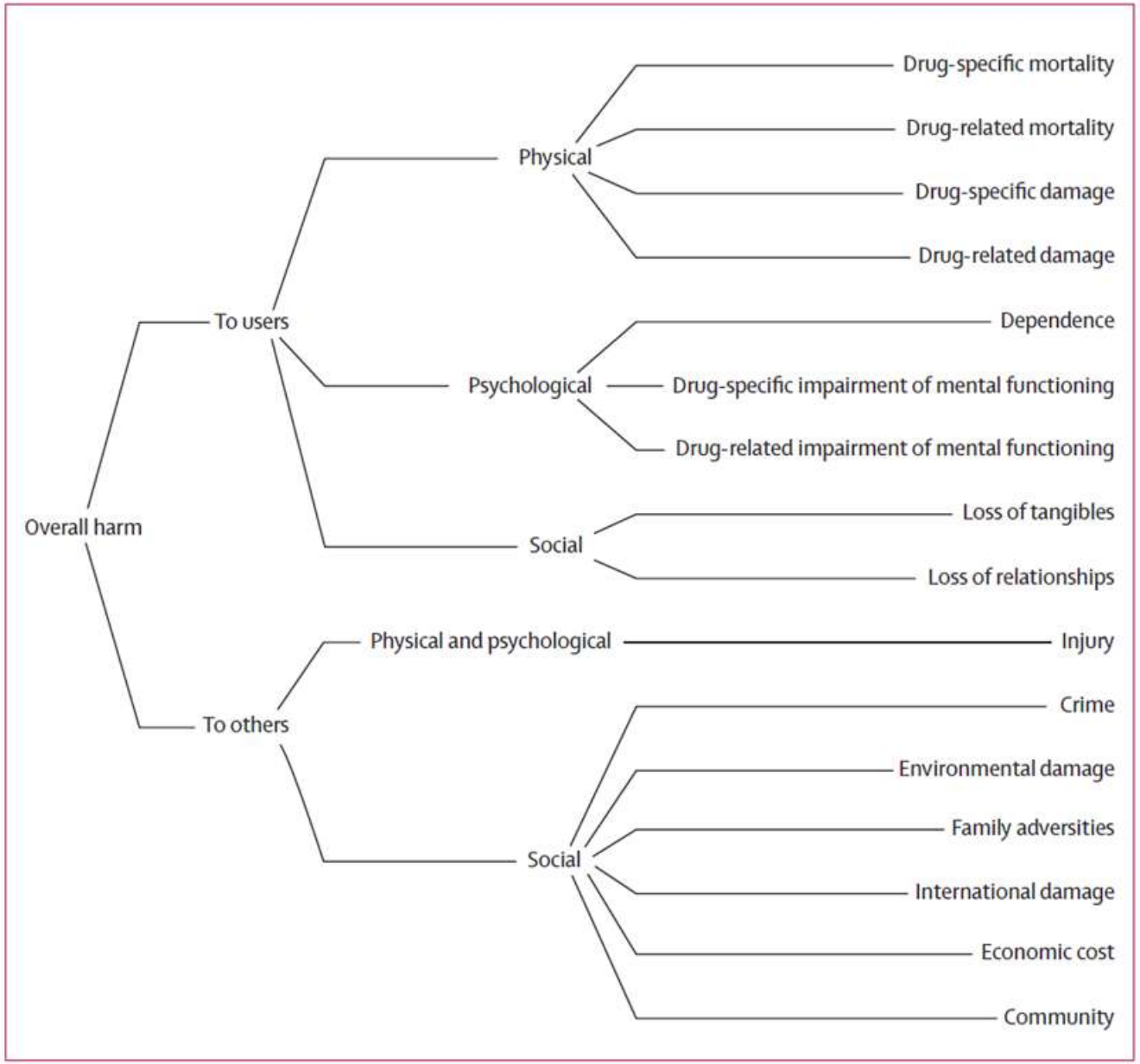
(DALY): Disability Adjusted Life Years

Degenhardt et al, Lancet 2013

Harm caused by drugs



Evaluation criteria organized by harms to users and harms to others



FDA-Approved medications for treating Alcohol Use Disorder

Typical Dose	Comment
666 mg three times daily	Dose reduction required with renal impairment
500 mg once daily for 1-2 weeks, then decrease to maintenance dose (range 125-500 once daily)	Not for use in persons actively drinking alcohol; avoid alcohol in other products
50 mg once daily	Cannot be given to patients taking opioids
380 mg IM every 4 weeks; administer in gluteal area with 1.5 inch 20-gauge needle	Cannot be given to patients taking opioids

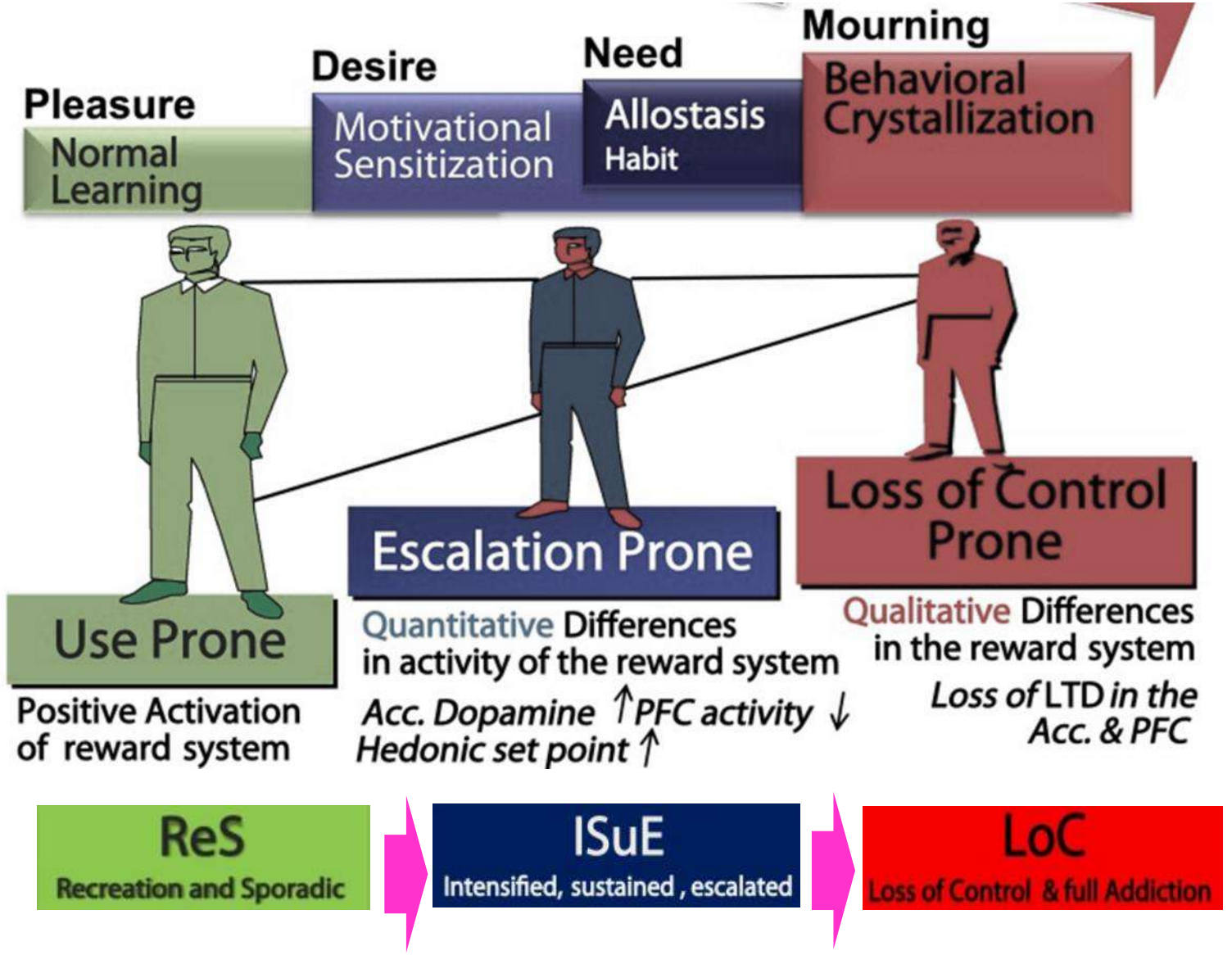
Medications for the treatment of Alcohol Use Disorder

	Original indication	Mechanism of action	Clinical implications
Baclofen	Spasticity	Agonist of GABA-B receptors	Particularly used for high severity dependence; meta-analyses based mostly on small studies have shown divergent results, but efficacy was robustly replicated in an adequately powered multicentre randomized controlled trial; because it is a direct (orthosteric) agonist at GABA-B receptors, baclofen results in tolerance and a need for dose escalation, in turn associated with a potential for serious adverse events
Gabapentin	Epilepsy or neuropathic pain	Complex molecular mechanisms of action; one major effect is inhibition of α2δ-subunit-containing voltage-dependent calcium channels	Gabapentin promoted abstinence and decreased relapse to heavy drinking; it also decreased alcohol-related insomnia, dysphoria, and craving; effects were dose-dependent and most pronounced at the dose of 1800 mg/day
Ondansetron	Nausea and vomiting	$5HT_3$ receptor antagonism	Potential for use in early-onset alcohol use disorder; prescriber should consider pharmacogenetics markers in serotonergic genes
Sodium oxybate	Narcolepsy	Unknown mechanism; a metabolite of GABA; interacts with GABA-B receptors, but unknown whether this mediates therapeutic actions in alcohol use disorder	Sodium oxybate was safe and effective in severe alcohol dependence; it has a high abuse liability, and use should be reserved for specialist treatment settings under a Risk Evaluation and Mitigation Strategy
Topiramate	Epilepsy	Complex molecular mechanisms of action; glutamatergic actions are likely to be key in alcohol use disorder treatment; Effectiveness is moderated by a polymorphism at the locus encoding the glutamatergic kainate receptor subunit GRIK1 (also known as GluK1).	Limited to specialist treatment because it needs to be carefully titrated over an extended period of time, and is initially associated with cognitive side-effects, including impairments of working memory
Varenicline	Smoking cessation	Partial agonist of the α4β2 isoform of the nicotinic acetyl choline receptor	Highest effectiveness seen with phosphatidylethanol as outcome, which is a biomarker for short and intermediate heavy alcohol intake; medication should start immediately after detoxification

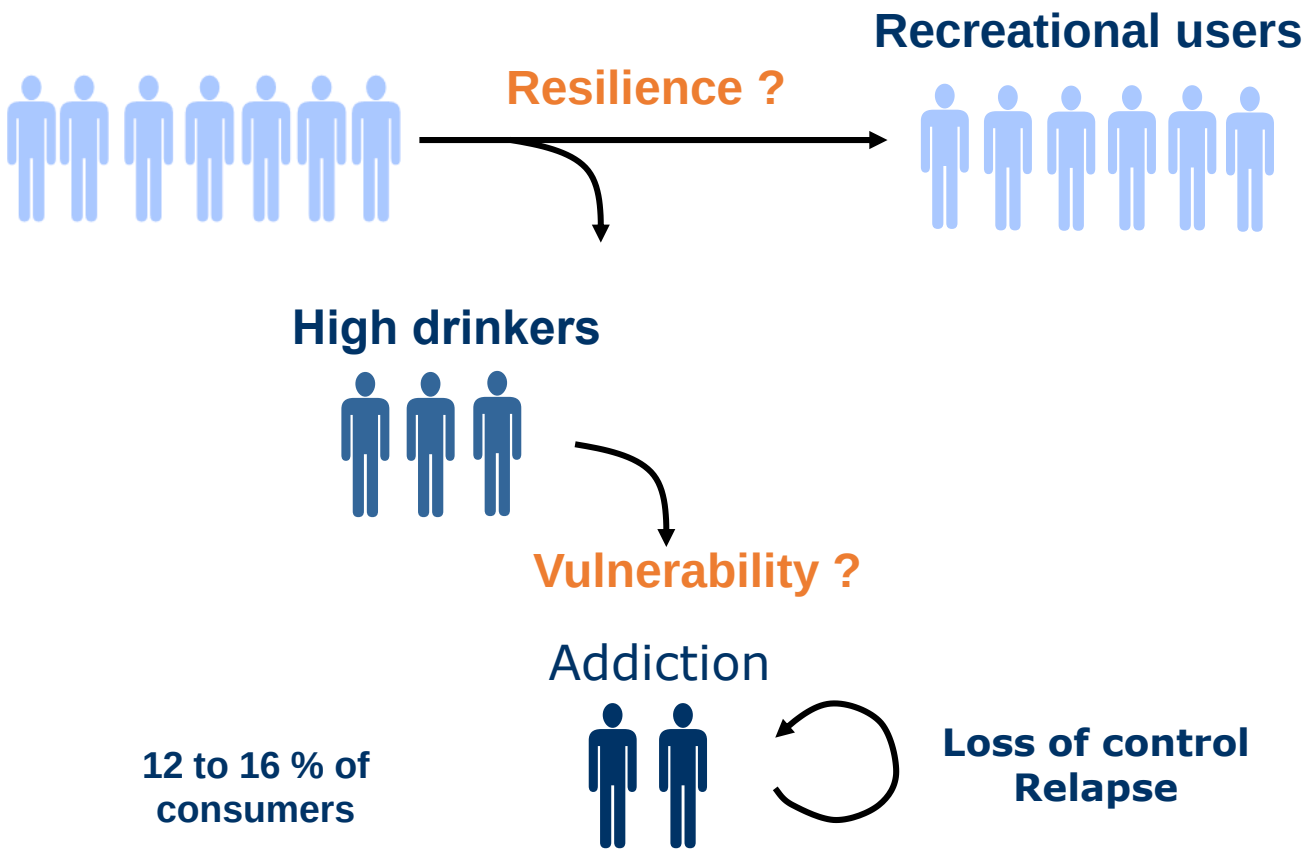
See the appendix (pp 8–9) for reference details. GABA=γ-aminobutyric acid.

Table 2: **Wave 2 medications for the treatment of alcohol use disorders**

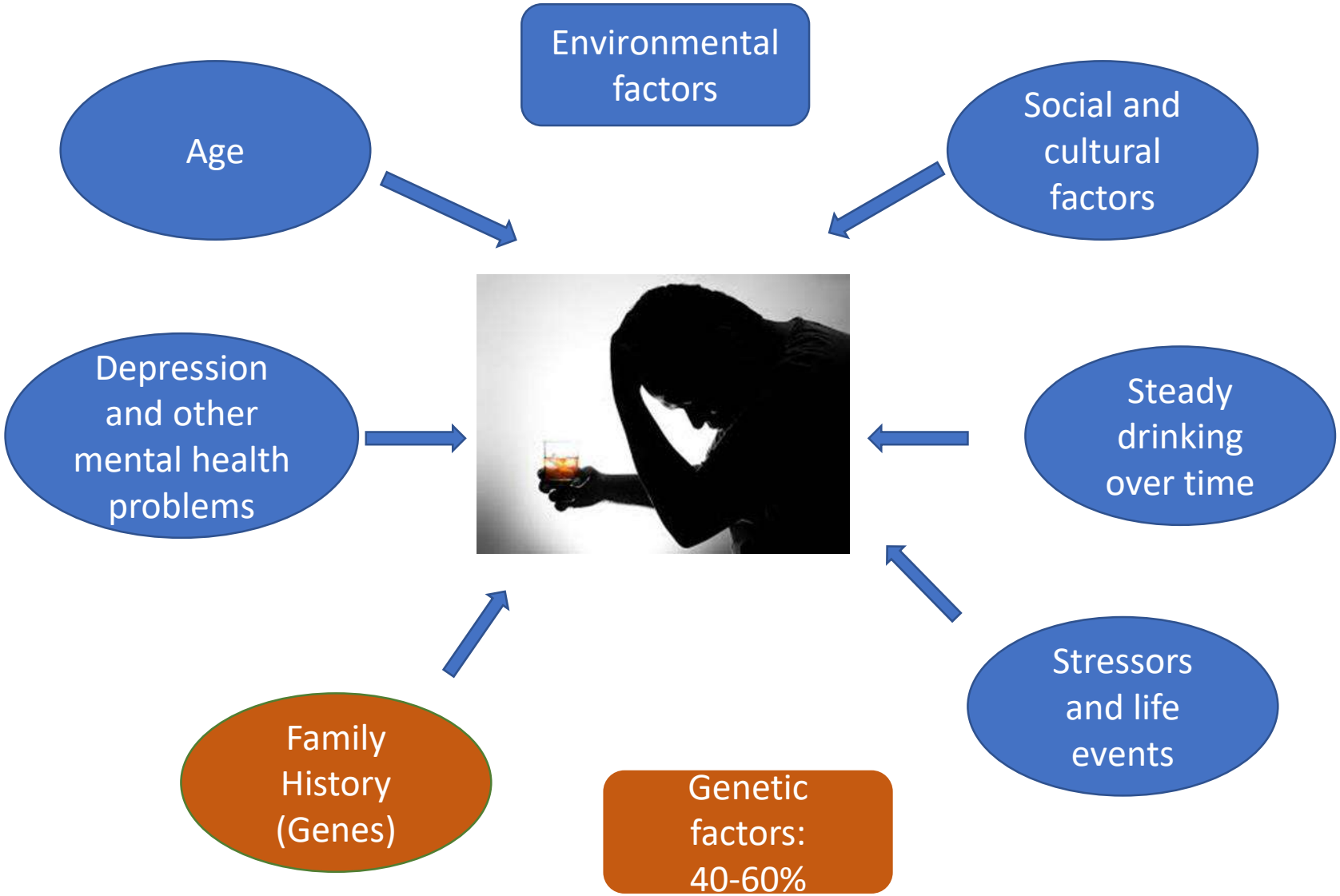
Transition to addiction



Transition to addiction

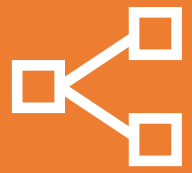


Vulnerability to Addiction



Comorbidity between Alcohol Use and Anxiety Disorders.

