

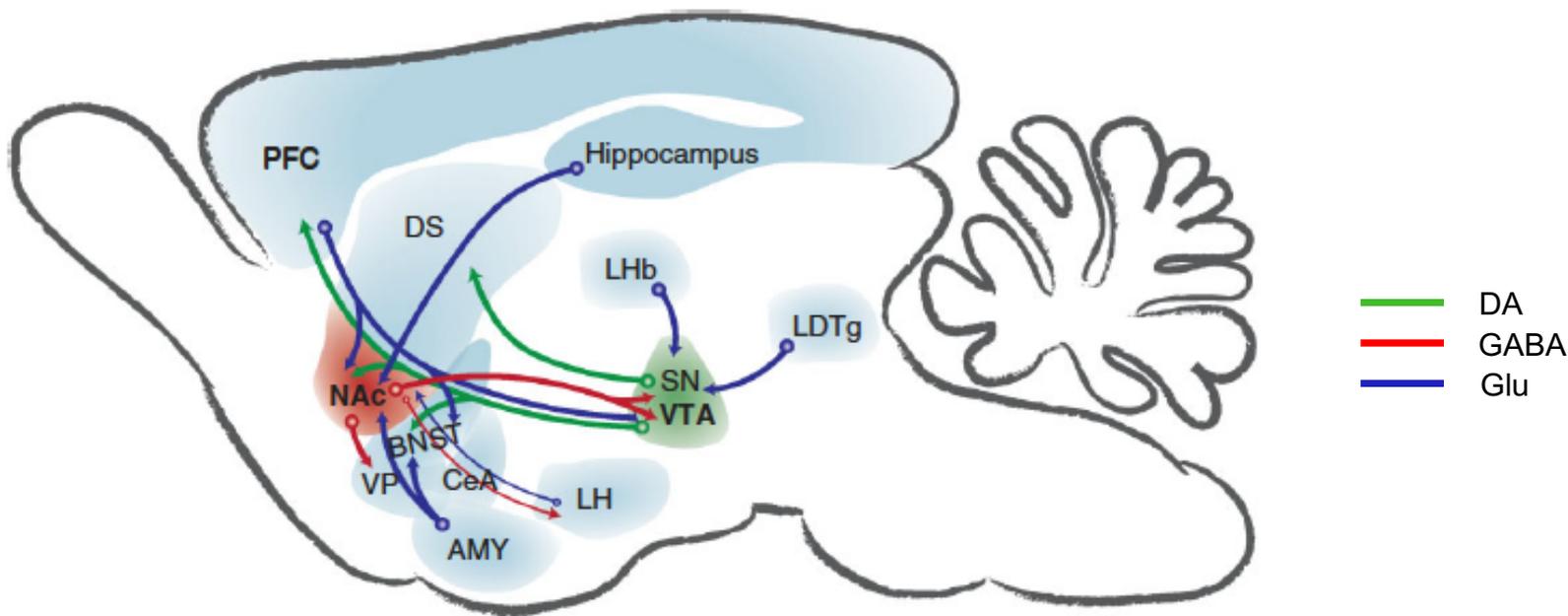
Neurobiologie et épigénétique des opiacés

Florence Noble, PhD

CNRS ERL3649, INSERM UMR1124
Université de Paris

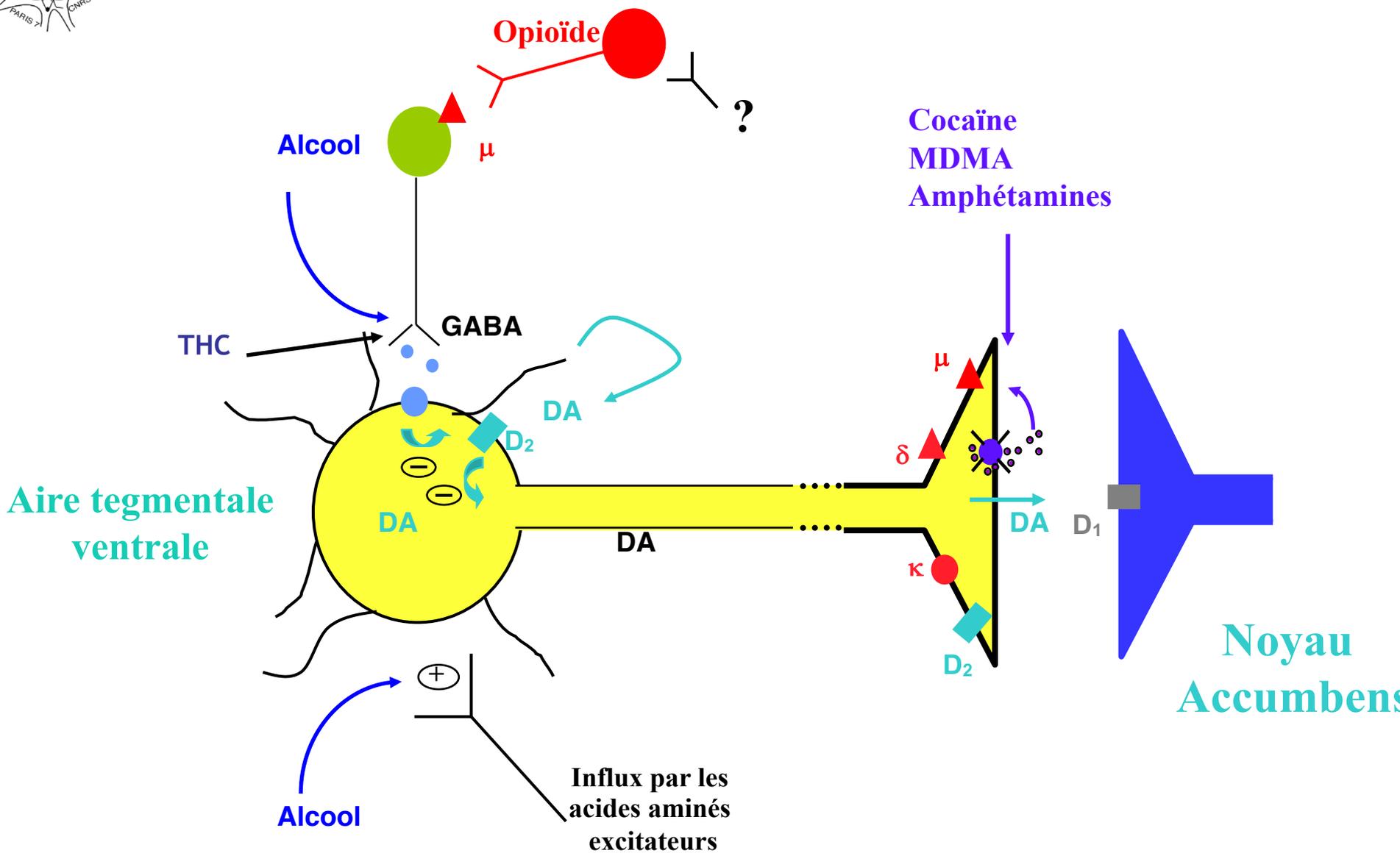
Pharmacologie et Thérapies des Addictions

Schéma simplifié du circuit de la récompense chez le rongeur



Joffe et al. (2014) *WIREs Cogn. Sci.* 5:151-171

AMY, amygdala; BNST, bed nucleus of the stria terminalis; CeA, central nucleus of the amygdala; DS, dorsal striatum; LDTg, laterodorsal tegmentum; LDb, lateral habenula; LH, lateral hypothalamus; PFC, prefrontal cortex; SN, substantia nigra; VP, ventral pallidum.