Among individuals with comorbid Alcohol Use et psychiatric disorders



35.8 alcohol-dependent men had a co-occurring anxiety disorder.

60.7 alcohol-dependent women had a co-occurring anxiety disorder.

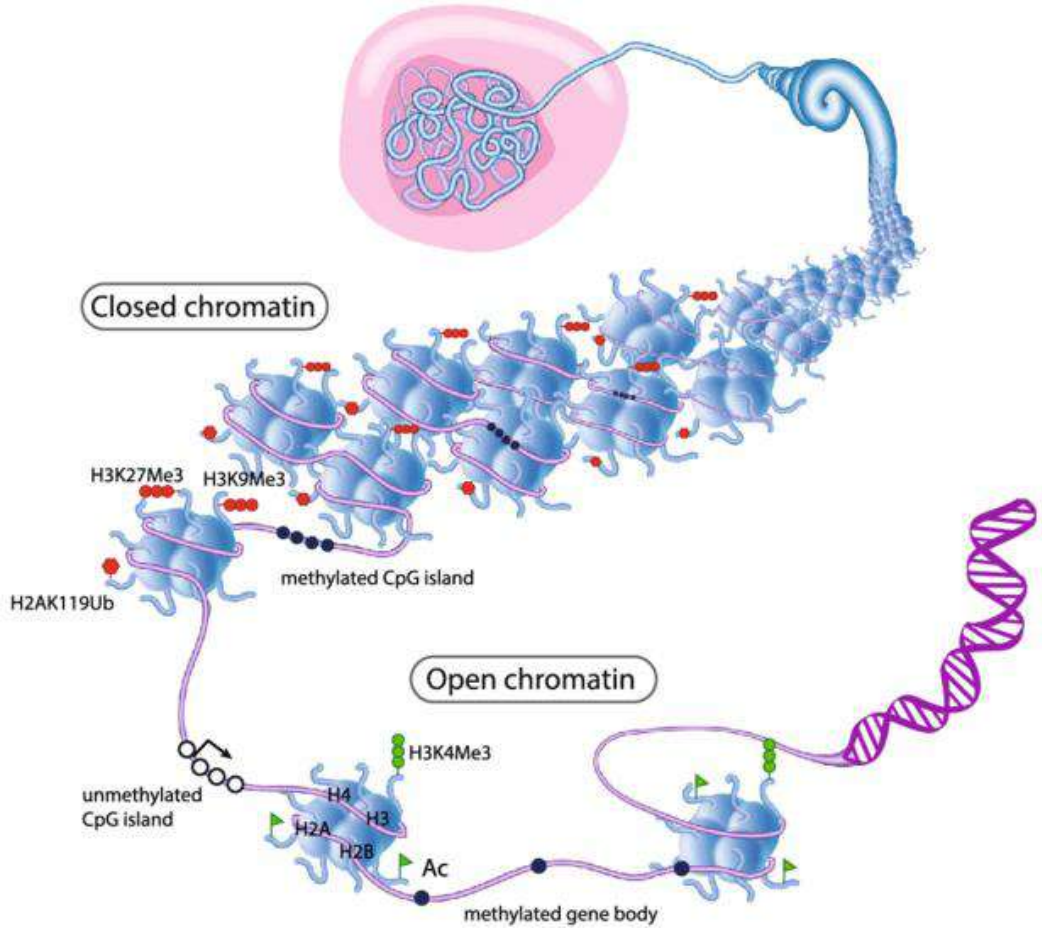
Hypothesis

Both Alcohol Use and Anxiety Disorders are characterized by long-term changes in gene expression.

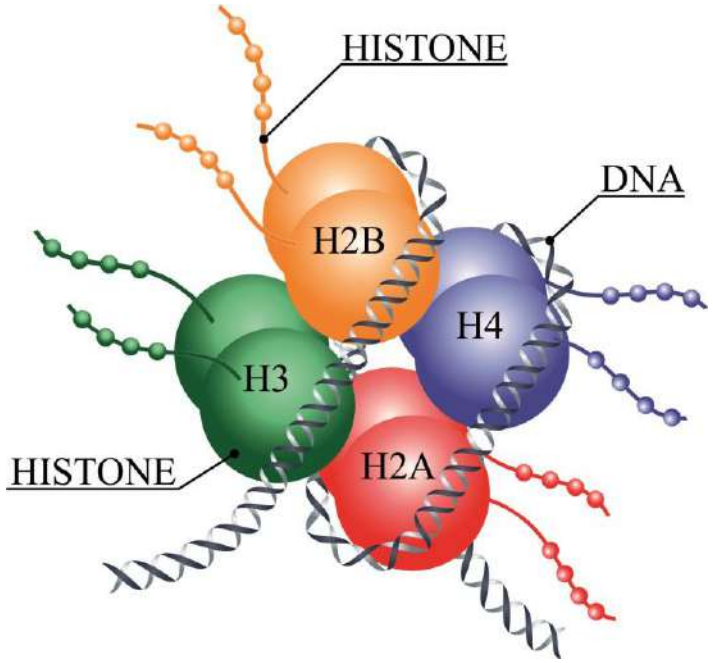
Epigenetic modifications may be one of the underlying mechanism.

Epigenetic : Definition

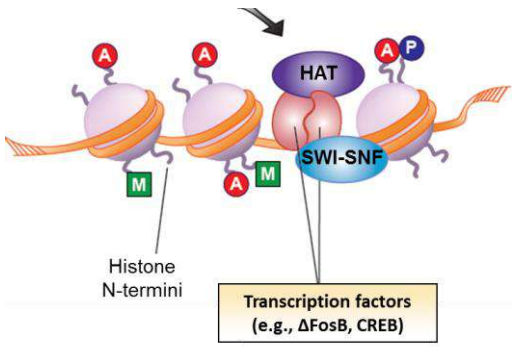
- Heritable and reversible mechanism that regulates gene expression without changing DNA sequence



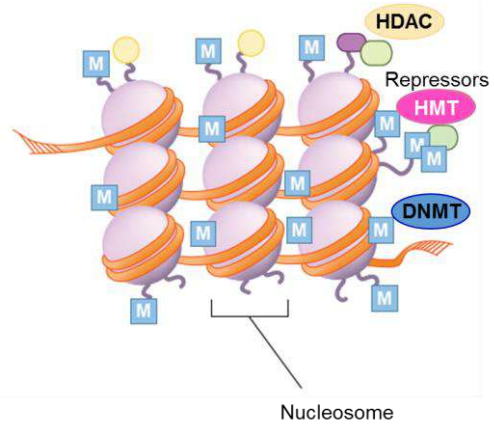
Nucleosome



Active (open)



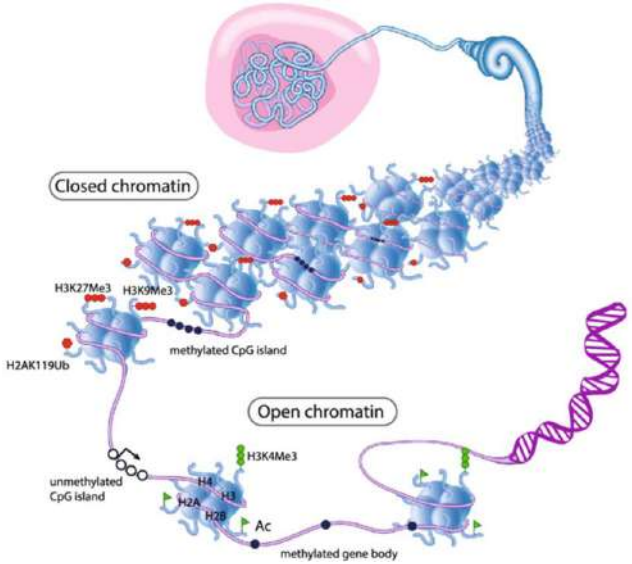
Inactive (condensed)

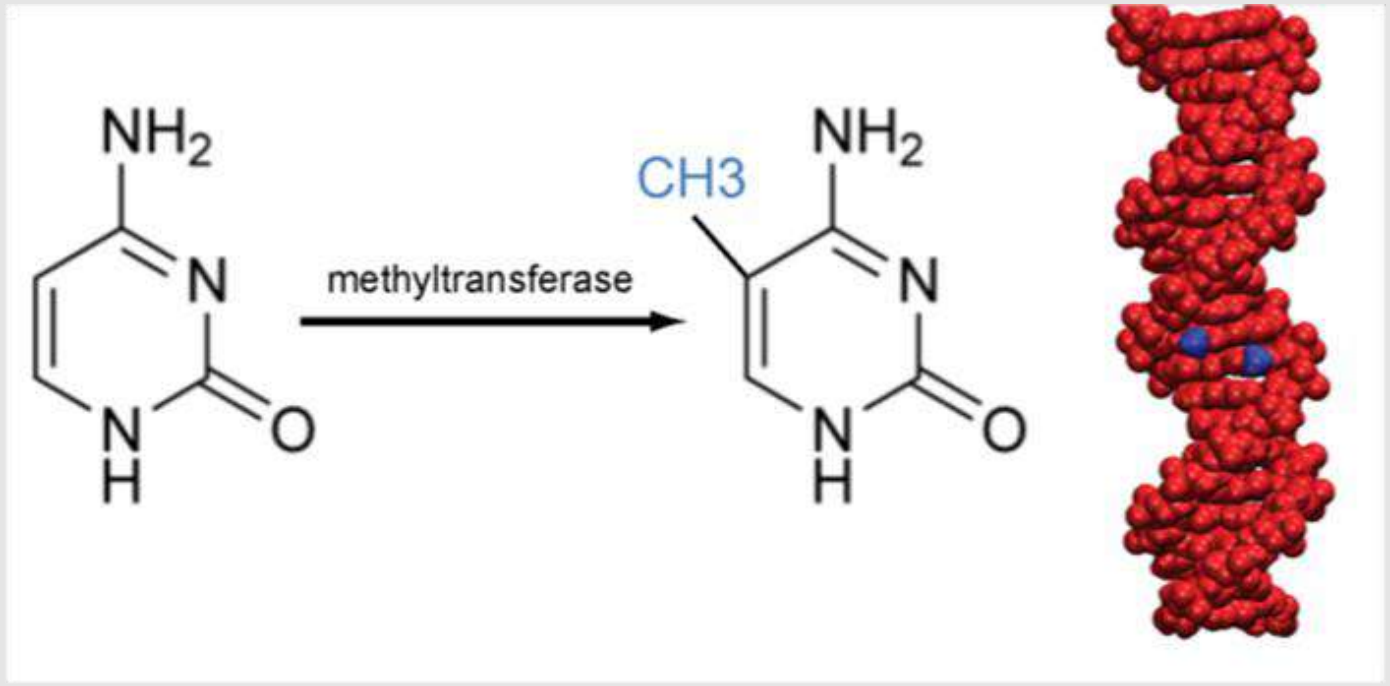


Epigenetic: Definition

- Heritable and reversible mechanism that regulates gene expression without changing DNA sequence