**Circuit mésocorticolimbique
+
Dopamine**

**Rôle clé dans les mécanismes
de dépendance**

**Circuit mésocorticolimbique
+
Dopamine**

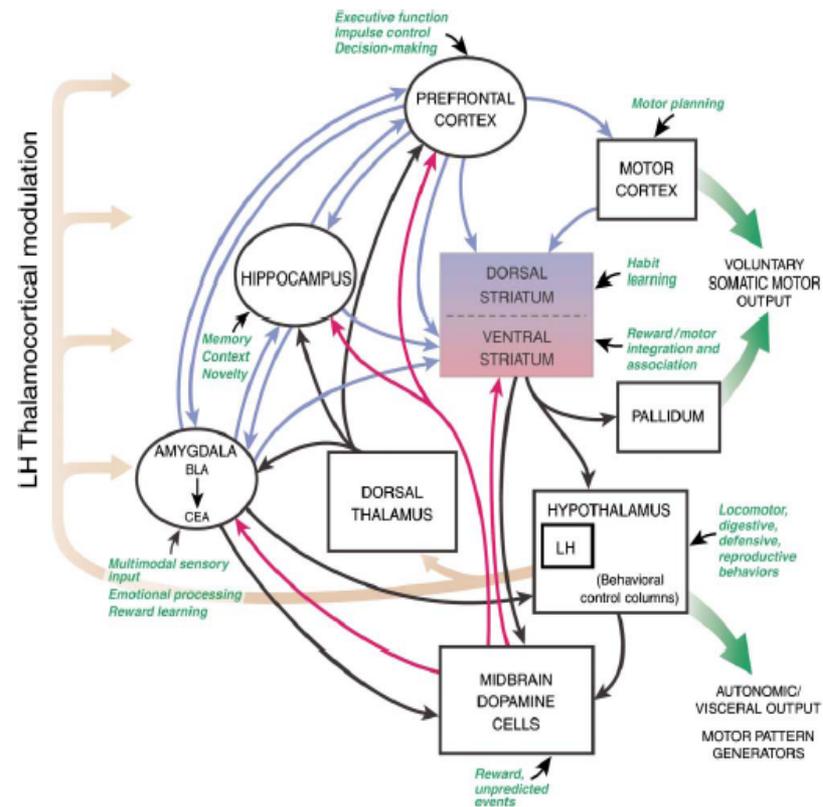
**Rôle clé dans les mécanismes
de dépendance**



Neurotransmetteurs et circuits impliqués dans les addictions

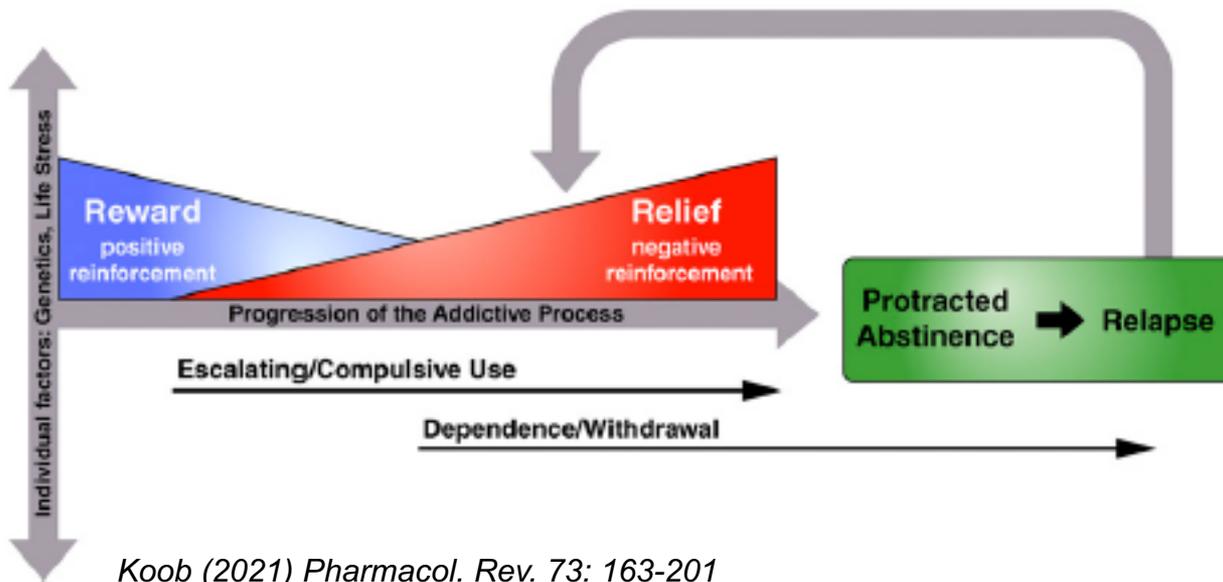
Noyau accumbens
Aire tegmentale ventrale
Cortex préfrontal
Striatum
Insula
Hippocampe
Amygdale
 ...

Sérotonine
GABA
Glutamate
CRF
Peptides opioïdes
Endocannabinoïdes
Orexine
 ...



Kelly A.E., 2004

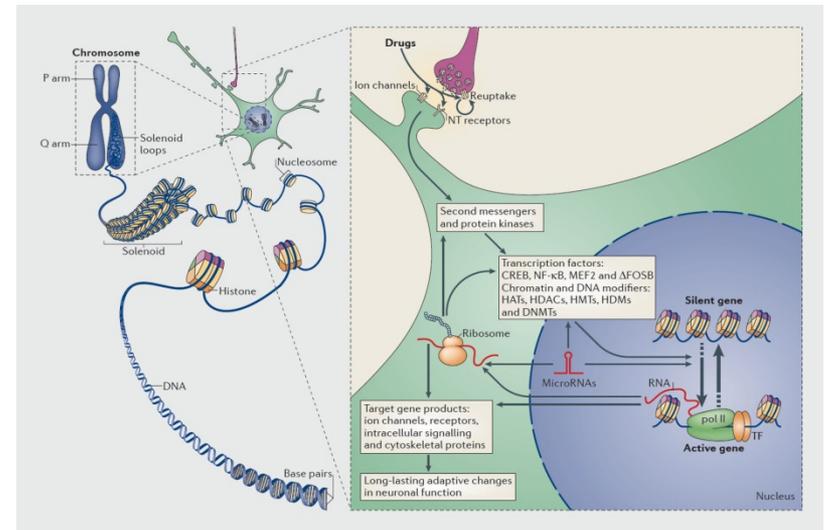
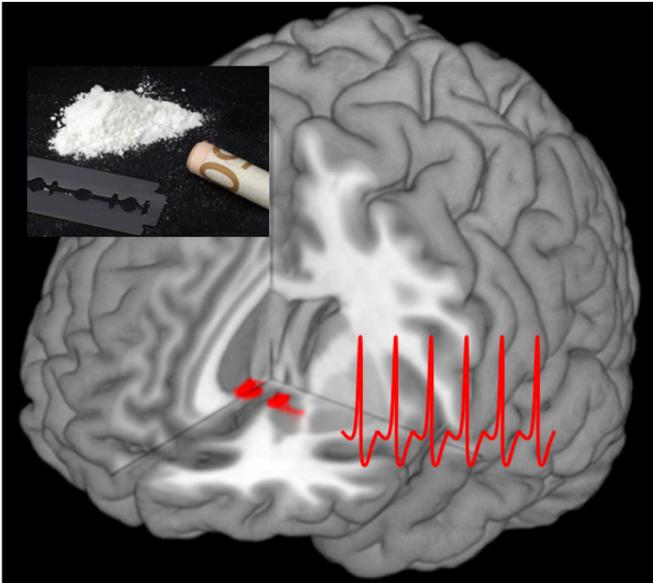
Les addictions: différentes étapes avec des processus de neuroadaptations