→ DNA methylation
→ Histone modifications
→ non coding RNAs

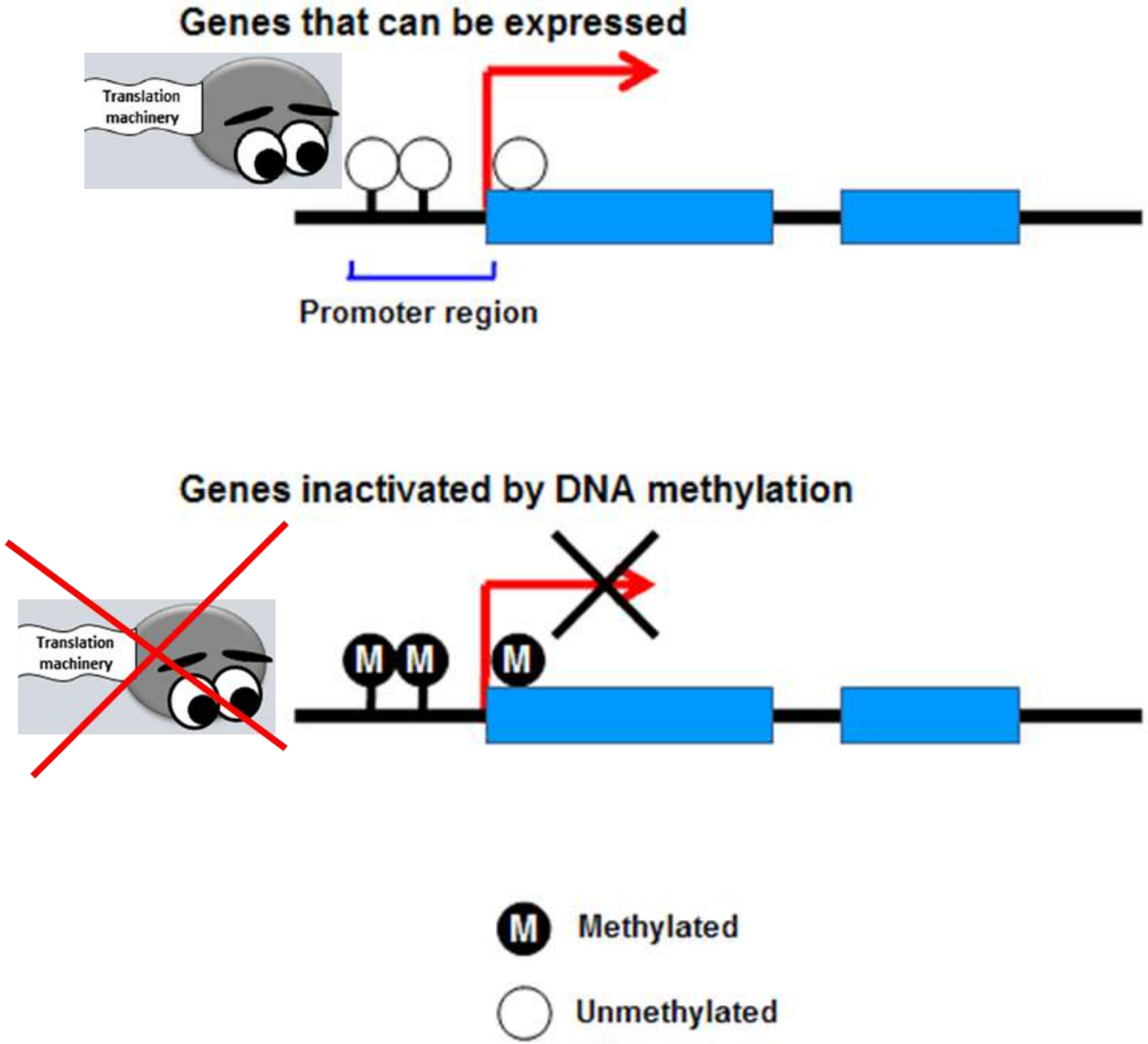




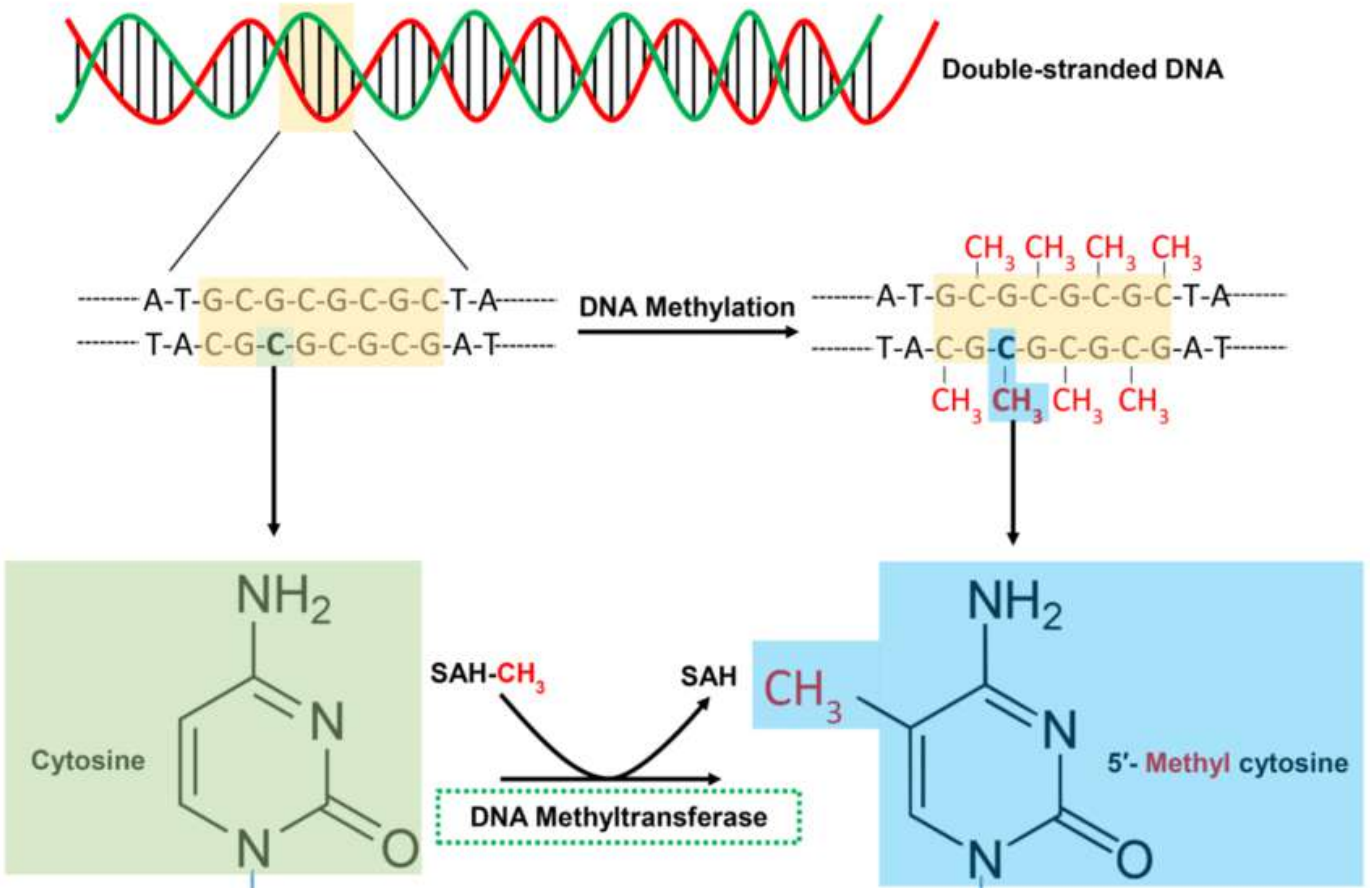
DNA methylation

- DNA methylation at C5 position of cytosine associated with a guanine: **CpG sites**.
- **CpG Island**: genomic region that contain high frequency of CpG sites

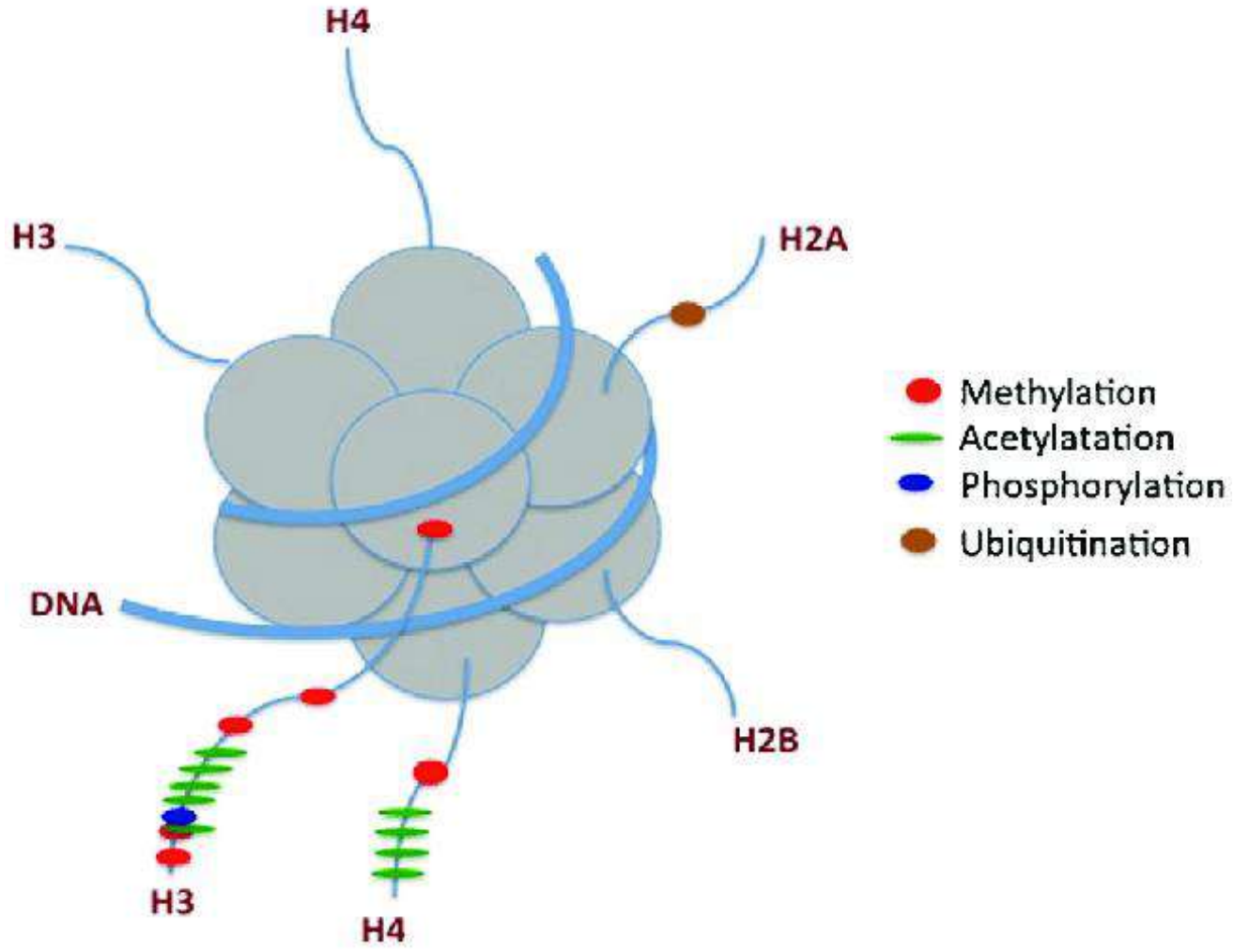
DNA methylation: gene expression silencing