Koob (2021) *Pharmacol. Rev.* 73: 163-201

Neuroadaptations

- Structural
- Moléculaire
- Cellulaire
- Epigénétiques



Alterations in amygdala in prescription opioid-dependent patients

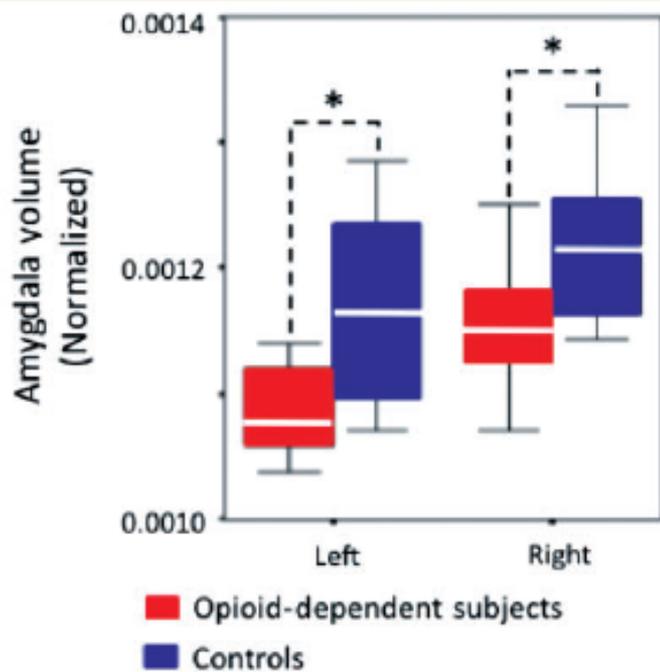
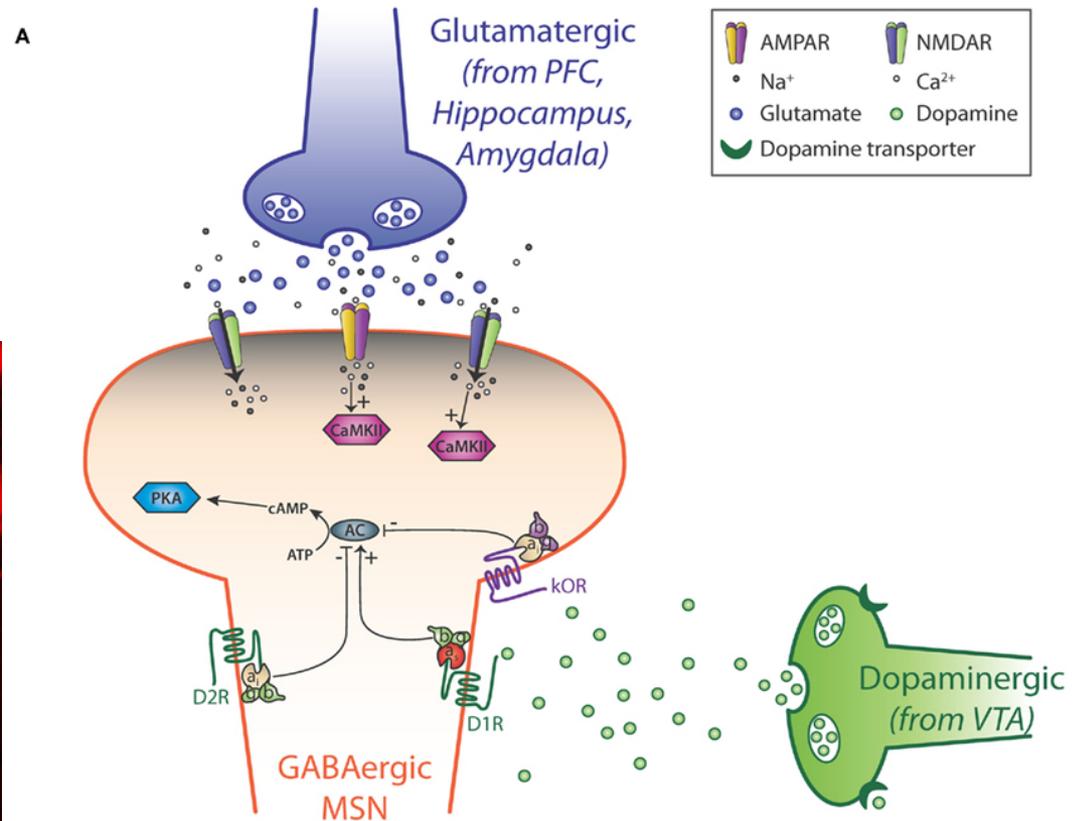
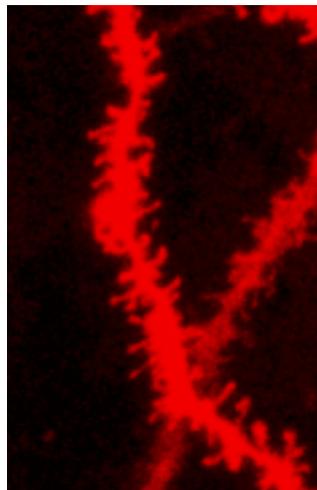
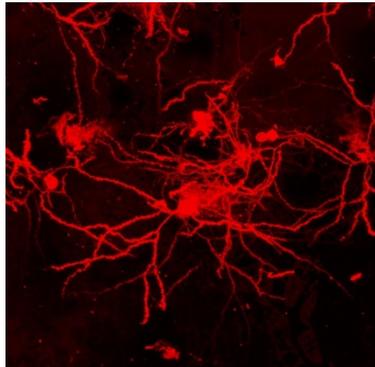
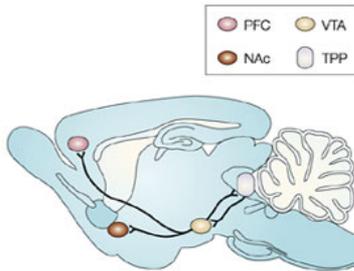


Figure 2 Decreases in mean amygdala volume in opioid-dependent subjects. Note that volumes have been normalized to the total intracranial volume to scale for brain volume. White lines represent the mean value for each volumetric measurement, while the length of each box represents the variance. Error bars represent the 95% confidence interval of the mean. $*P < 0.05$.

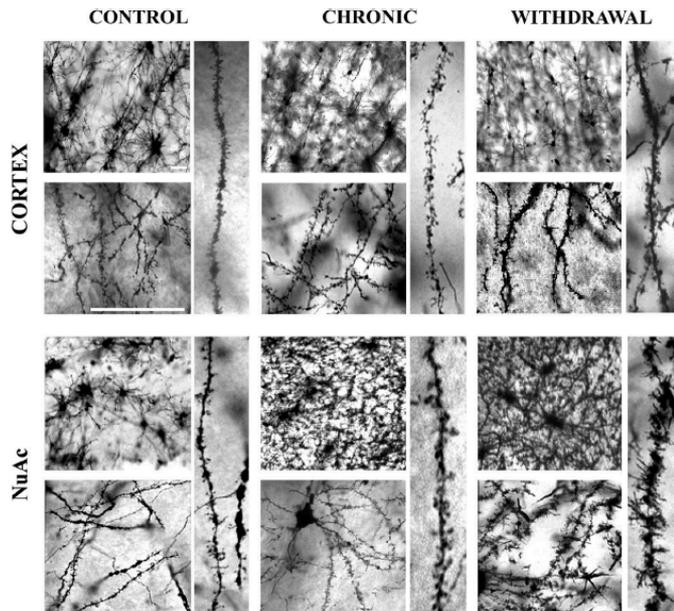
Upadhyay et al., 2010, Brain 133: 2098-2114

Neurones épineux dans le noyau accumbens



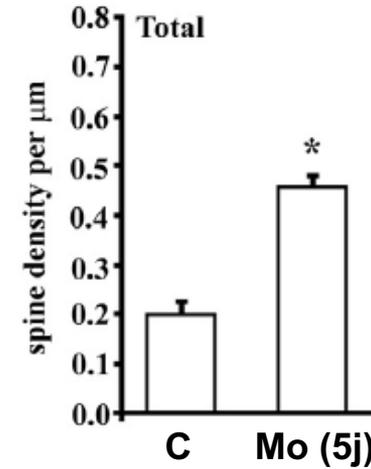
(Eipper-Mains, 2012)

Augmentation de la densité des épines dendritiques après traitement à la morphine chez la souris

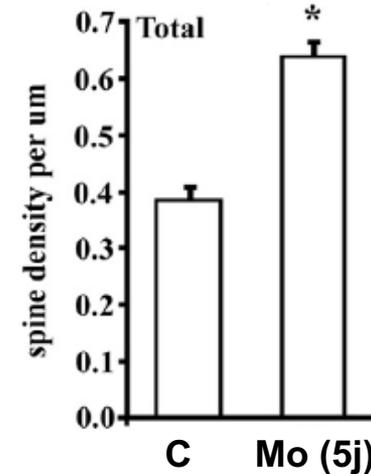


Pal and Das (2013) *Neurochem. International* 62: 956-964

CORTEX

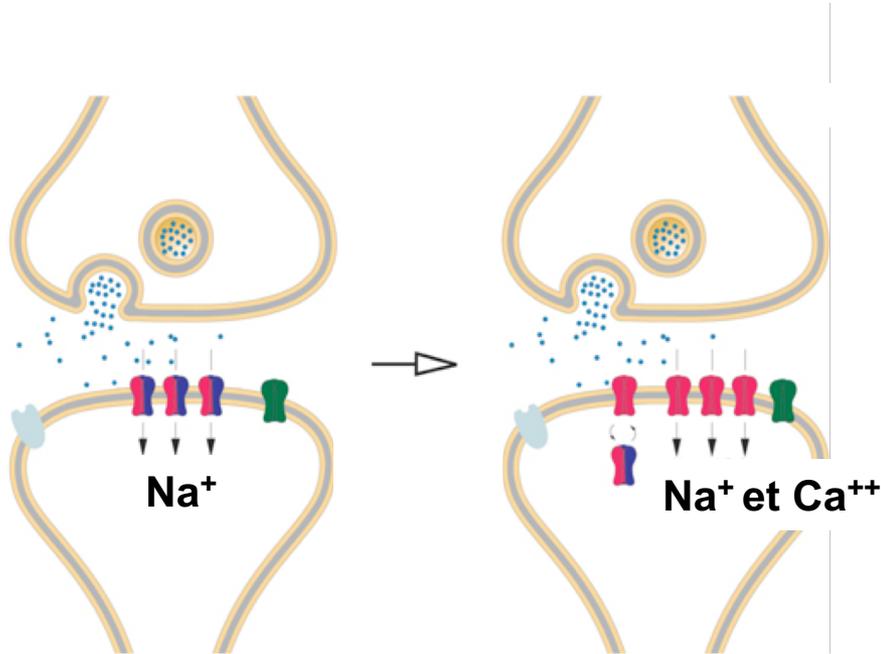


NuAc



Modification dans la composition des récepteurs AMPA et entrée de Ca^{2+}

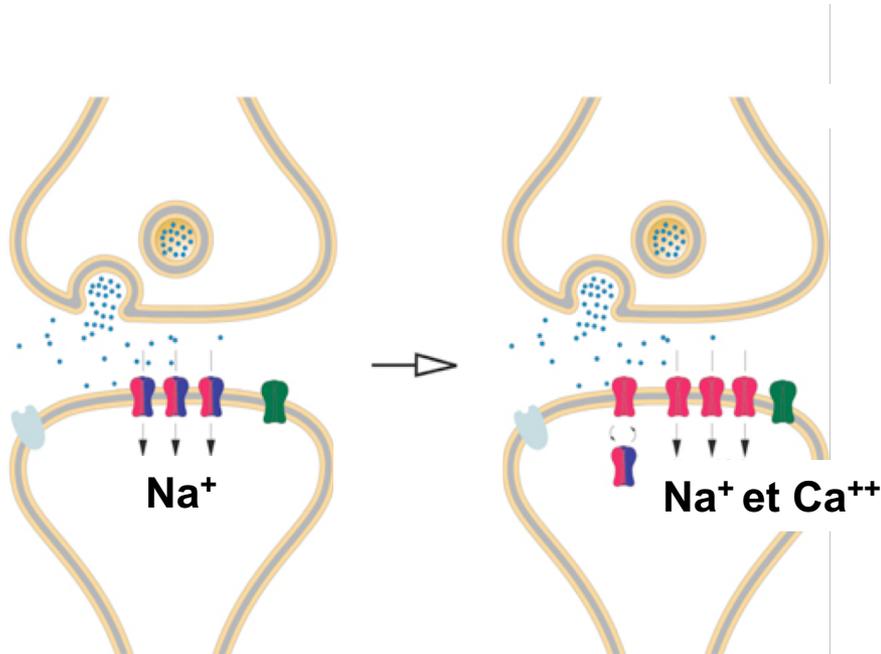
- GluA1/2 heteromers 
- GluA1 homomers 
- NMDARs 
- Group 1 mGluRs 