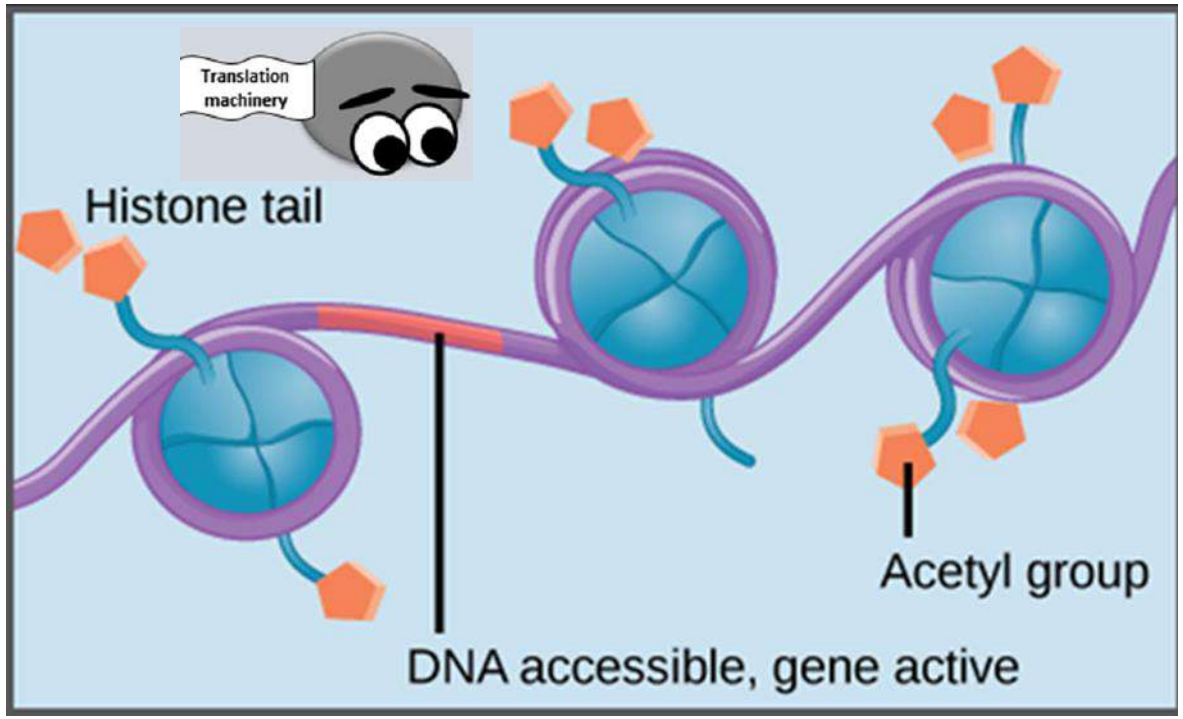


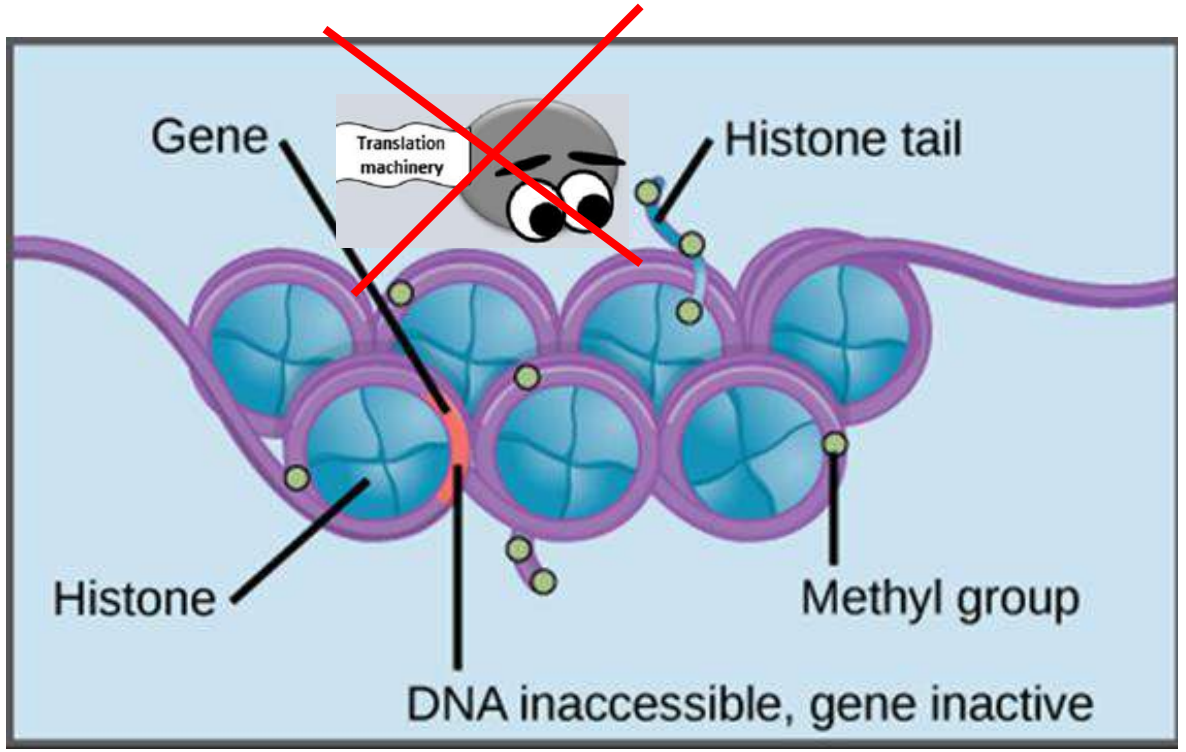
DNA methyltransferases



Histone modifications

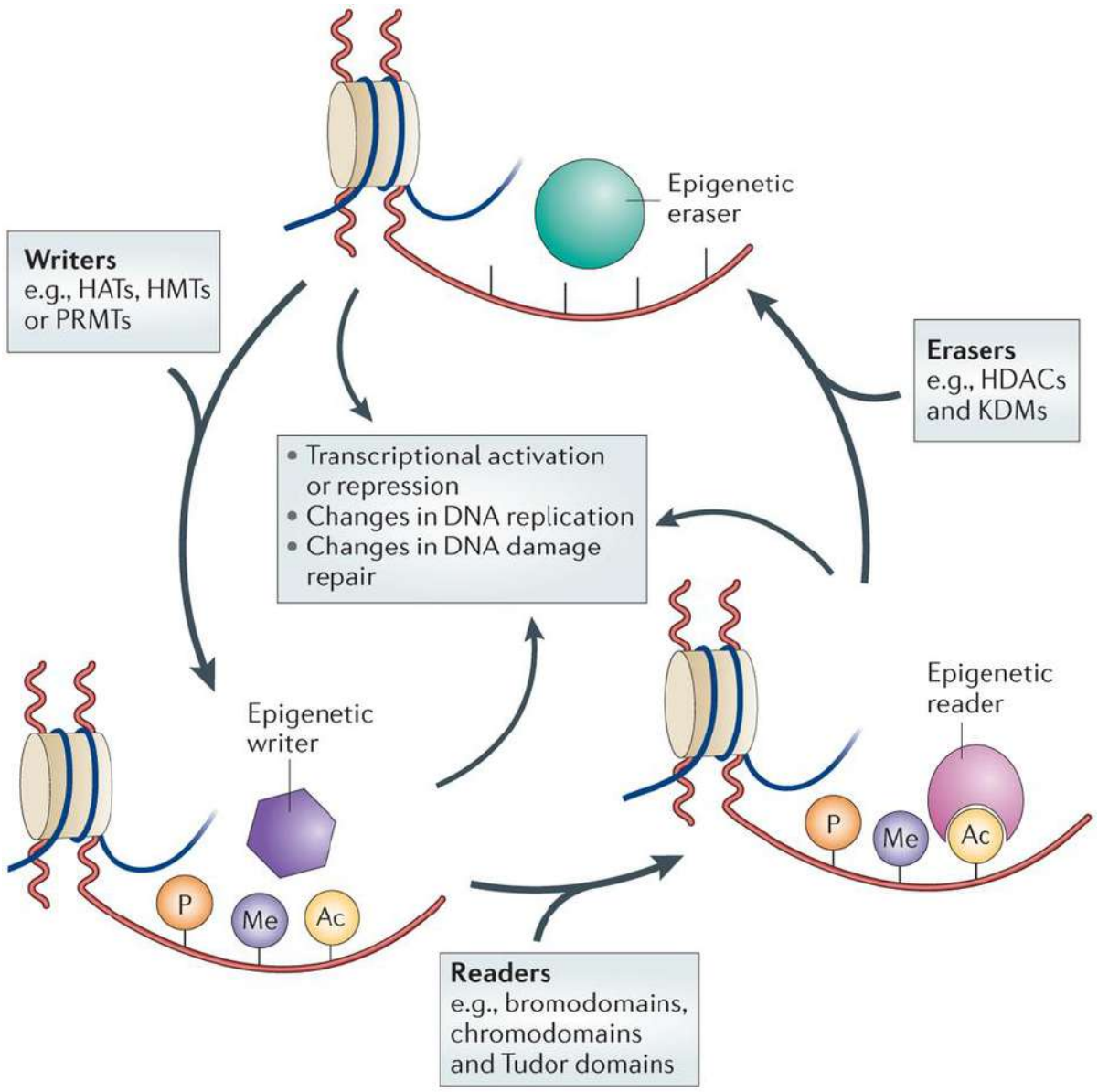






Histone acetylation

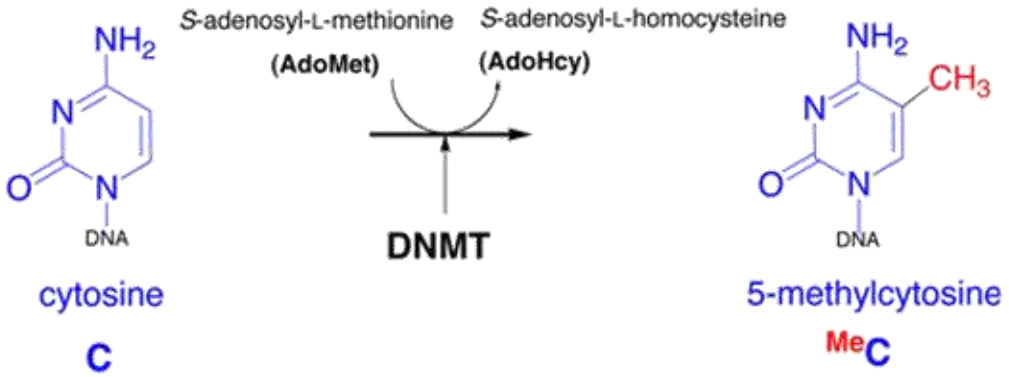
Histone modifications



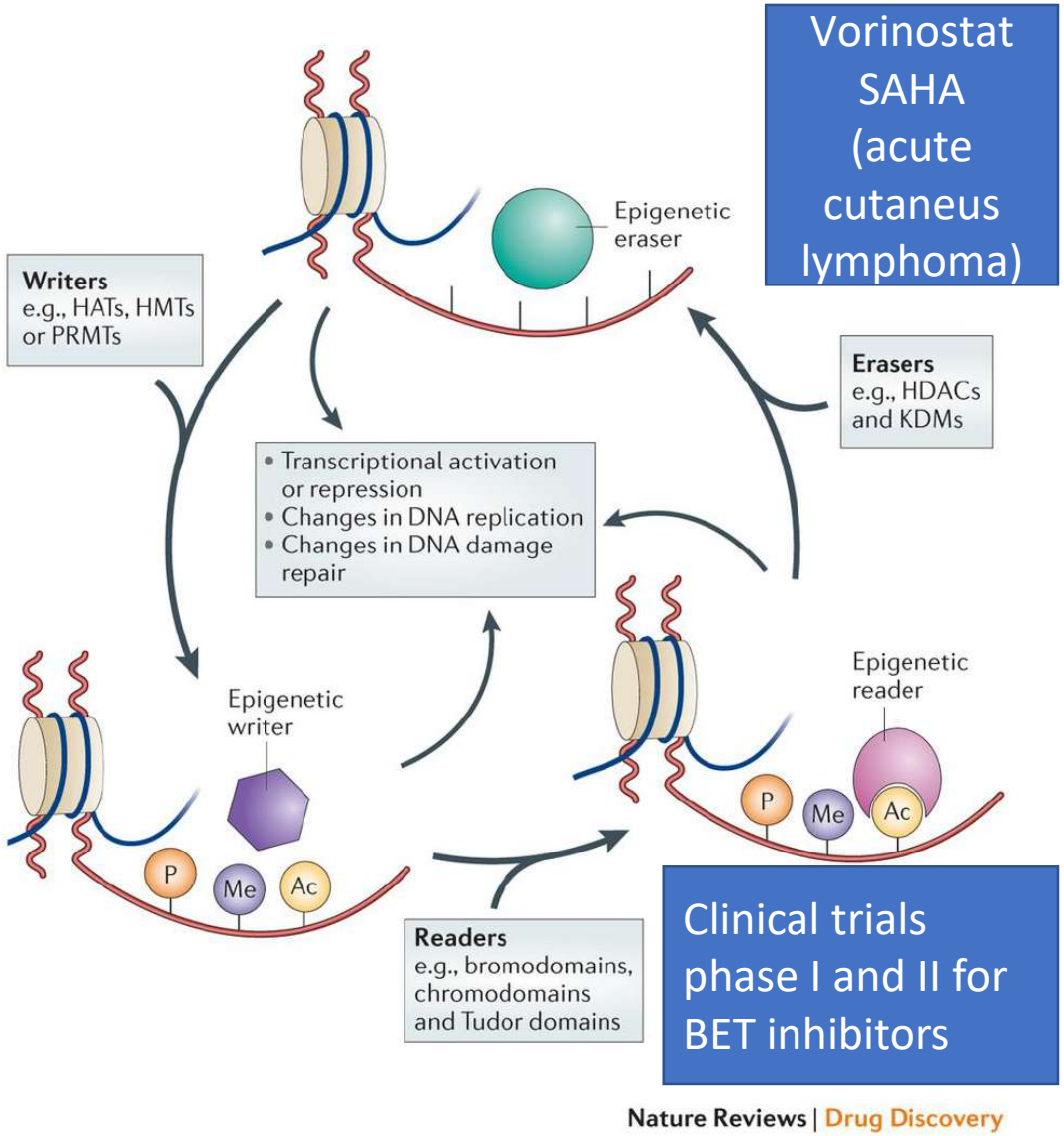
Nature Reviews | Drug Discovery

Katrina J. Falkenberg & Ricky W. Johnstone

FDA approved drugs that target epigenetic enzymes



5-Azacytidine (myelodysplastic syndrome)



Vorinostat SAHA (acute cutaneus lymphoma)

Clinical trials phase I and II for BET inhibitors

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Examples of known epigenetic modifiers with therapeutic implications

Class	Modifier	Type of epigenetic modification
Prescribed drugs	Valproate	HDAC inhibitor (increases H3 and H4 acetylation)
	Lithium	HDAC inhibitor (increases H3 acetylation and phosphoacetylation)
	Imipramine	Inhibition of HDAC5 (increases histone acetylation of BDNF III and IV promoters)
	Amitriptyline	Inhibition of HDACs, H3 acetylation, and DNA demethylation by the inhibition of DNMT activity
	Fluoxetine	Reverses the stress-induced decreases in H3K9me3 as well as H3 acetylation in dentate gyrus and hippocampus, respectively
	Escitalopram	Reduces the mRNA levels of *DNMT1* and *DNMT3A*, and DNA methylation of *S100A10* gene promoter
	Tranylcypromine (MAOI)	Inhibition of *LSD1* (lysine-specific demethylase 1 that demethylates H3K4me1, H3K4me2, H3K9me1, and H3K9me2) inducing transcription
	Haloperidol	H3 phosphorylation at serine 10, H3K14 phosphoacetylation
	Clozapine	Increases H3K4me3 and DNA demethylation of *RELN* and *GAD67* promoters
	Sulpiride, amisulpiride (and MS-275)	H3K9 and H3K14 acetylation and DNA demethylation of *RELN* and *GAD67* promoters
	Lurasidone	Increases HDAC1, HDAC2, and HDAC5 expression

Abdolmaleky et al., 2013

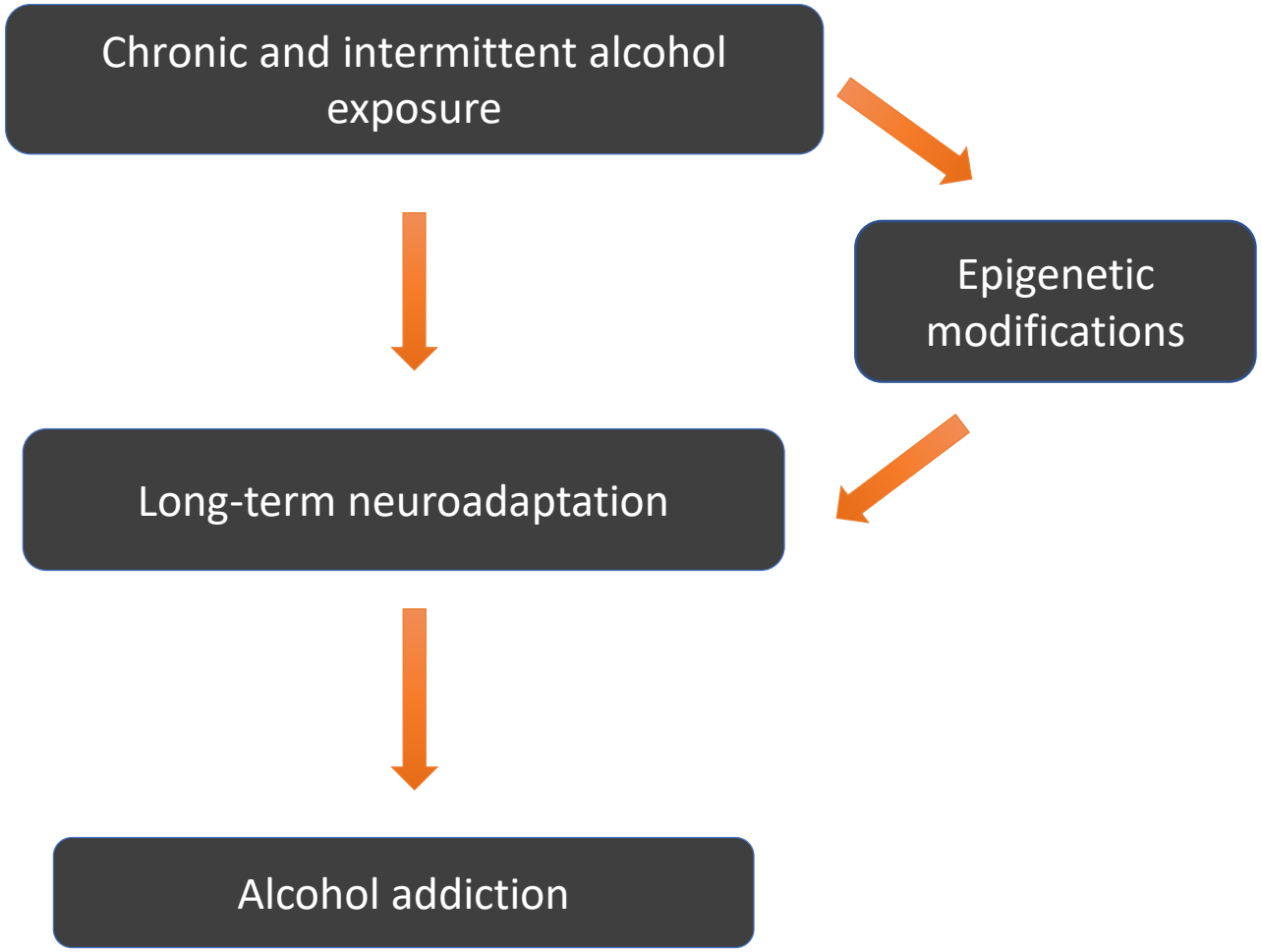
Epigenetic aberrations reported in psychiatric diseases

Histone modification	Direction of change	Affected gene or histone codes	Morbidity
Acetylation	▲	HDAC1	Schizophrenia
	▲	HDAC2, HDAC5	MDD (phase)
	▲	HDAC4	Depressive phase of bipolar disorder
	▼	HDAC6, HDAC8	Depressive and remission phase of bipolar disorder
Methylation	▲	H3K9me2	Schizophrenia
	▼	H3K4me of *GAD1* promoter	Schizophrenia
DNA modification	**Direction of change**	**Affected gene**	**Morbidity**
DNA methylation	▲	*RELN, SOX10*	Schizophrenia
	▲	*WDR18*	Schizophrenia (male)
	▲	*GABAA*	Suicide
	▲	*NR3C1*	Child abuse
	▲	*DAT1, HERP*	Alcoholism
	▼	Atrial natriuretic peptide	Alcoholism
	▲	*5-HTT*	MDD
	▲	*HTR2A*	Schizophrenia and bipolar disorder
	▲	*DAT1, DRD2*	Anorexia nervosa
	▲	*PER1, CRY1, SORBS3, S100A2*	Alzheimer disease
	▼	*MB-COMT*	Schizophrenia and bipolar disorder
	▼	*RPL39*	Bipolar disorder (females)
	▼	Genome-wide DNA	Autism
	▼	Genome-wide DNA	Schizophrenia
	▼	*PPIEL*	Bipolar II
	▼	*MAOA*	Smoking (tobacco)
RNA modification	**Direction of expression change**	**Affected miRNA**	**Morbidity**
miRNAs	▲	miR-15a, miR-15b, miR-195, miR-107, miR-181b; exosomal miR-497, miR-29c	Schizophrenia
	▼	miR-24, miR-26b, miR-30e, miR-92, miR-346	Schizophrenia and schizoaffective disorder
	▼	Circulating miR-134	Manic phase of bipolar disorder

HDAC, histone deacetylase; miRNA, microRNA.

Abdolmaleky et al., 2013

Hypothesis


Chronic and intermittent alcohol exposure
Epigenetic modifications
Long-term neuroadaptation
Alcohol addiction

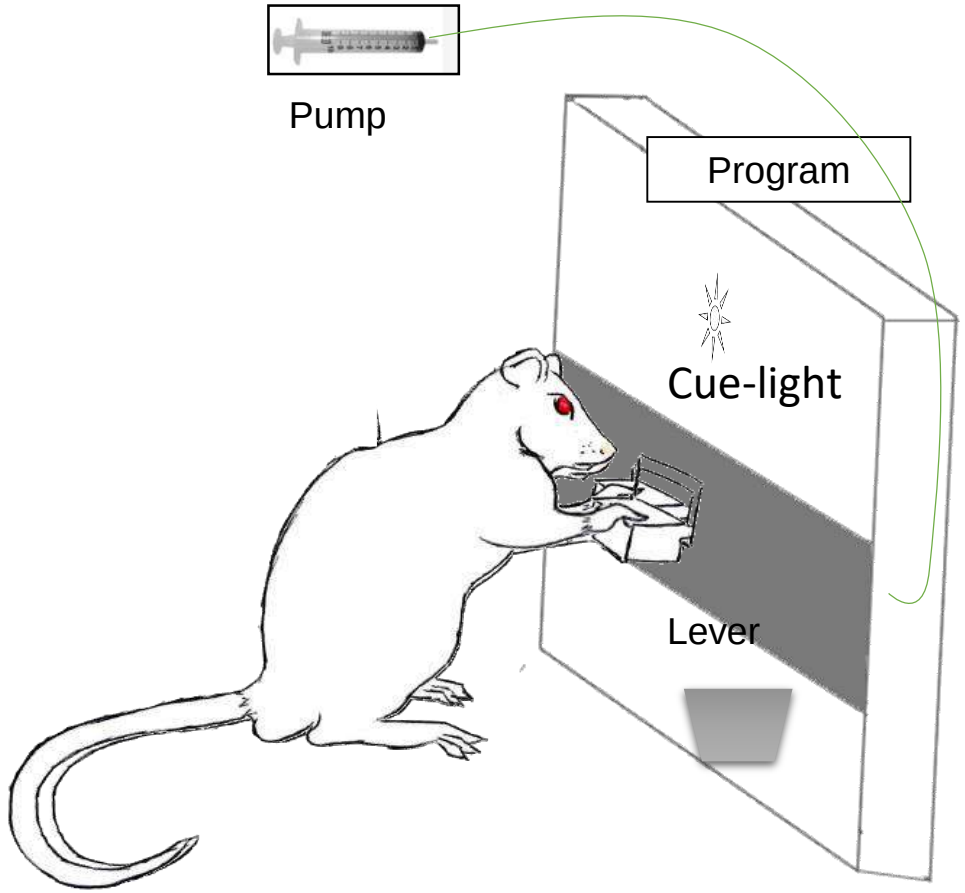
A translational prospective of our research

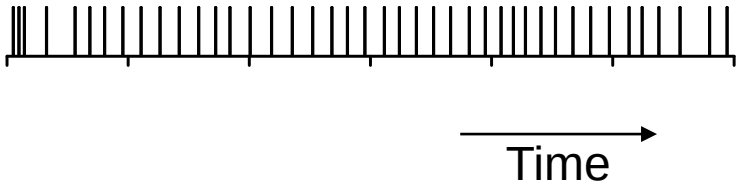
- Animal research has continued to provide novel insights about how genes influence individual differences in addiction risk and consequences

How do we model addiction and related behaviours in rats?