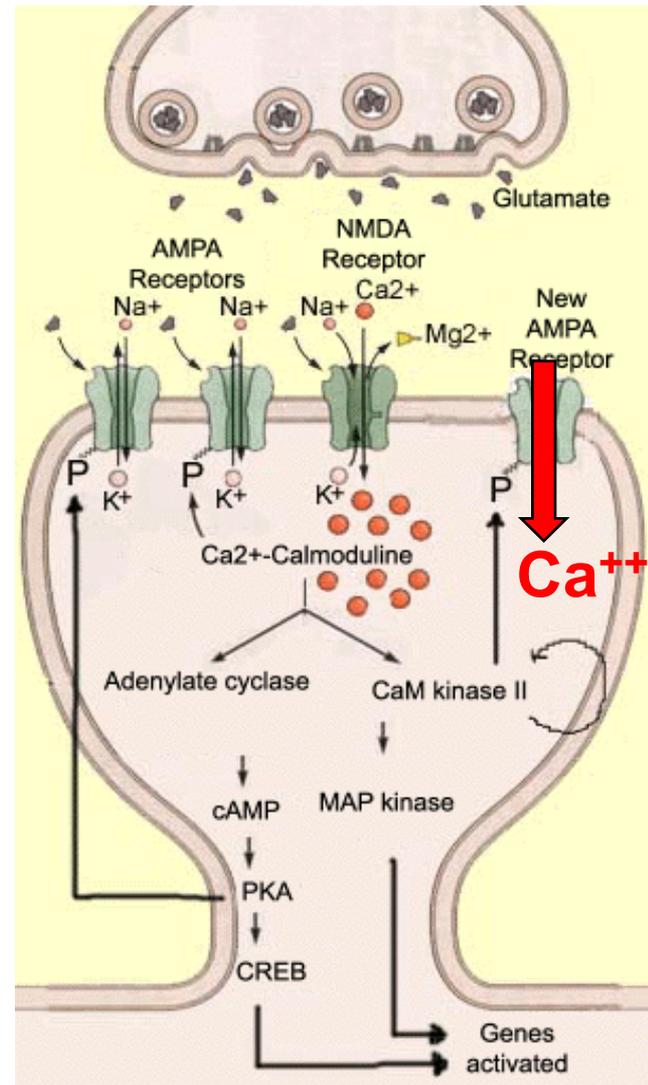
Traitement chronique opioïde 

Modification dans la composition des récepteurs AMPA et entrée de Ca^{2+}

- GluA1/2 heteromers
- GluA1 homomers
- NMDARs
- Group 1 mGluRs

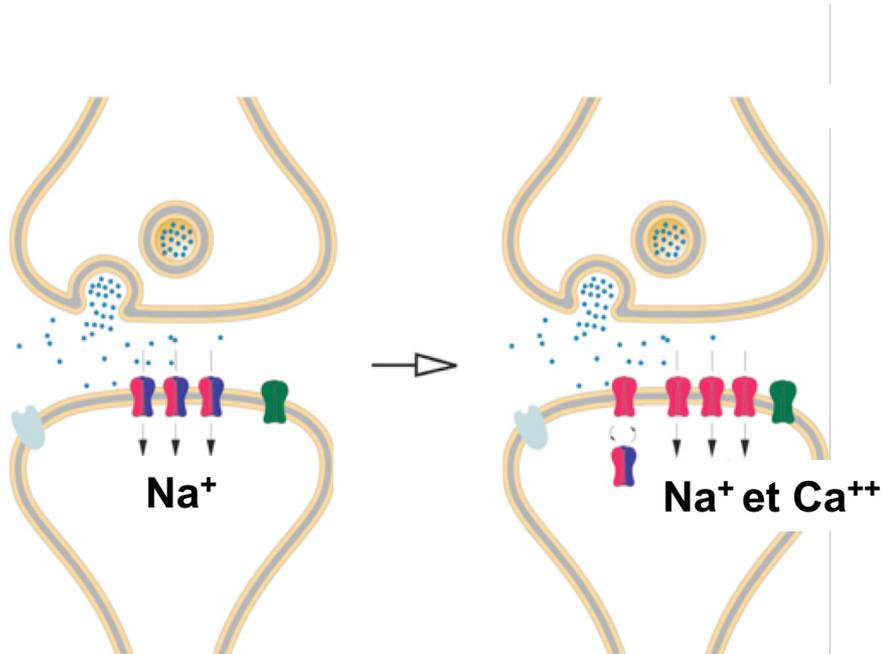


Traitement chronique opioïde

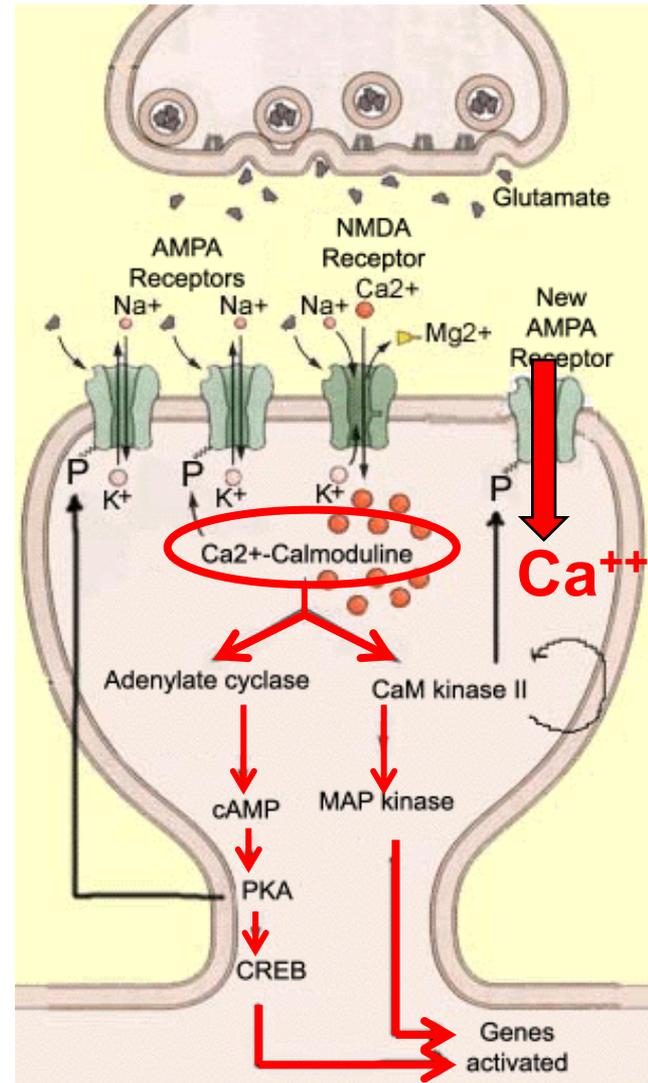


Modification dans la composition des récepteurs AMPA et entrée de Ca^{2+}

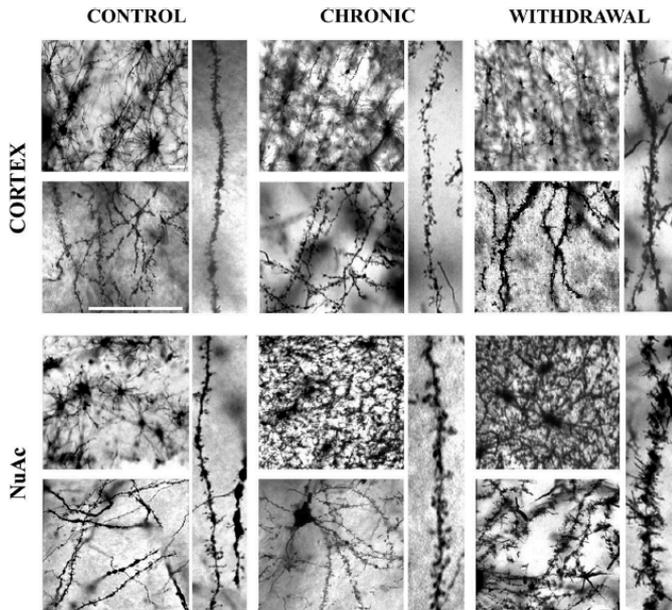
- GluA1/2 heteromers
- GluA1 homomers
- NMDARs
- Group 1 mGluRs



Traitement chronique opioïde



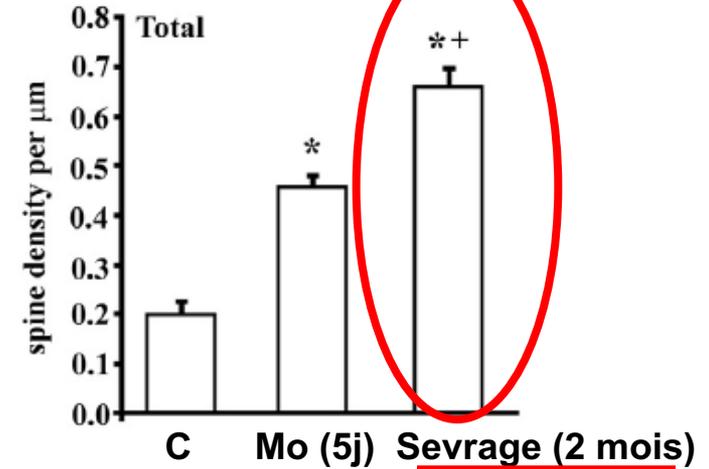
Augmentation de la densité des épines dendritiques après traitement à la morphine chez la souris



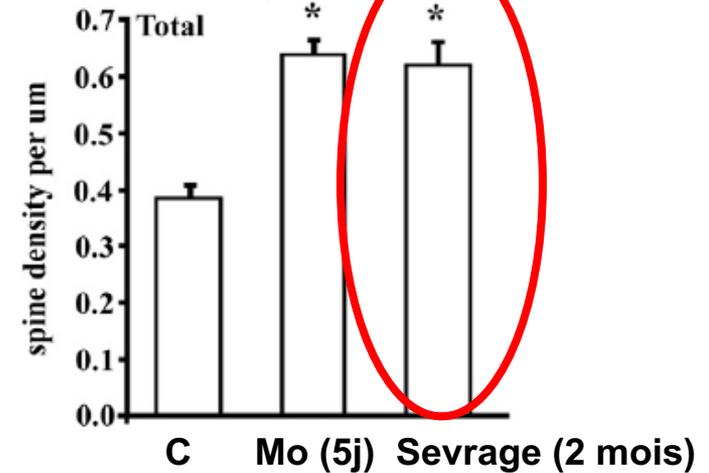
Pal and Das (2013) *Neurochem. International* 62: 956-964

👉 Modifications épigénétiques

CORTEX

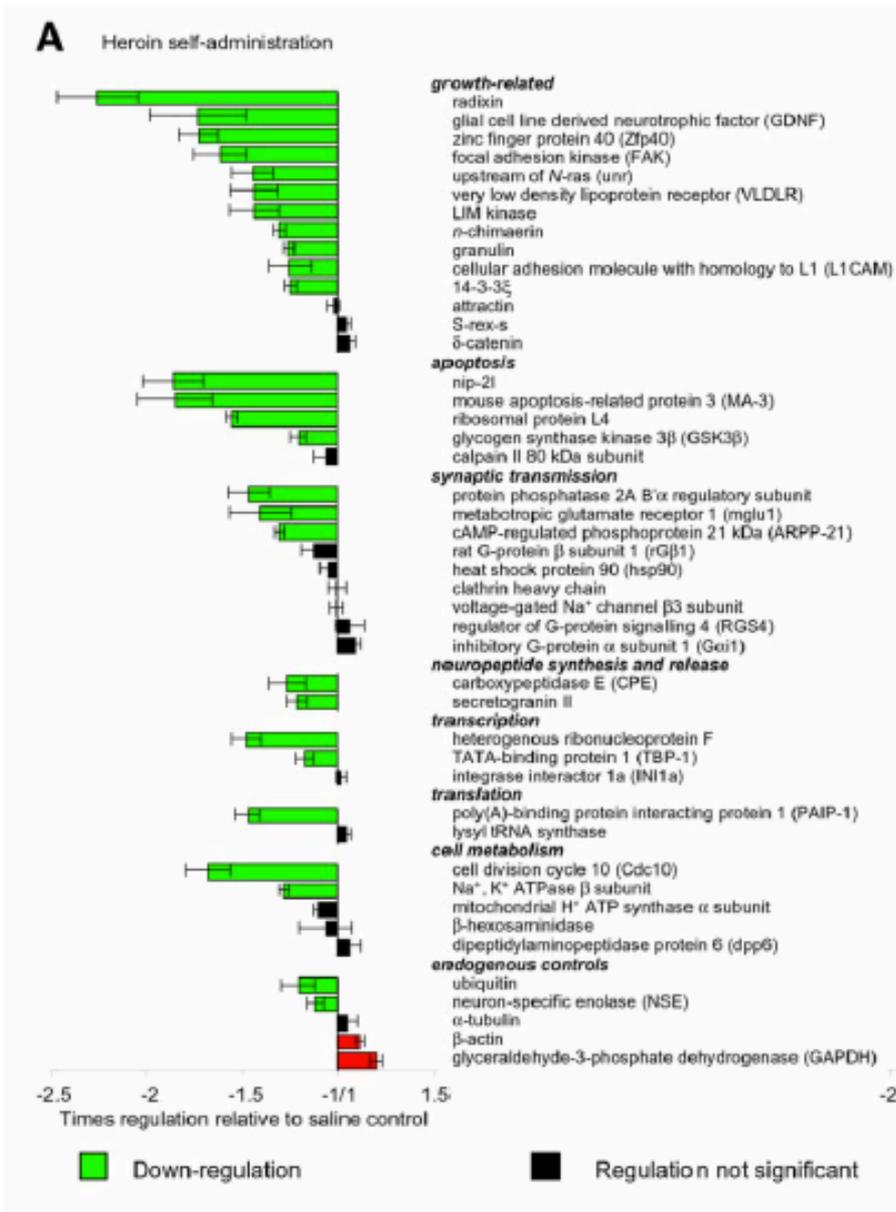


NuAc



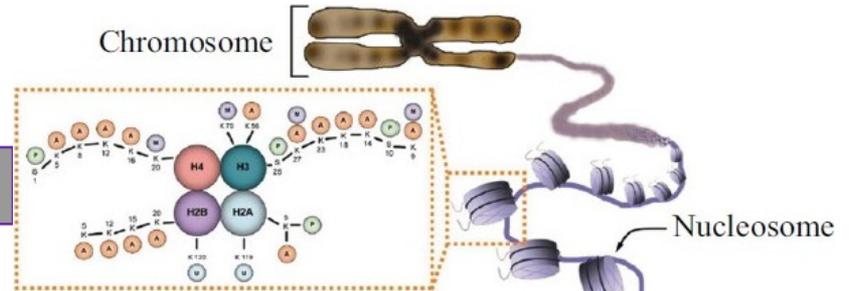
Régulation génique après auto-administration d'héroïne chez le rat

Jacobs et al. (2002) *Faseb J.* 16(14):1961-3

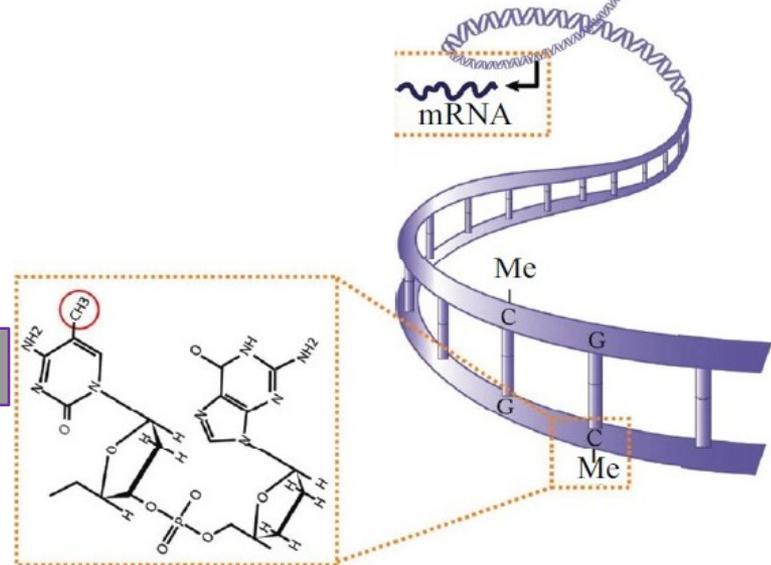


Régulations épigénétiques

Modification de l'histone

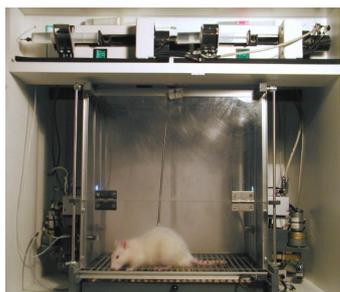
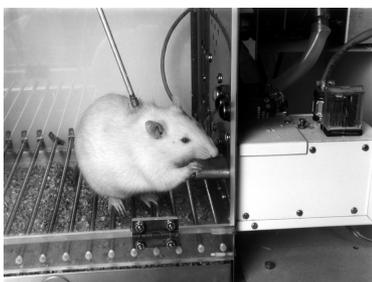


Méthylation de l'ADN

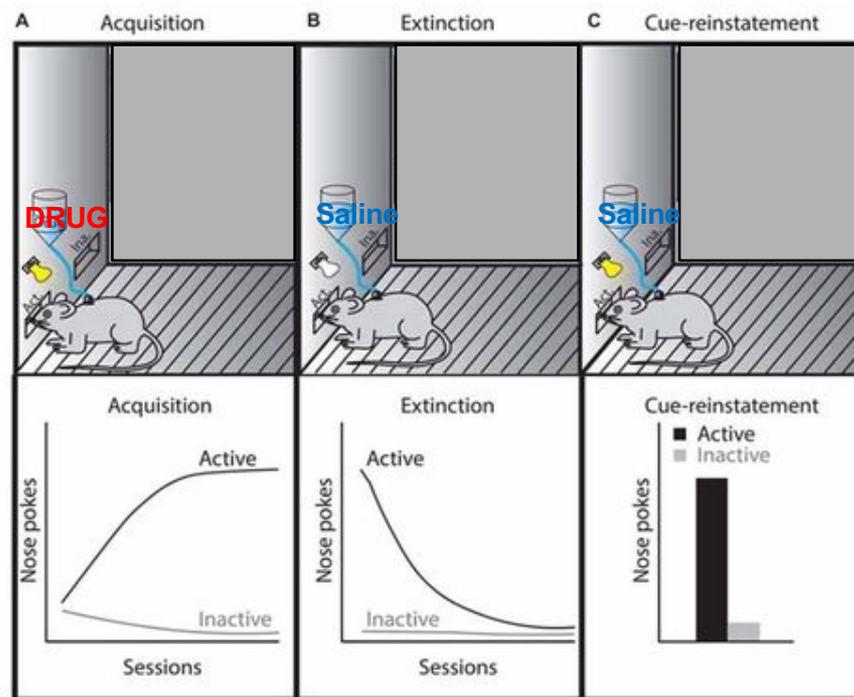


Auto-administration par voie intraveineuse chez le rat

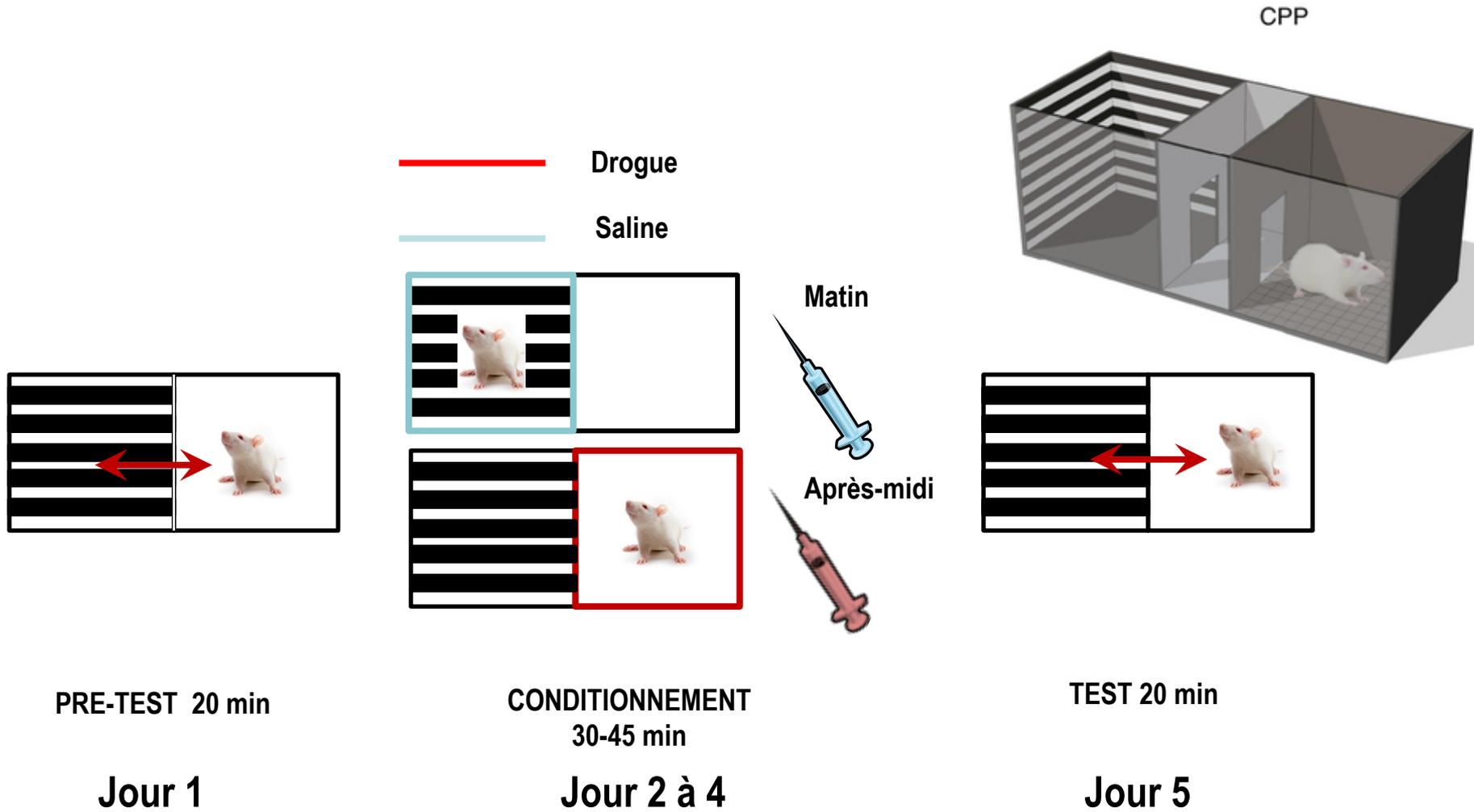
Insertion du cathéter dans la veine jugulaire