Preclinical research on addiction

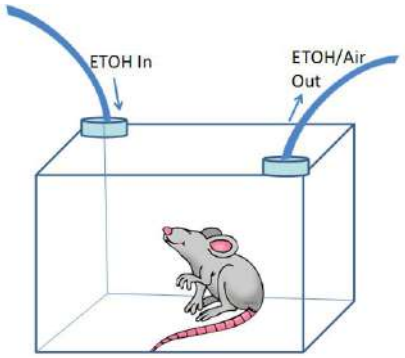
- Operant self-administration

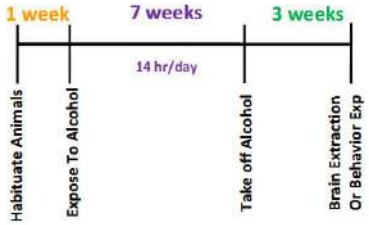




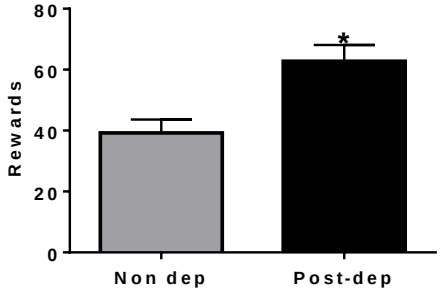
- ✓ Drug consumption
- ✓ Motivation
- ✓ Compulsive-like behavior

Model: alcohol post-dependent

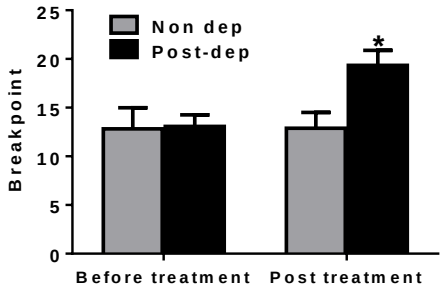




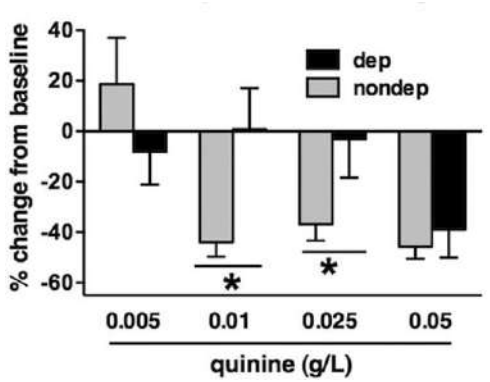
Alcohol self-administration



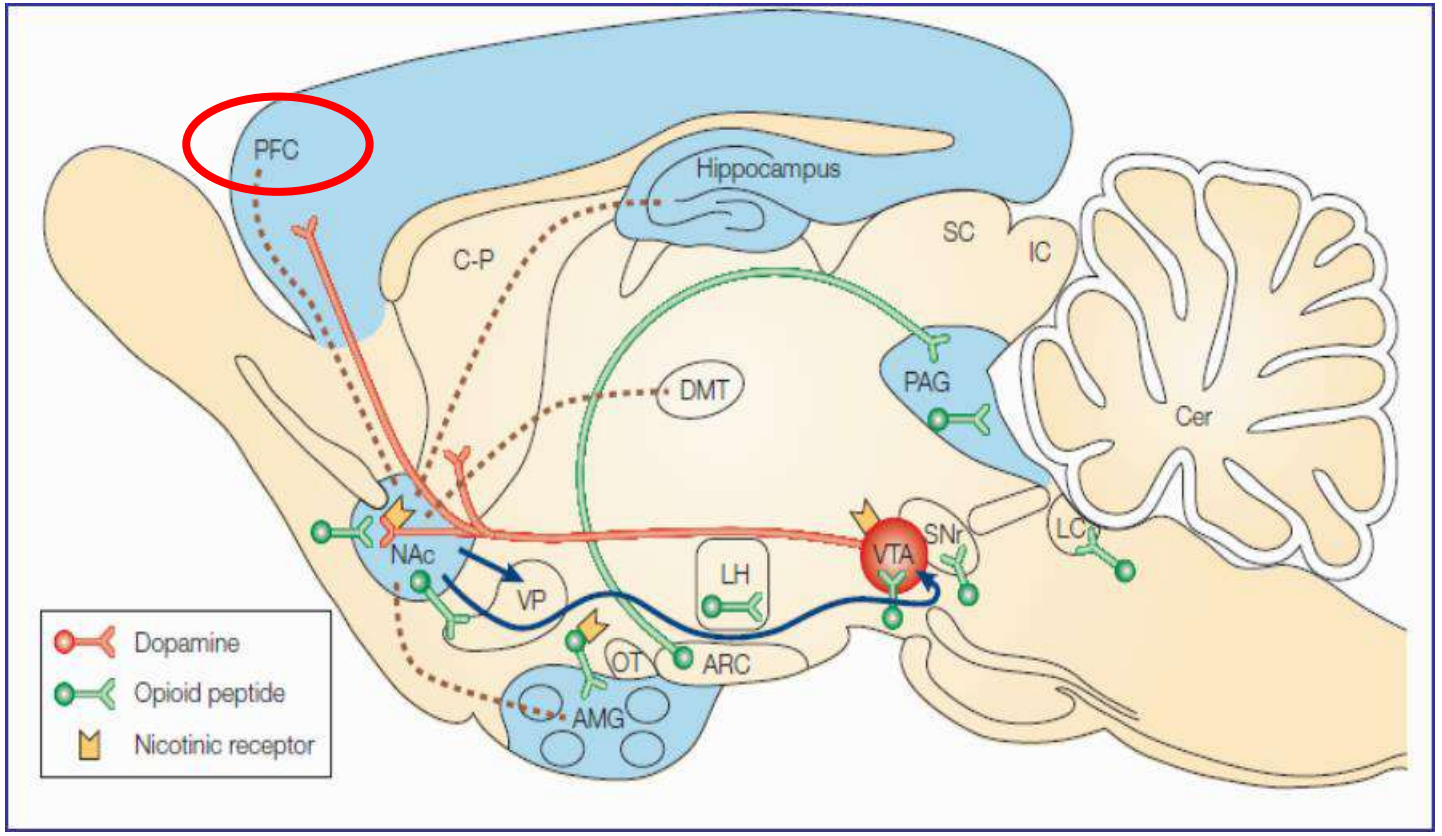
Progressive ratio



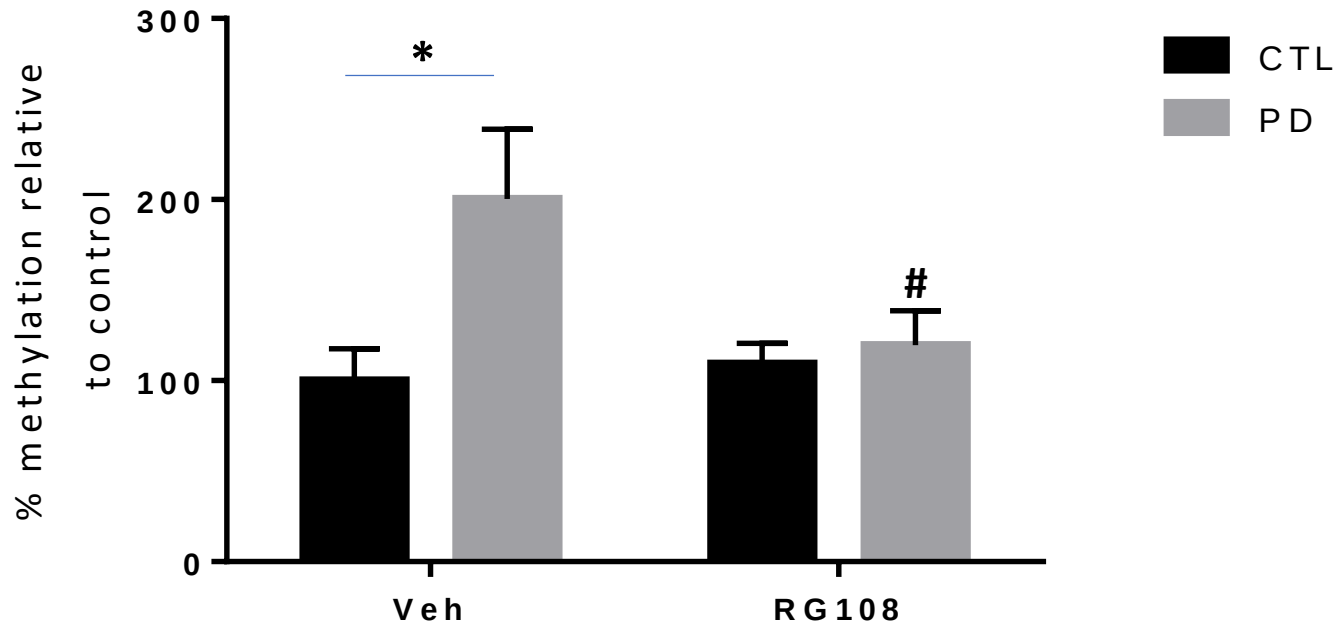
Quinine adulteration



ADDICTION: Neuronal Substrates

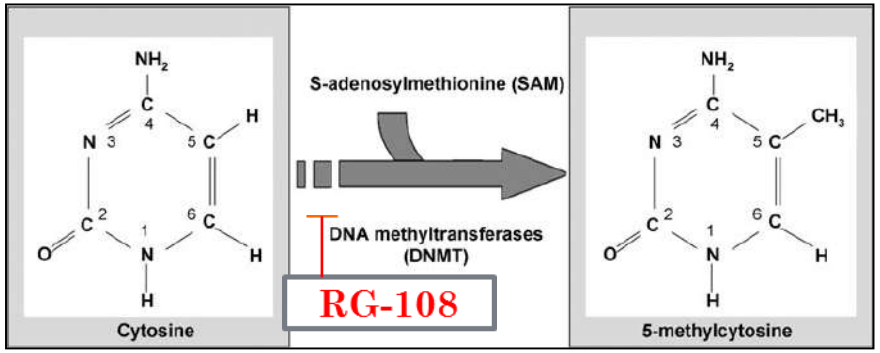


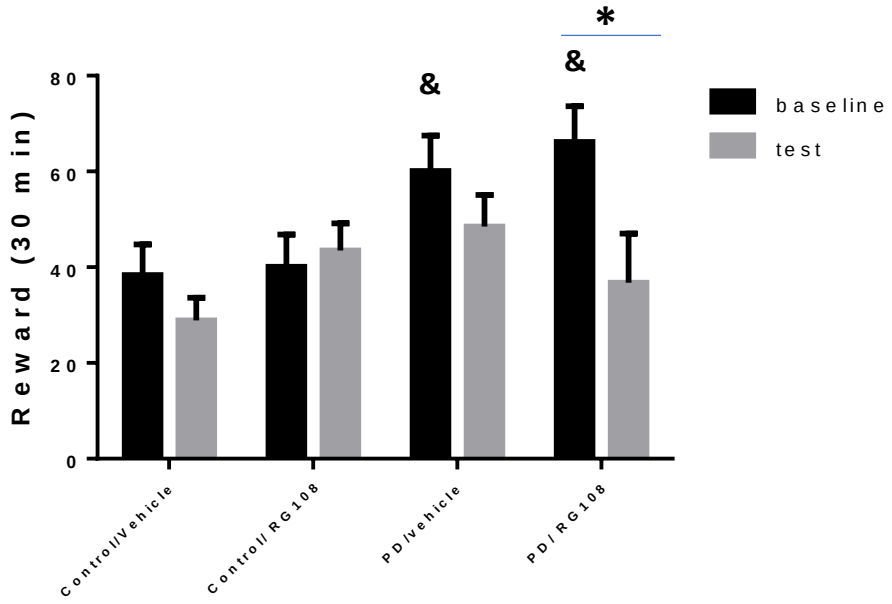
Chronic intermittent alcohol exposure increases DNA methylation in the mPFC



Barbier et al., J. of Neuroscience 2015

Injection of RG-108 in mPFC decreases alcohol consumption in post-dependent rats





Barbier et al., J. of Neuroscience 2015

History of alcohol dependence dysregulates expression of epigenetic enzymes

Gene Symbol	Epigenetic Class	Target Site or Gene Function	Entrez Id	Probe Id	Fold Change	p-value(%)
Dot1l	Histone Methyltransferase	H3K79	362831	ENSRNOG00000032546	-2,09	0,03
Mll5	Histone Methyltransferase	H3K4	311968	ENSRNOG00000021614	-1,39	0,03
Prdm2	Histone Methyltransferase	H3K9	313678	ENSRNOG00000033522	-1,51	0,03
Prmt5	Histone Methyltransferase	H3R8, H4R3, H2A, non-histone targets	364382	ENSRNOG00000012046	1,31	0,05
Kdm6b	Histone Demethylase	H3K27	363630	ENSRNOG00000037613	-2,33	0,03
Phf2	Histone Demethylase	H3K9	306814	ENSRNOG00000016816	-1,95	0,01
Brpf1	Histone Acetyltransferase	MOZ/MORF complex, H2AK5, H4K12, H3K14	679713	ENSRNOG00000008142	-1,55	0,02
Ep300	Histone Acetyltransferase	All core histones, non-histone targets, CREB binding	170915	ENSRNOG00000000190	-1,33	0,04
Myst3	Histone Acetyltransferase	MOZ/MORF complex, H3 acetylation	306571	ENSRNOG00000025174	-1,77	0,04
Hdac7	Histone Deacetylase	Class IIa HDAC	84582	ENSRNOG00000008308	-2,44	0,02
Tet1	DNA Demethylase	5hmc	309902	ENSRNOG00000000277	-2,06	0,01
Tet3	DNA Demethylase	5hmc	680576	ENSRNOG00000011387	-1,73	0,03
Brd3	Bromodomain Reader	H4K5/8/12/16	362092	ENSRNOG00000007681	-1,48	0,02
Brd4	Bromodomain Reader	H4K5/8/12/16	362844	ENSRNOG00000006770	-1,95	0,01
Trim33	Bromodomain Reader	E3 Ub ligase, H3K9me3, H3K18ac	365894	ENSRNOG00000018946	1,35	0,02
Chd4	Chromodomain Reader	NuRD chromatin remodeling complex	117535	ENSRNOG00000018309	-1,49	0,04
Chd8	Chromodomain Reader	DNA helicase	65027	ENSRNOG00000025011	-1,36	0,02
Dpf2	PhD Domain Reader	Ac histones	361711	ENSRNOG00000020892	-1,53	0,03
Chmp1b	Chromatin Remodeling	ESCRT-II complex	689364	ENSRNOG00000038673	1,41	0,03
Ino80	Chromatin Remodeling	SNF2/SWI2 helicase family	296084	ENSRNOG00000014483	-1,35	0,04
Smarce1	Chromatin Remodeling	SWI/SNF complex	303518	ENSRNOG00000010676	-3,02	0,00
Srcap	Chromatin Remodeling	SWI/SNF complex, H2AZ/H1AZ exchange	361652	ENSRNOG00000018637	-1,69	0,02

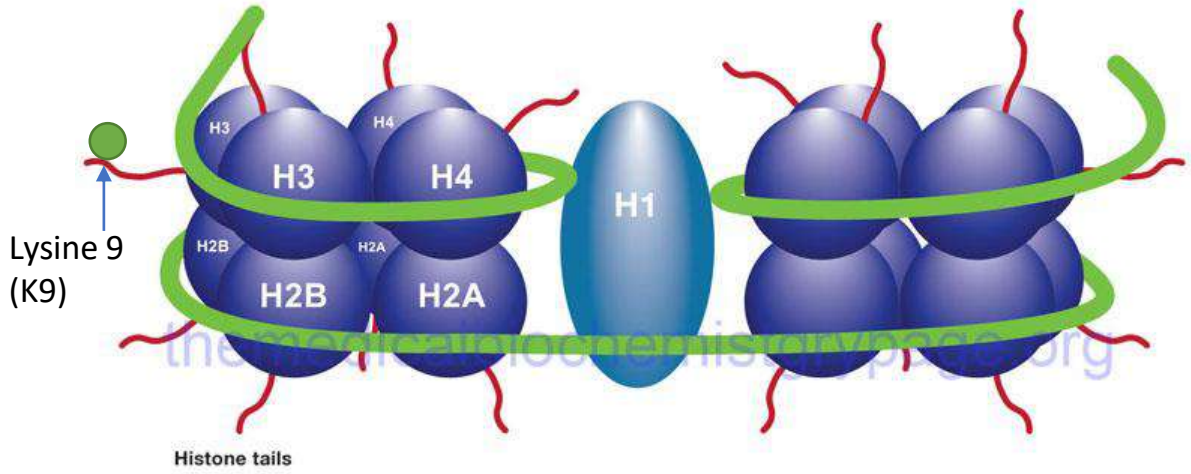
Barbier et al., Mol. Psychiatry 2016

PRDM2?