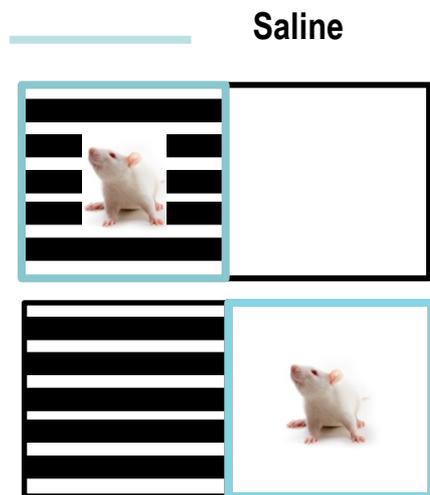
Cage opérante



Préférence de place conditionnée

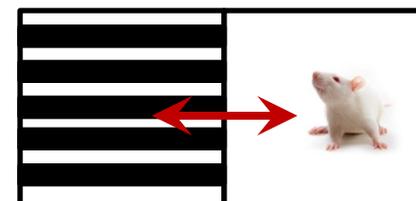
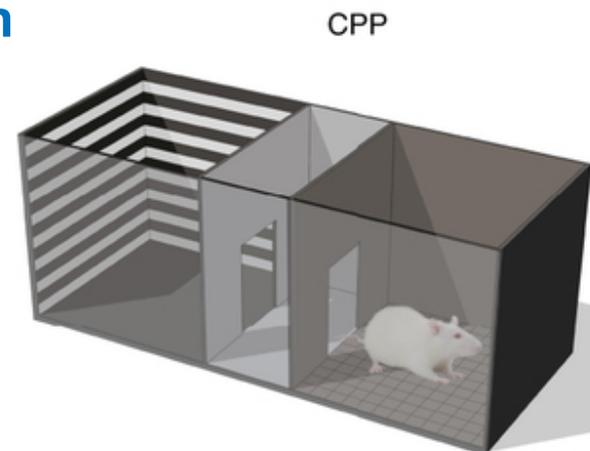


Préférence de place conditionnée Extinction et ré-installation



CONDITIONNEMENT
30-45 min

Quelques jours

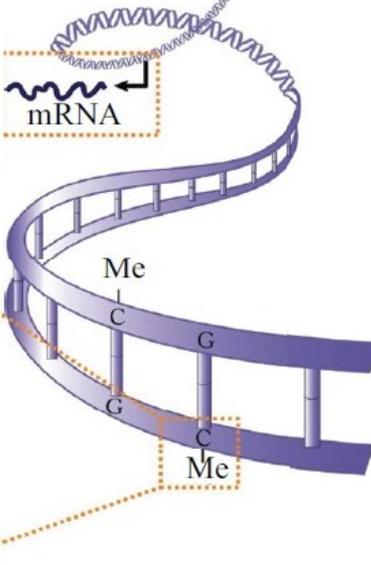
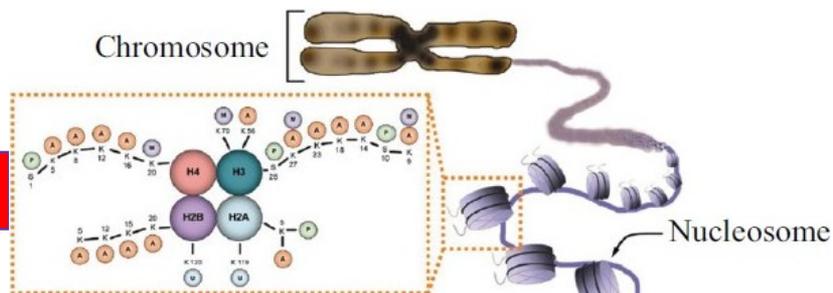


TEST 20 min

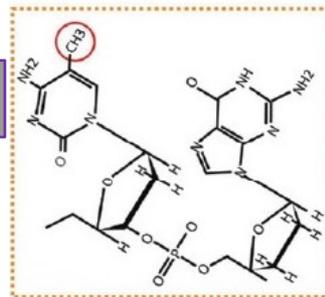
Denier jour (injection d'une faible dose de drogue)

Régulations épigénétiques

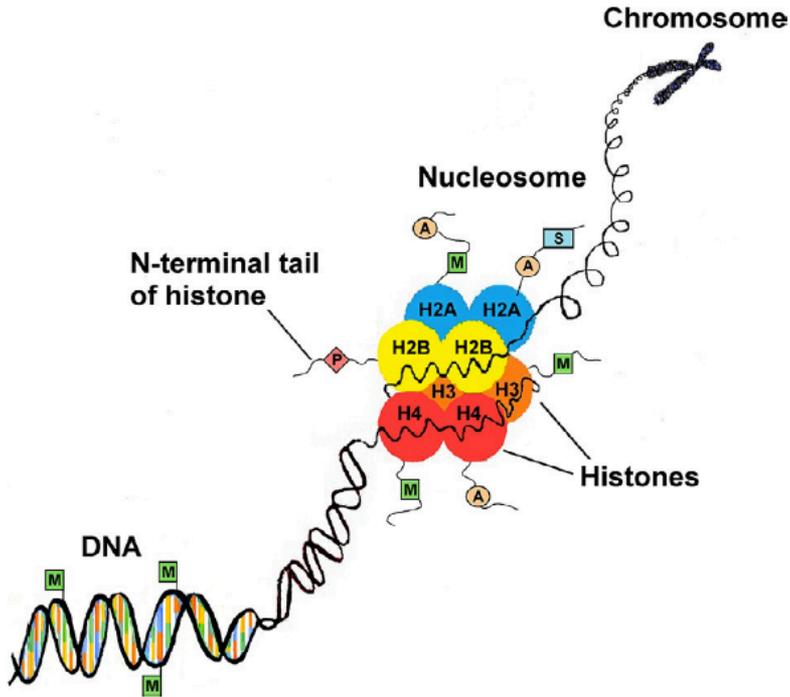
Modification de l'histone



Méthylation de l'ADN



Modifications au niveau des histones



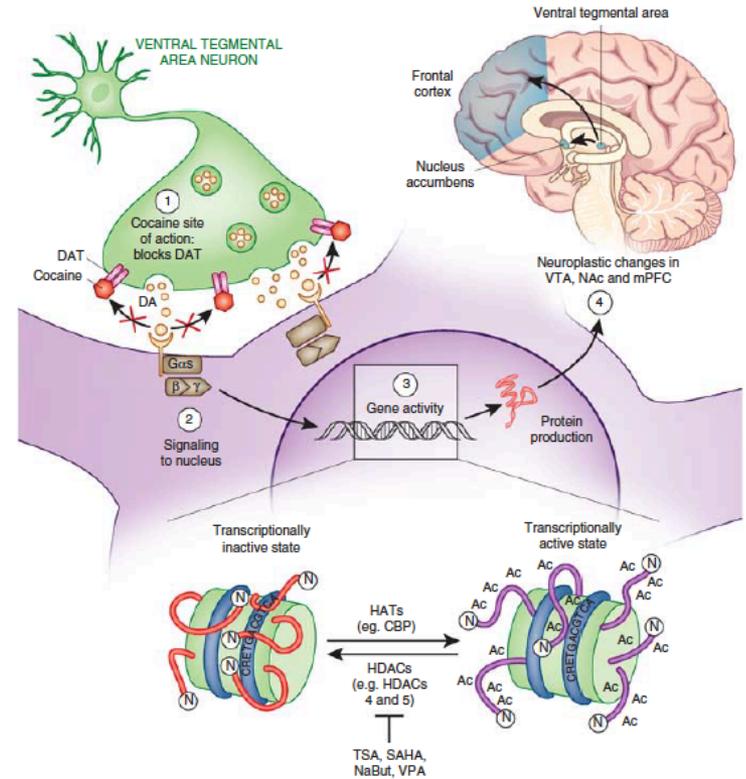
A: acétylation (Histone acetyltransferases [HATs]; histone deacetylases [HDACs])

M: méthylation

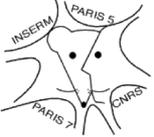
P: phosphorylation

S: sumoylation

Kalda and Zharkovsky (2015) Intern. Rev. Neurobiol.