- PRDM2: PR domain containing 2, with ZNF domain.
- The PRDM2 gene has 2 promoters producing the isoforms RIZ1 and RIZ2
- PRDM2 is evolutionarily conserved
- PRDM2/RIZ1: a tumor suppressor that can arrest the cell cycle and induce apoptosis

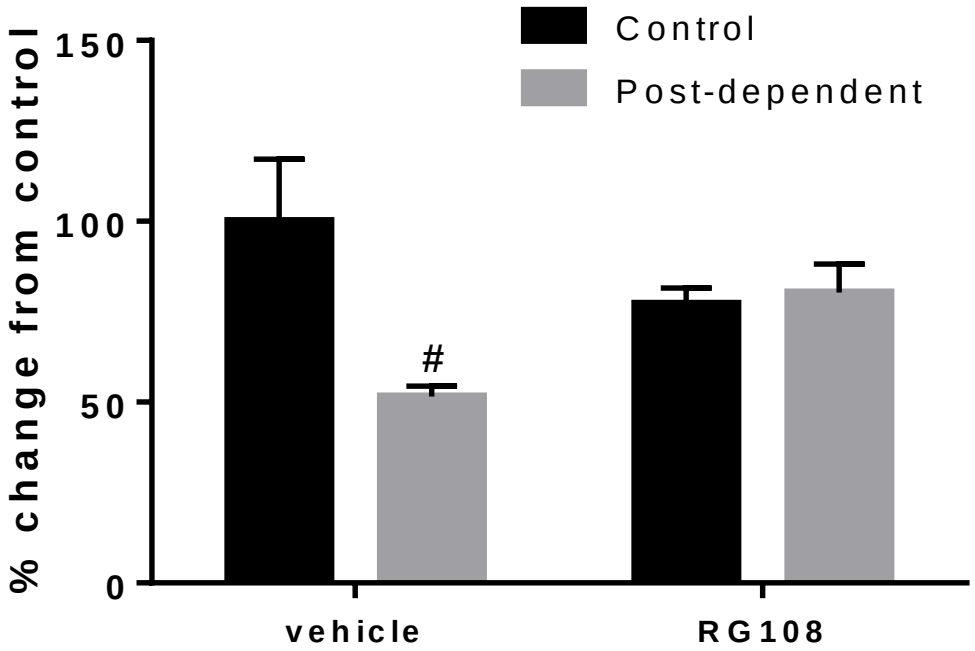
1706 aa in rat, 1718 in humans

Histone methylation: H3 lysine 9 methylation



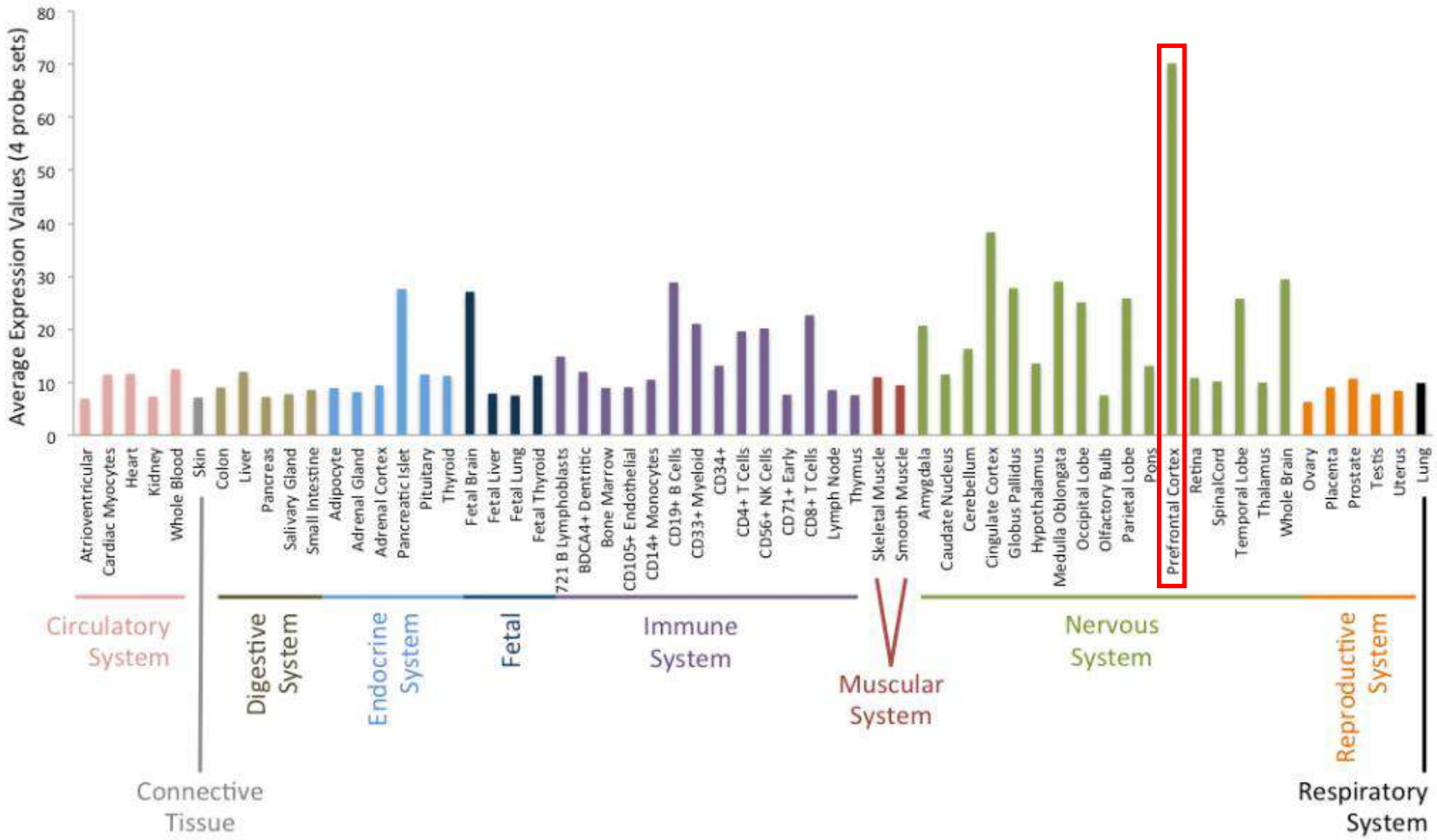
Methyl group

DNMT inhibition blocks alcohol-induced *Prdm2* downregulation in the mPFC



Barbier et al., Mol. Psychiatry 2016

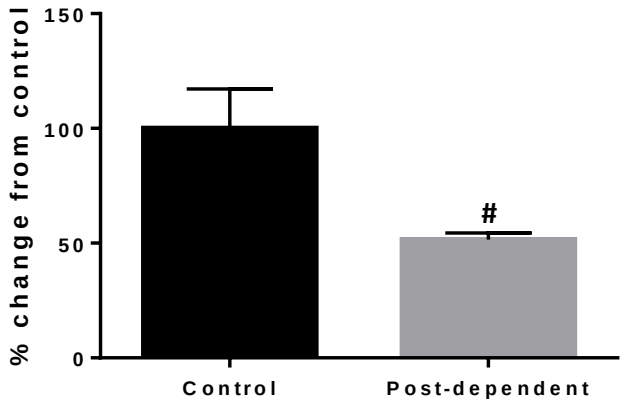
PRDM2 is highly expressed in the mPFC



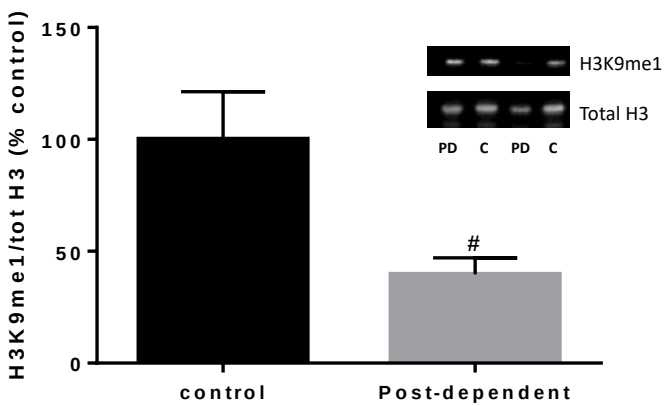
Barbier et al., Mol. Psychiatry 2016

Alcohol exposure decreases the expression of PRDM2 in mPFC of post-dependent rats

↓ Prdm2 mRNA in post-dependent rats



↓ H3K9me1 in post-dependent rats



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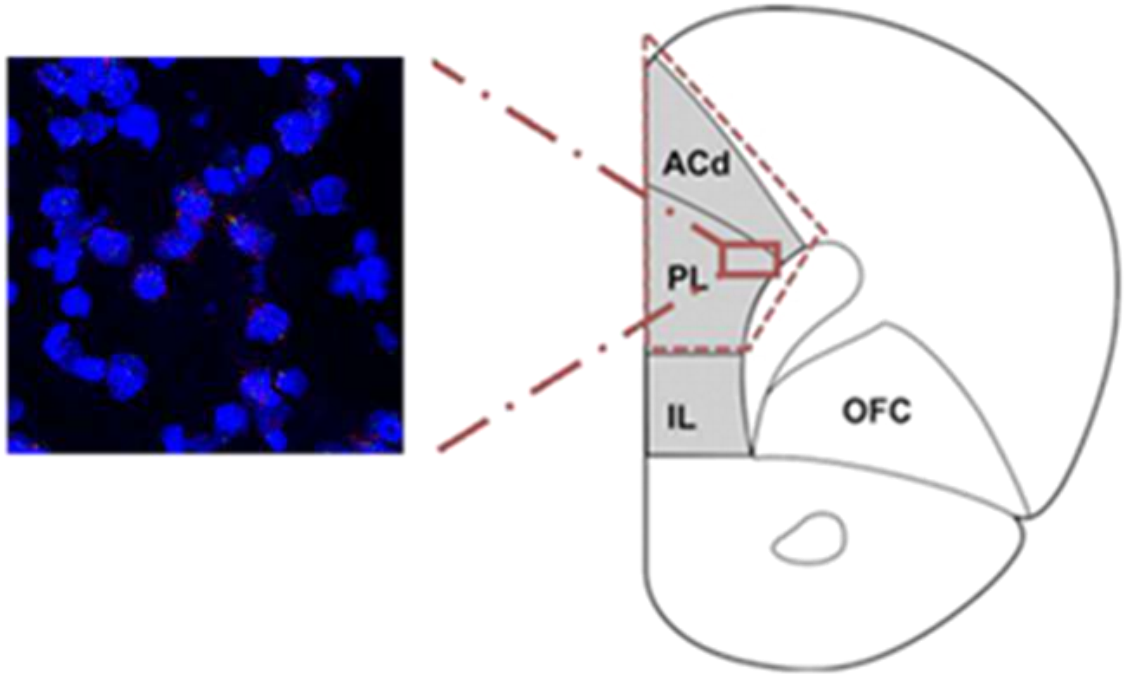
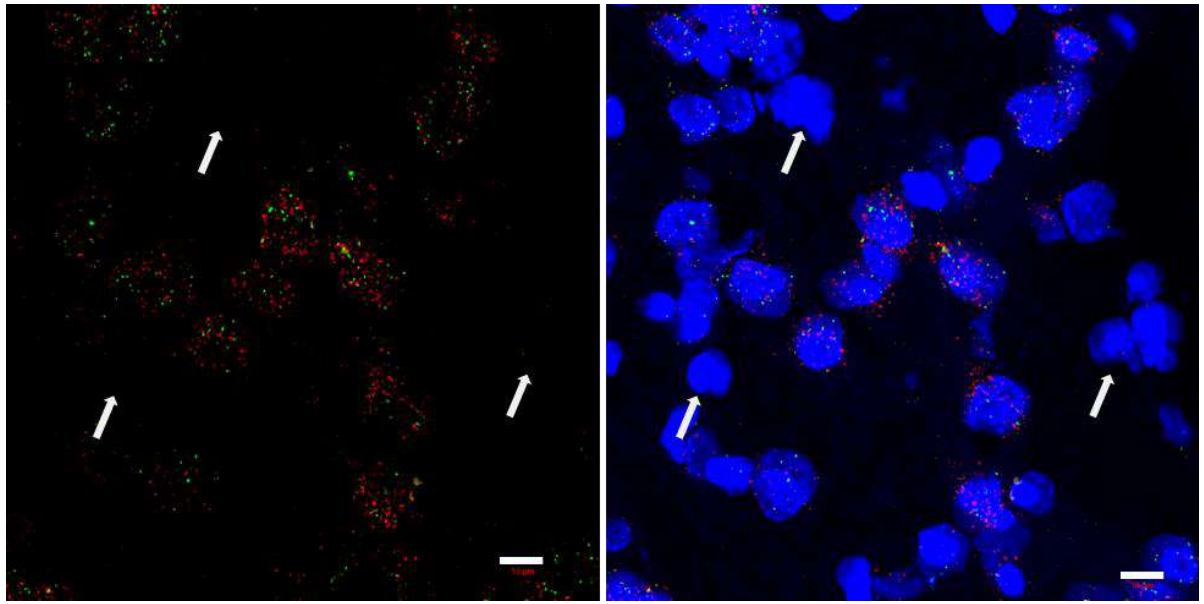
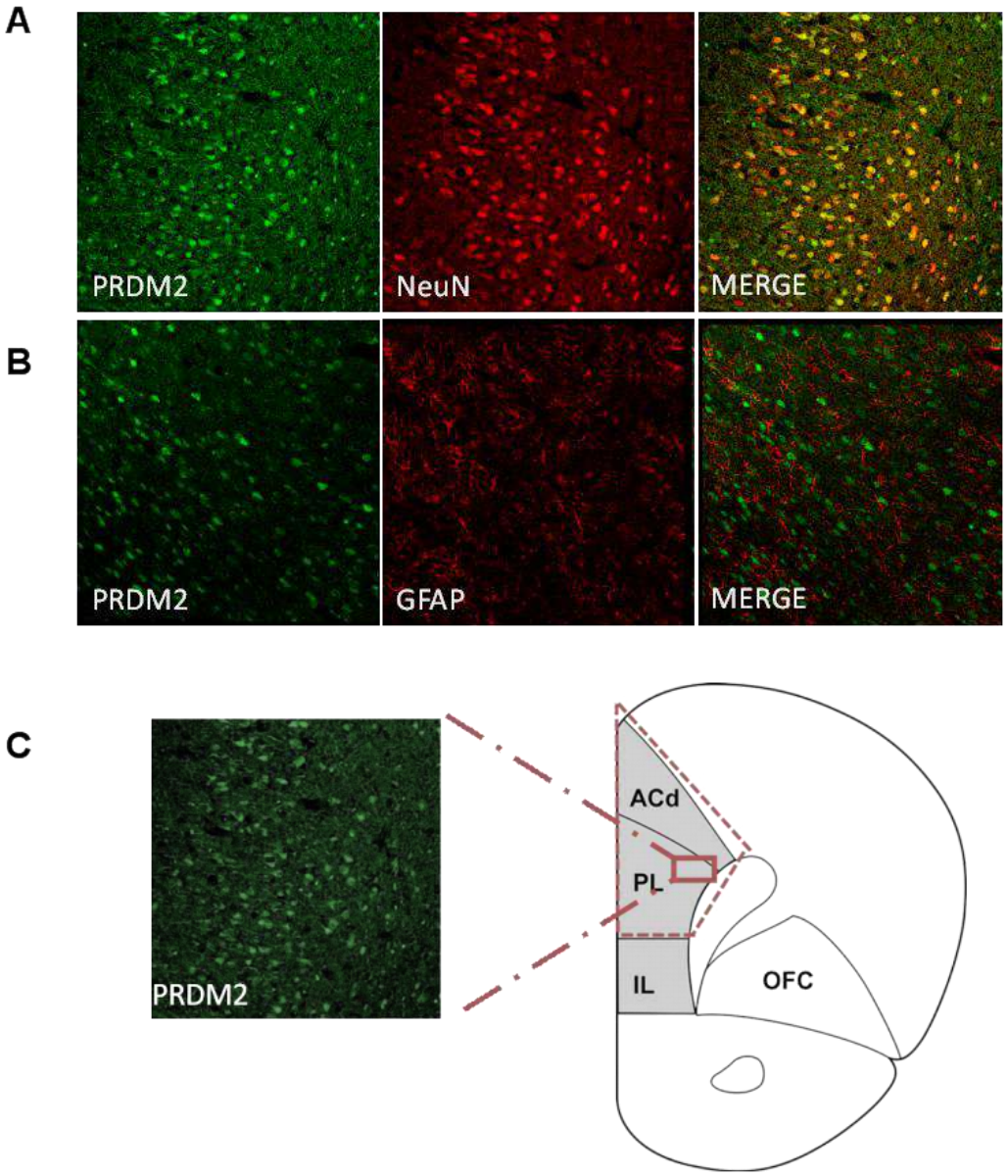
Expression of PRDM2 in the rat mPFC



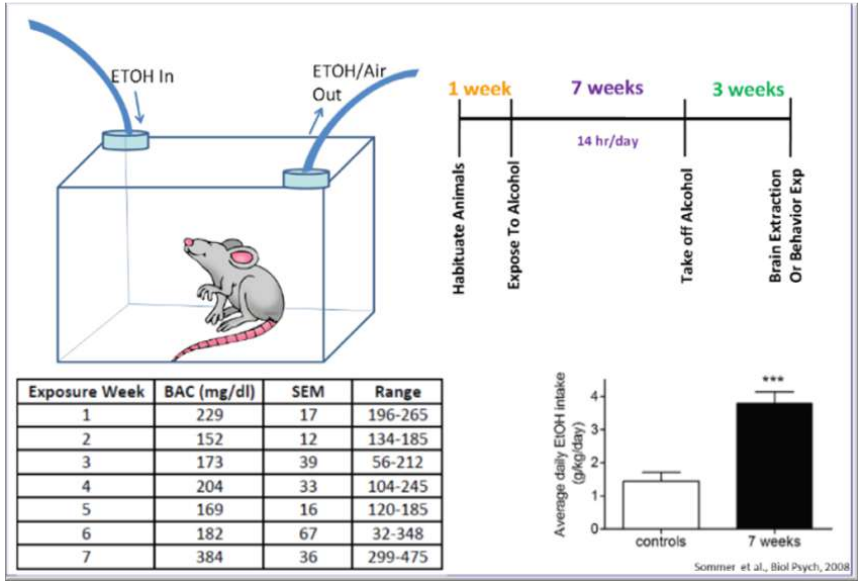


Expression of PRDM2 in the rat mPFC



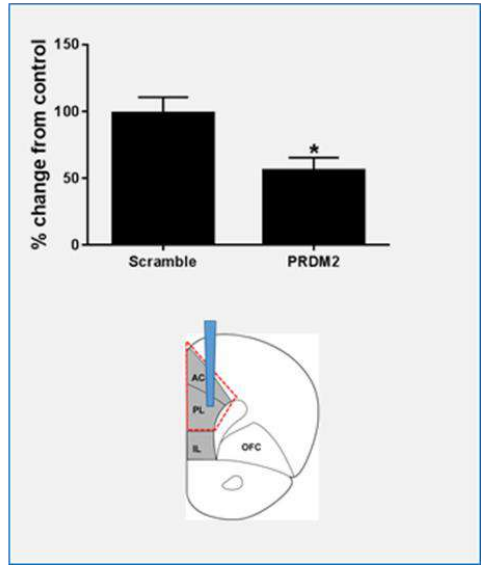
Animal models

Post-dependent rats

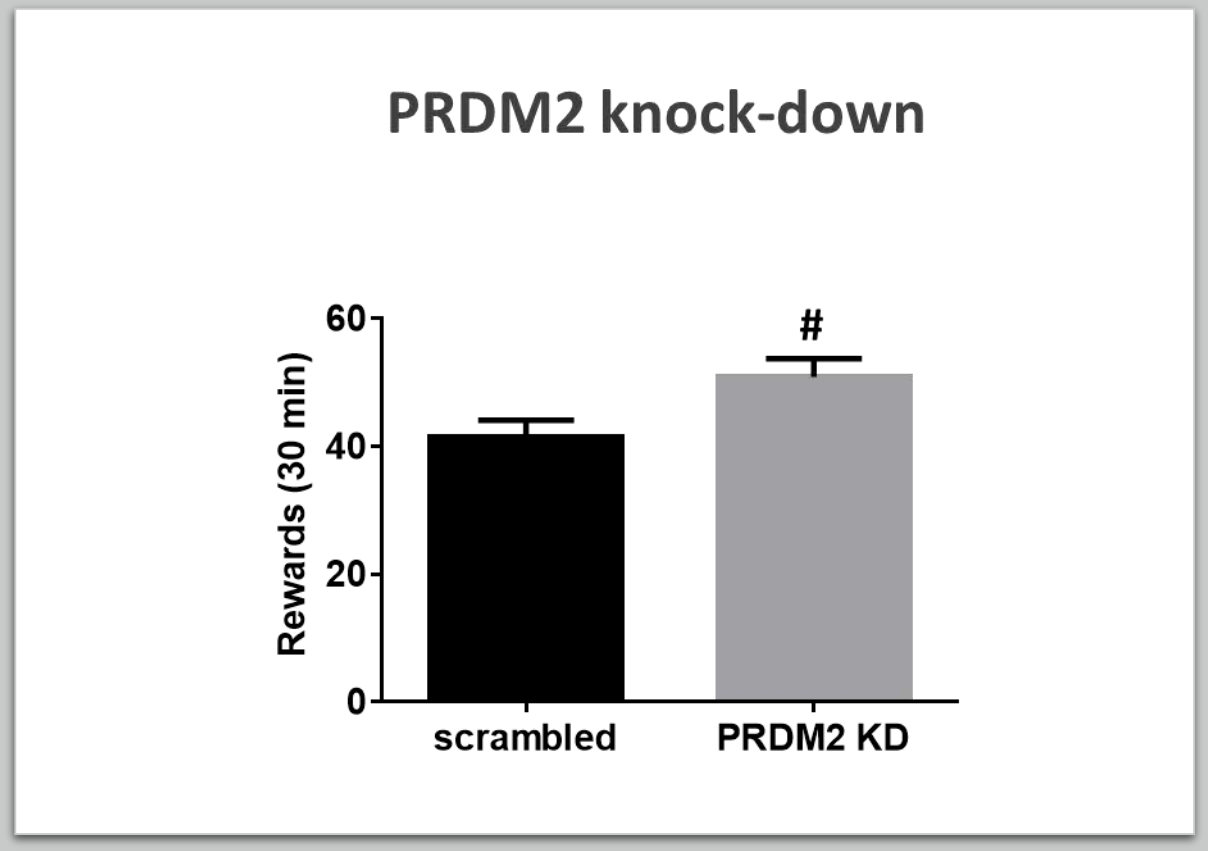


Exposure Week	BAC (mg/dl)	SEM	Range
1	229	17	196-265
2	152	12	134-185
3	173	39	56-212
4	204	33	104-245
5	169	16	120-185
6	182	67	32-348
7	384	36	299-475

Prdm2 knock-down



PRDM2 plays a role in the escalation of alcohol intake



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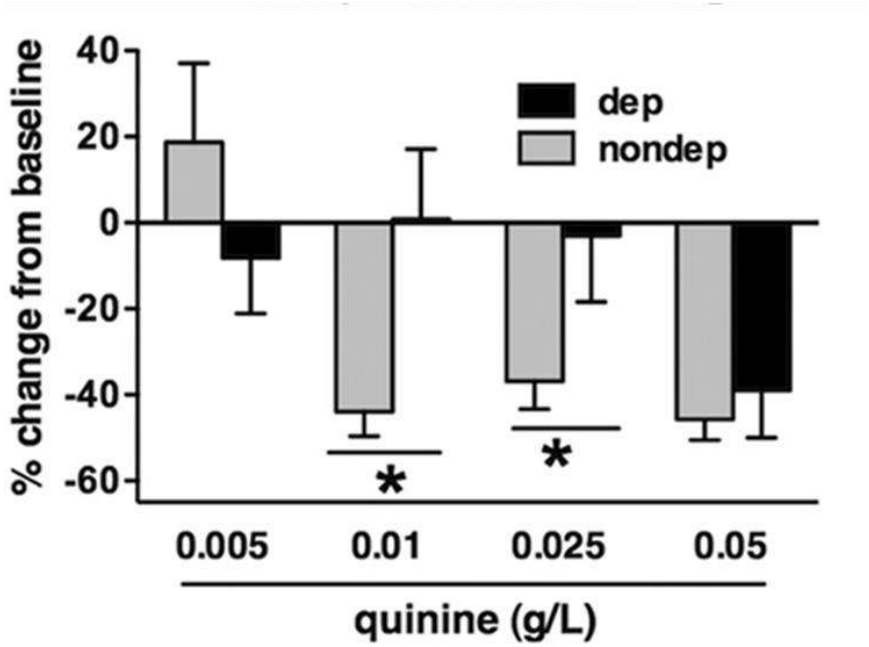
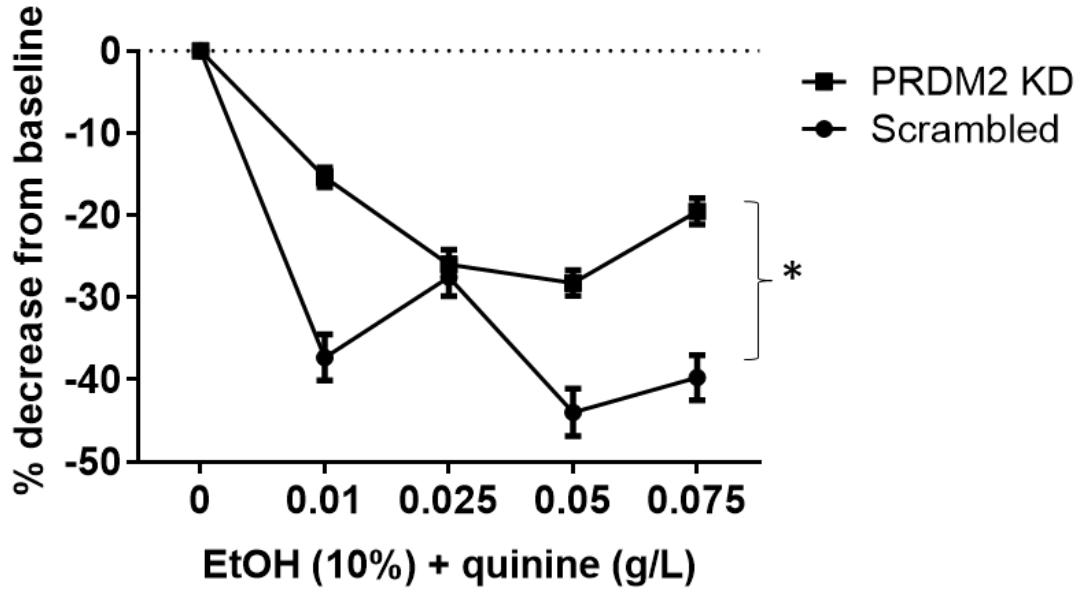
PRDM2 plays a role in compulsive alcohol drinking

Post-dependent rats

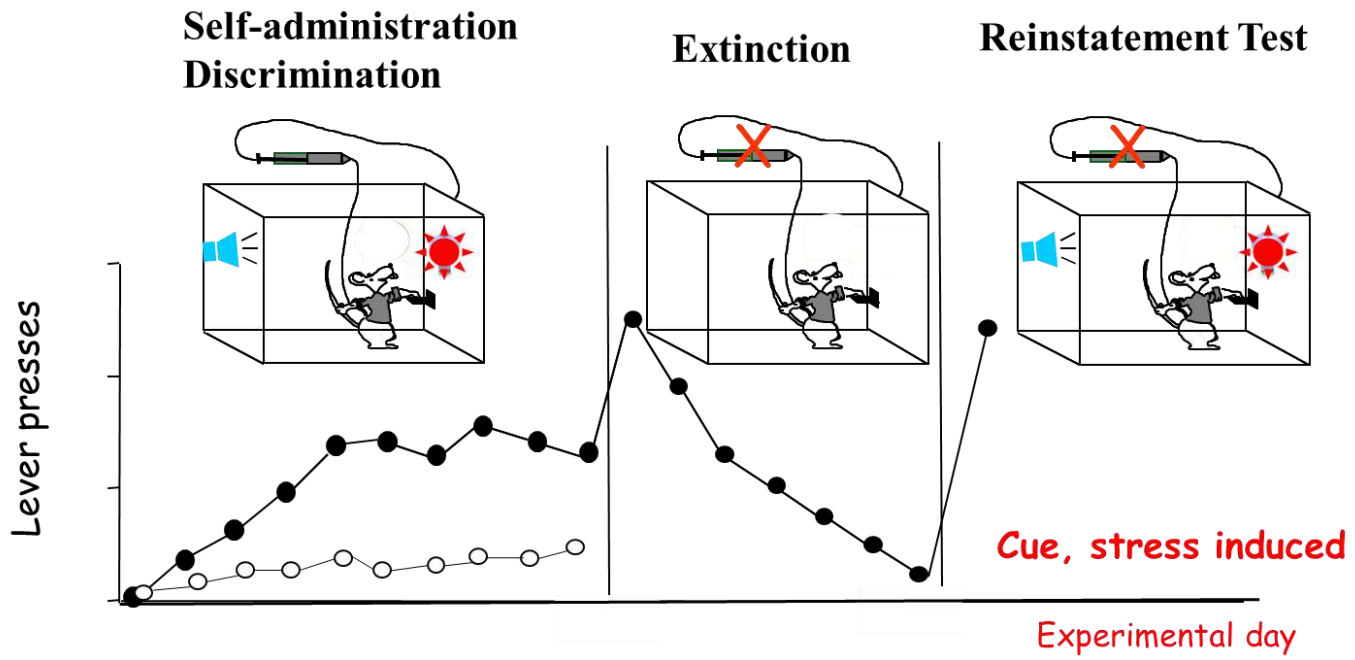


PRDM2 knock-down

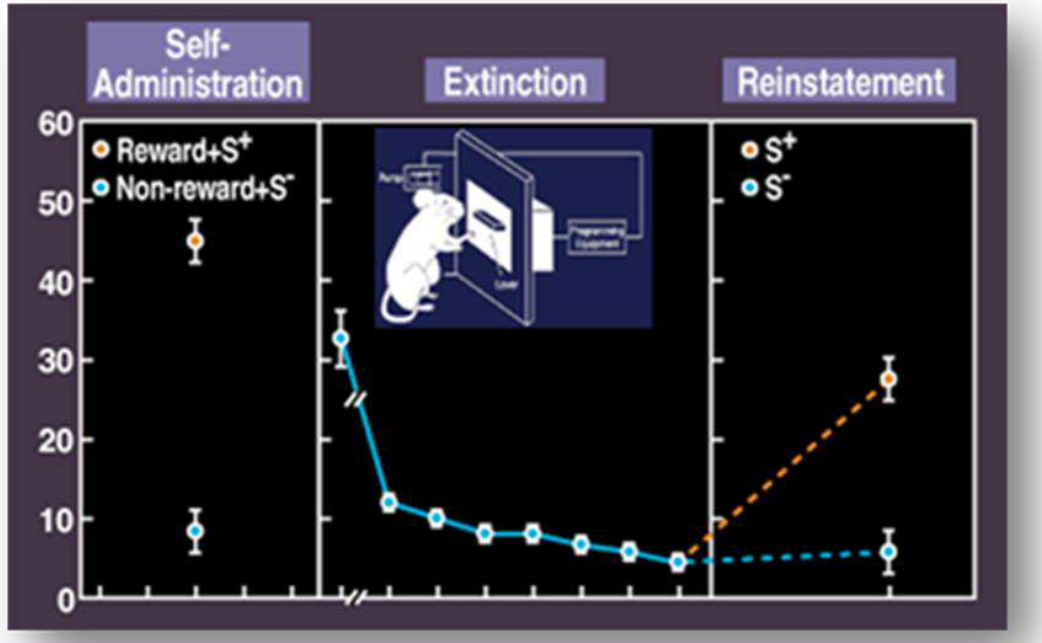


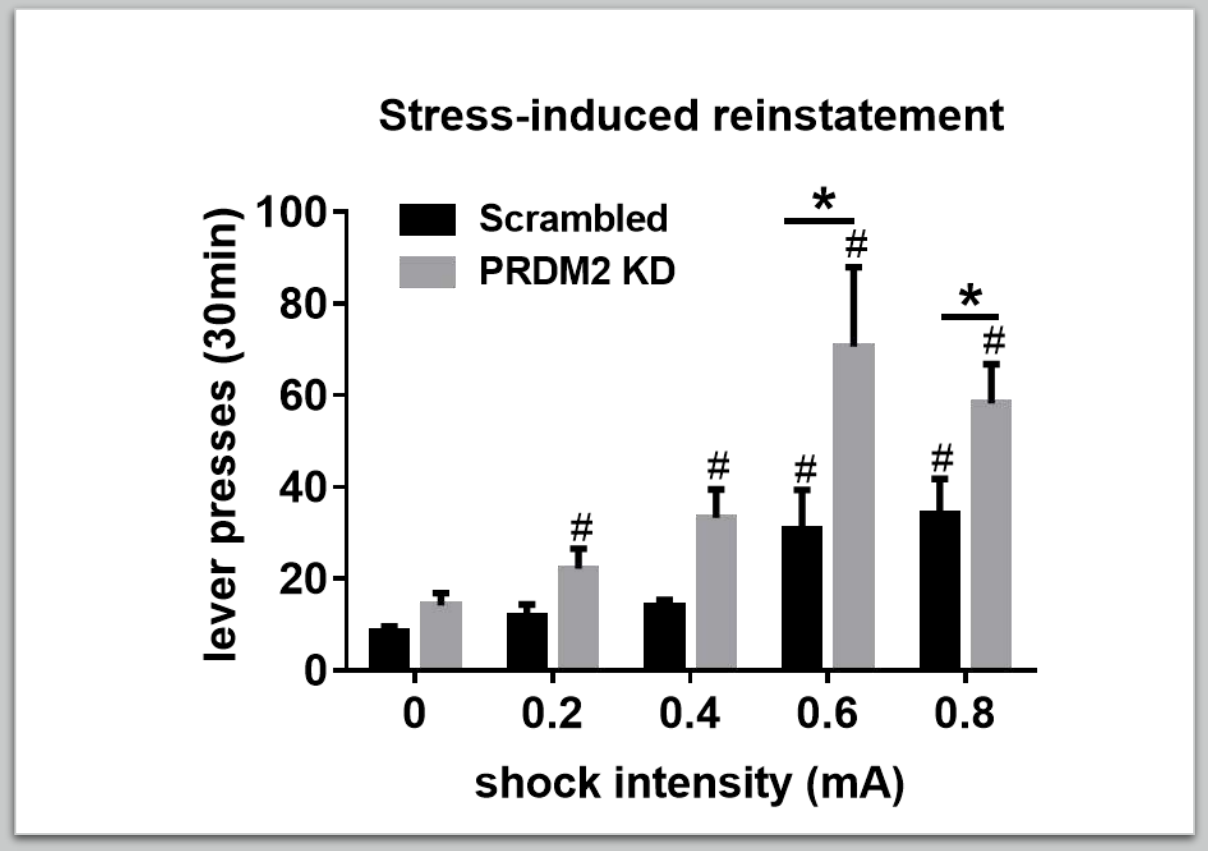
Barbier et al., Mol. Psychiatry 2016

Reinstatement models



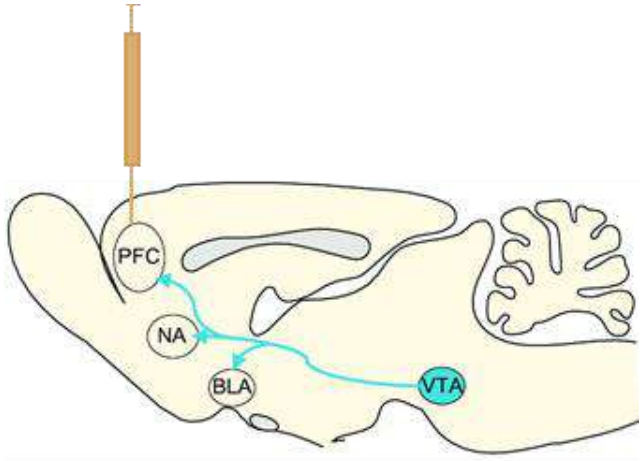
PRDM2 plays a role in stress-induced relapse



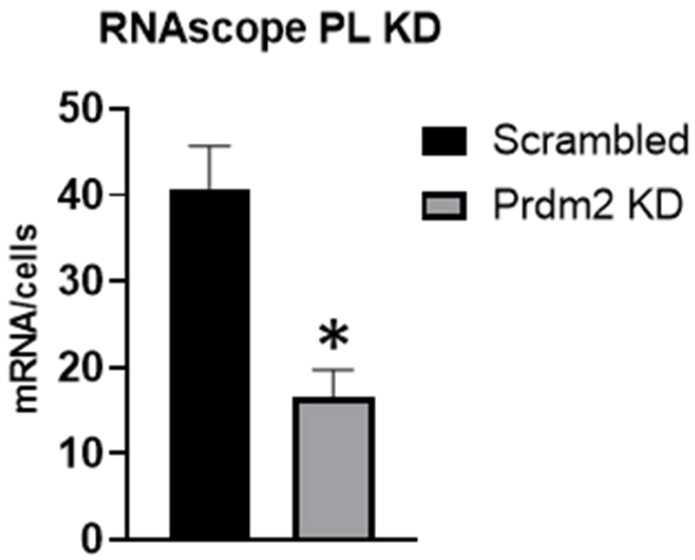


Barbier et al., Mol. Psychiatry 2016

Role of PRDM2 in fear memory

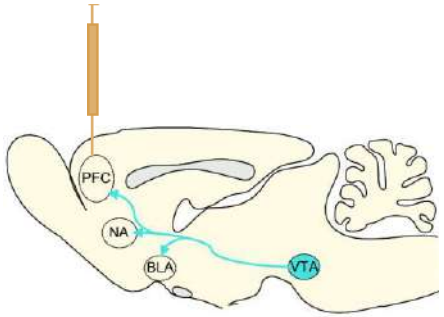


AAV9-shRNA-Prdm2

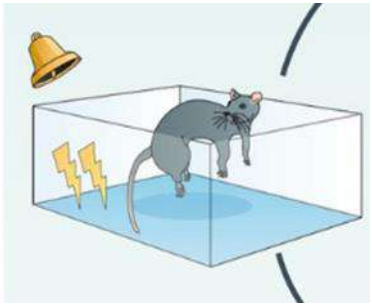


Role of PRDM2 in fear memory

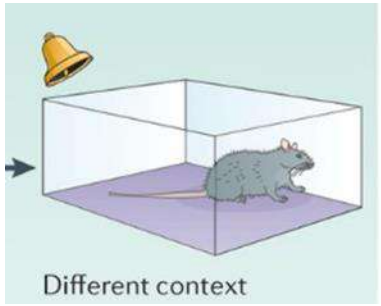
4 weeks | Day 1 | Day 2 | Day 3/4



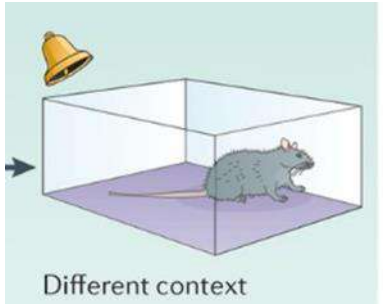
AAV9-shRNA-Prdm2



Fear Conditioning

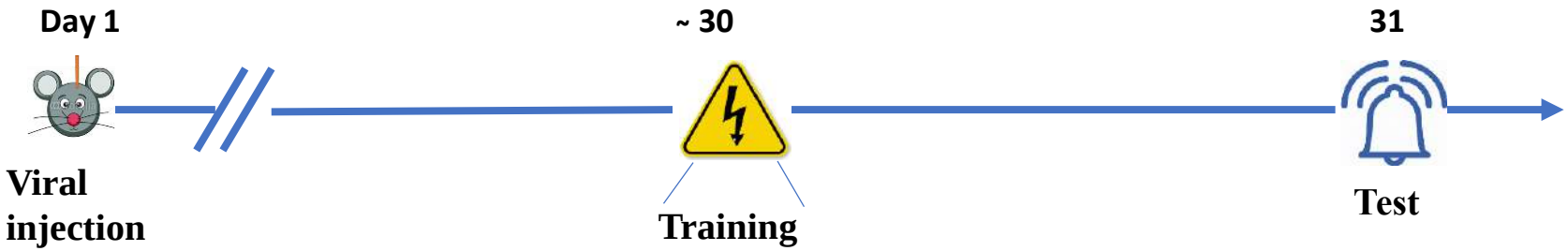


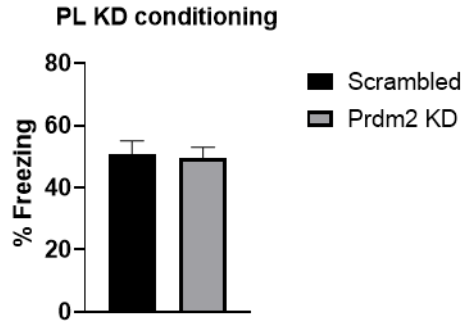
Conditioned Fear expression



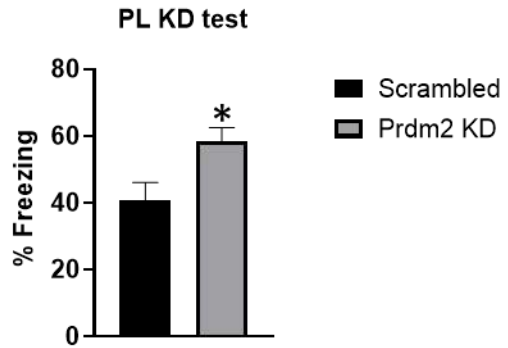
Conditioned fear extinction

Role of PRDM2 in fear expression



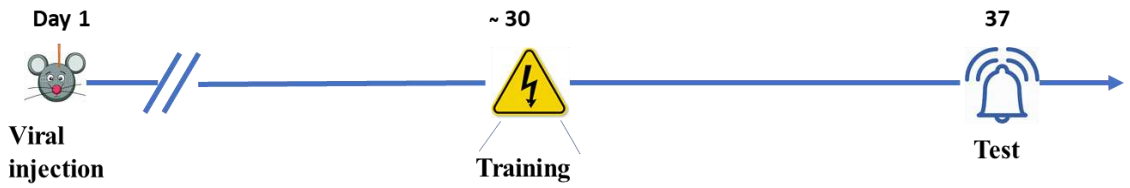


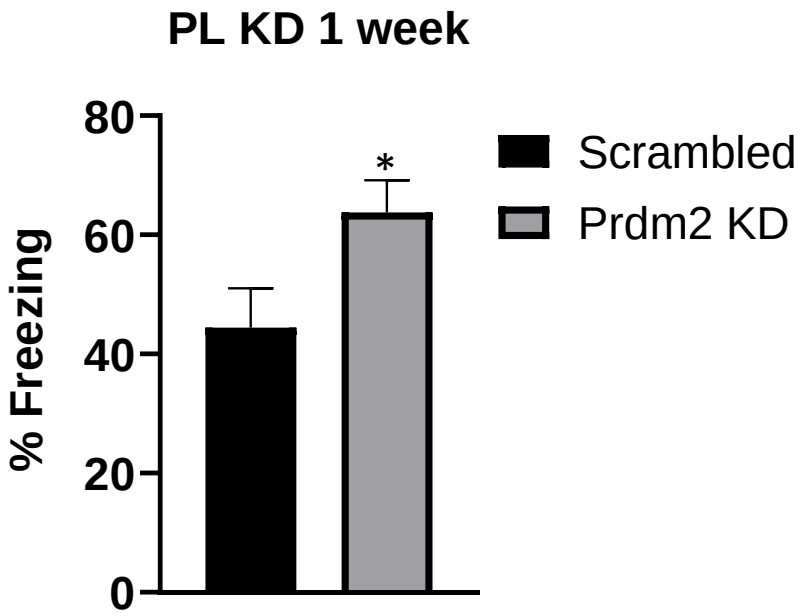
Acquisition



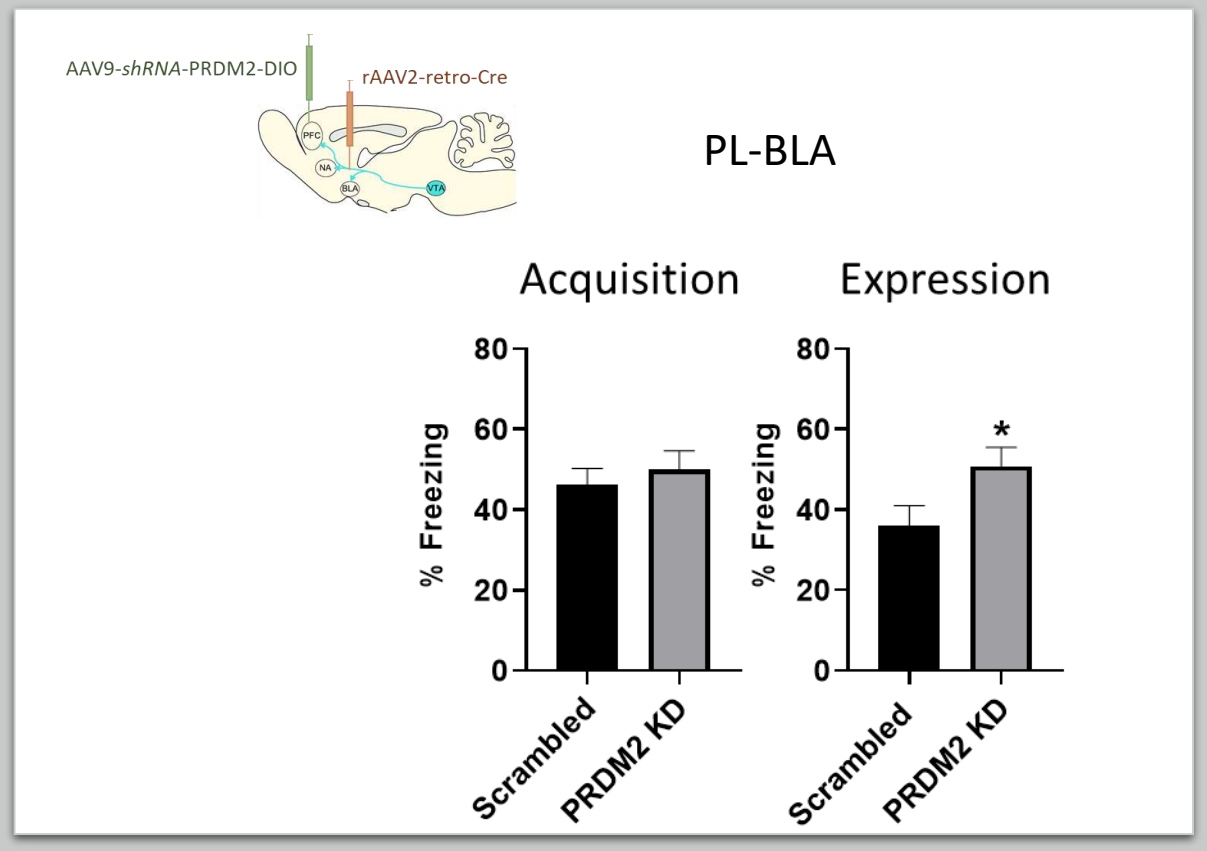
Expression

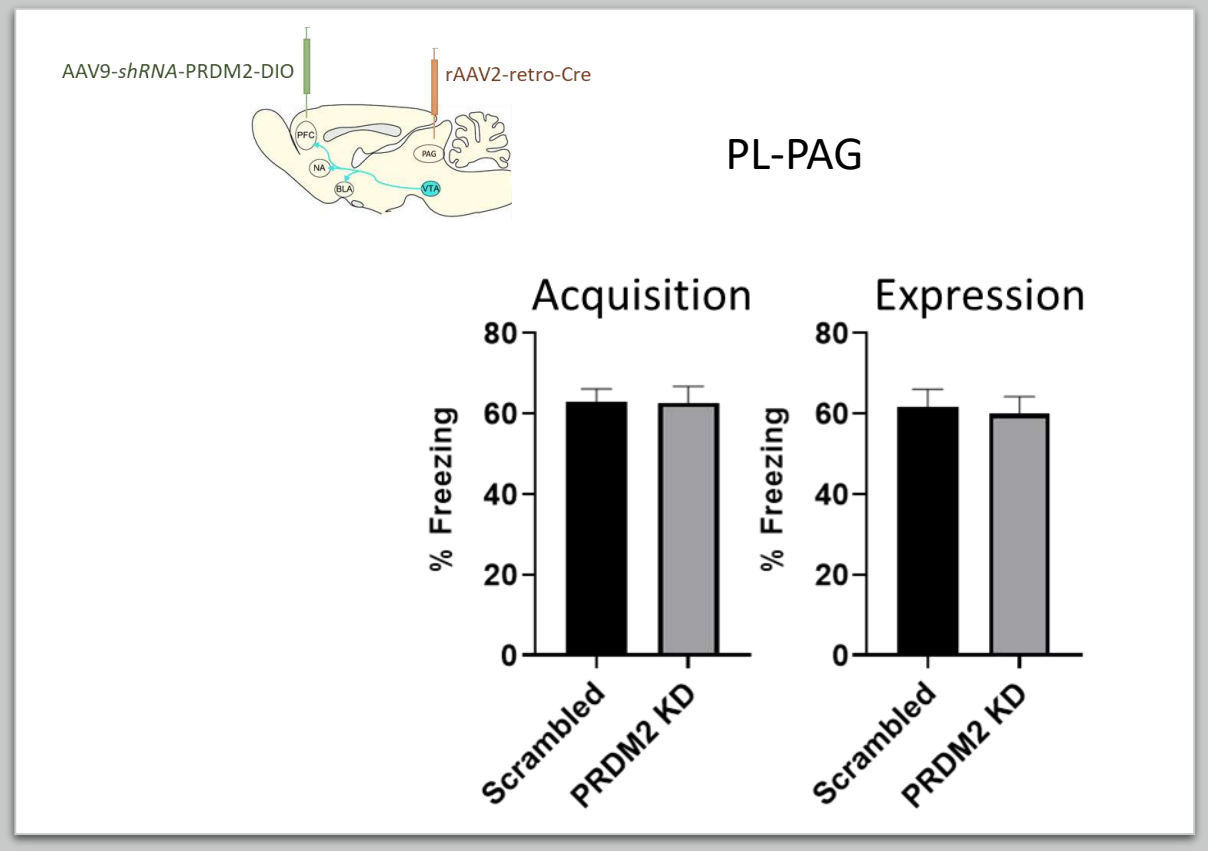
Role of PRDM2 in fear expression



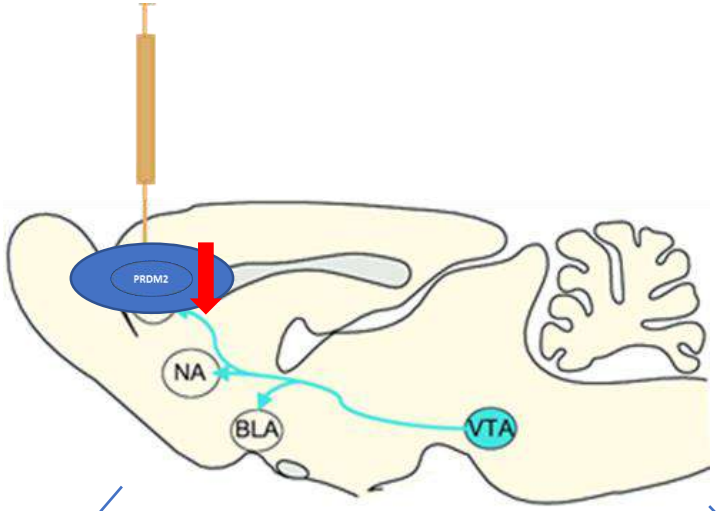


PRDM2 KD PL-BLA increases fear expression





Summary



Alcohol associated behaviors

↑ alcohol consumption

↑ Stress-induced relapse

↑ Fear expression

↑ Freezing

Thank you!!!

Collaborators

- Louise Adermark, Göteborg University (Sweden)
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Kanat Chanthongdee