Rogge and Wood (2013) Neuropsychopharmacol 38:94-110

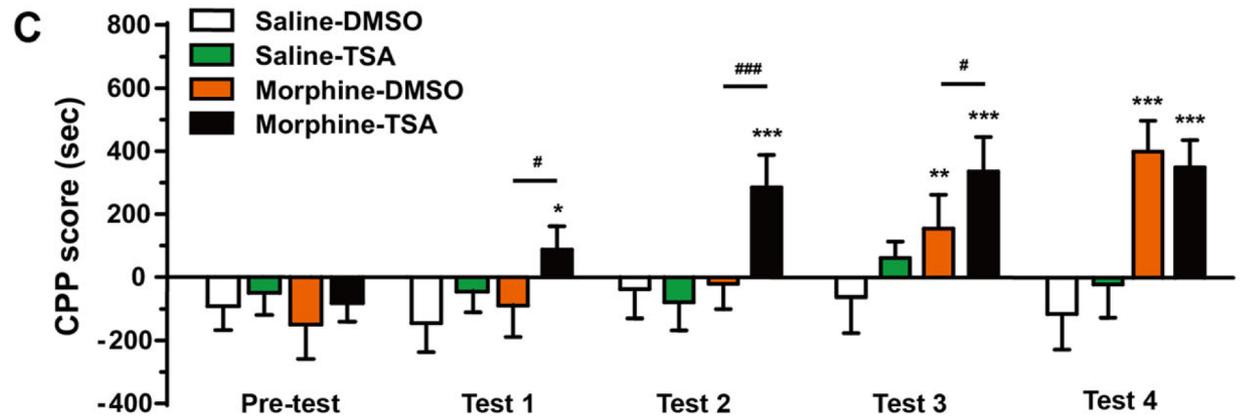
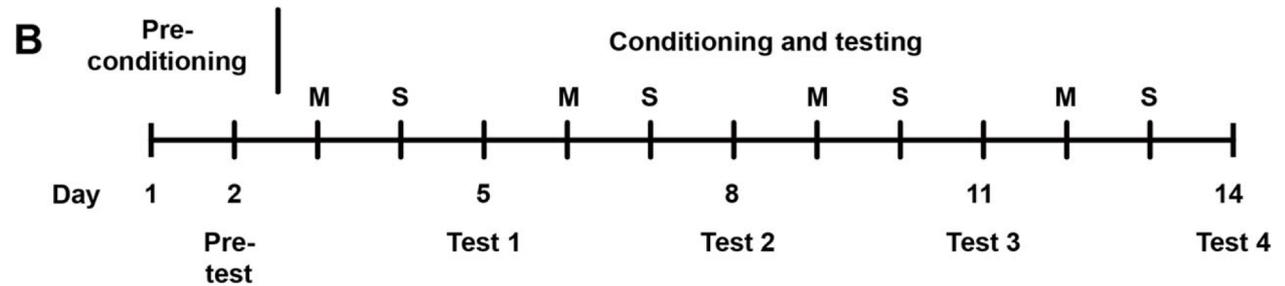
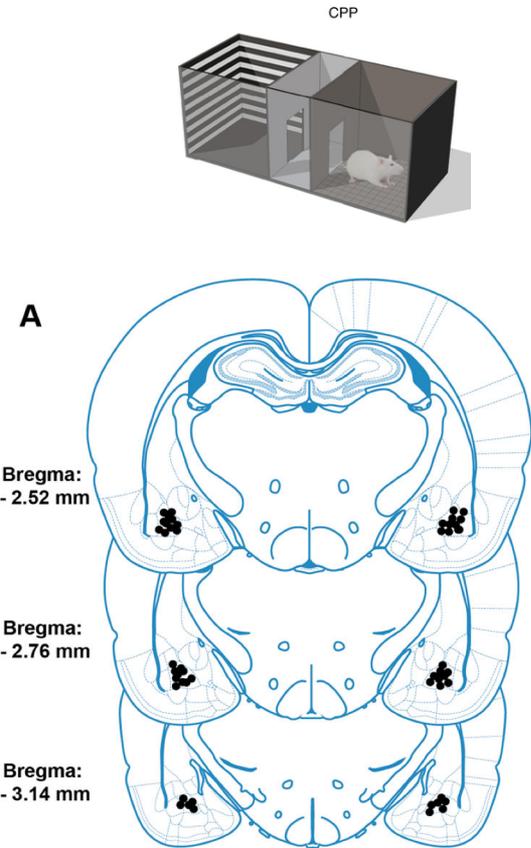


Modifications d'acétylation des histones et régulations des gènes après exposition aux opioïdes

Epigenetic Mark	Change	Brain Region	Model Examined	Gene targets identified?	Reference
Pan-H3ac	↑	Striatum	Human heroin addicts Rat IVSA		(36)
Pan-H3phosphoac	↑	NAc (not PFC)	Mouse, repeated heroin in CPP		(34)
H3K9ac	↑	LC, VLO	Rat, repeated morphine	<i>Bdnf</i>	(37; 38)
H3K14ac	↑	BLA	Mouse, repeated morphine in CPP, normalized during extinction	<i>Bdnf, FosB, Creb</i>	(39)
H3K18ac	↑	NAc	Rat, drug-primed reinstatement of heroin-seeking		(40)
H3K23ac	↑	NAc/dStri	Human heroin addicts (not correlated with use history)		(36)
H3K27ac	↑	NAc/dStri	Human heroin addicts Rat IVSA	Glutamate signaling, <i>GRLAI</i>	(36)
H4K5ac	↑	NAc	Rat, drug-primed reinstatement of heroin-seeking		(40)
H4K8ac	↑	NAc	Rat, drug-primed reinstatement of heroin-seeking		(40)
H3K9me1	No Δ	NAc	Mouse, repeated morphine		(41)
H3K9me2	↓	dStri	Mouse, repeated morphine	<i>FosB, Bdnf, glutamate signaling genes</i>	(41)
	↓	CeA	Mouse, repeated morphine in CPP	<i>Bdnf, glutamate signaling genes</i>	(42)

Browne et al. (2020) *Biol. Psychiatry* 87(1):22-33.

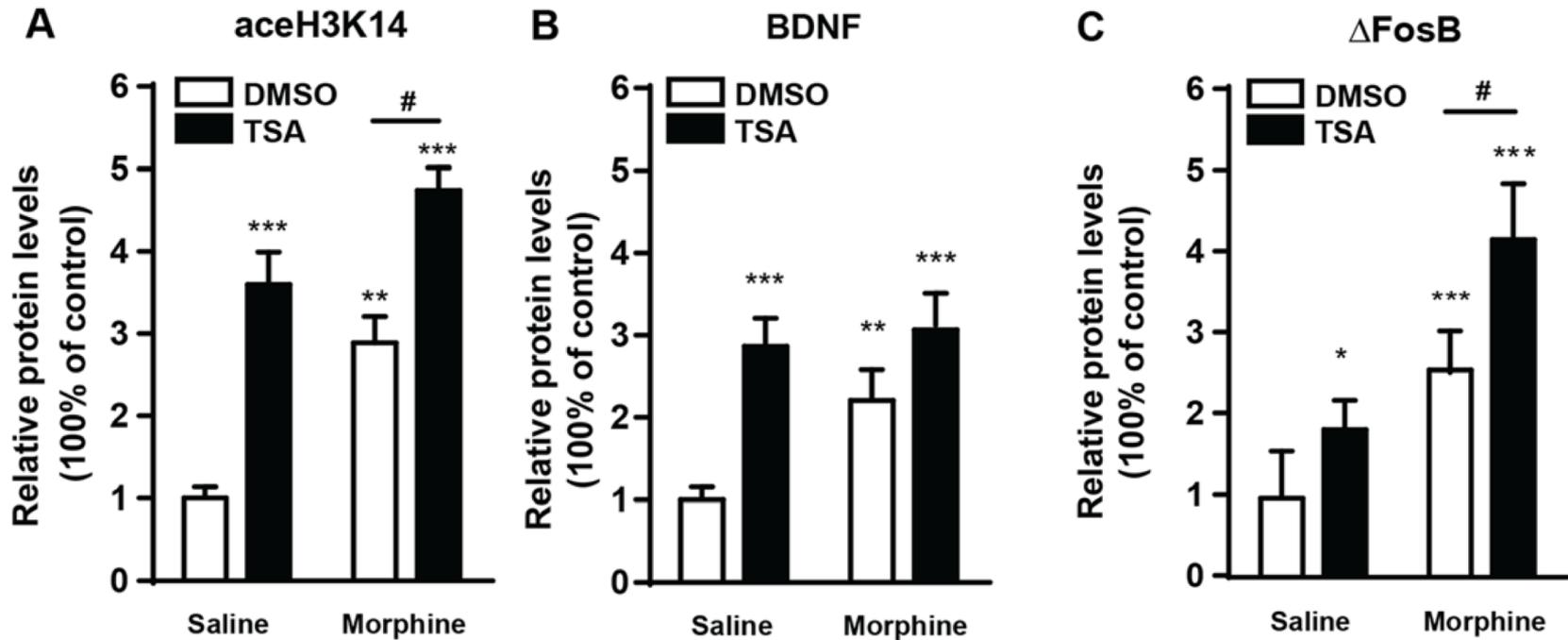
Inhibiteur de HDAC dans l'amygdale basolatérale: facilitation de la mémoire associant le contexte à l'injection de morphine chez le rat



TSA: trichostatin A (HDACi)
(injectée 30 min avant la morphine)

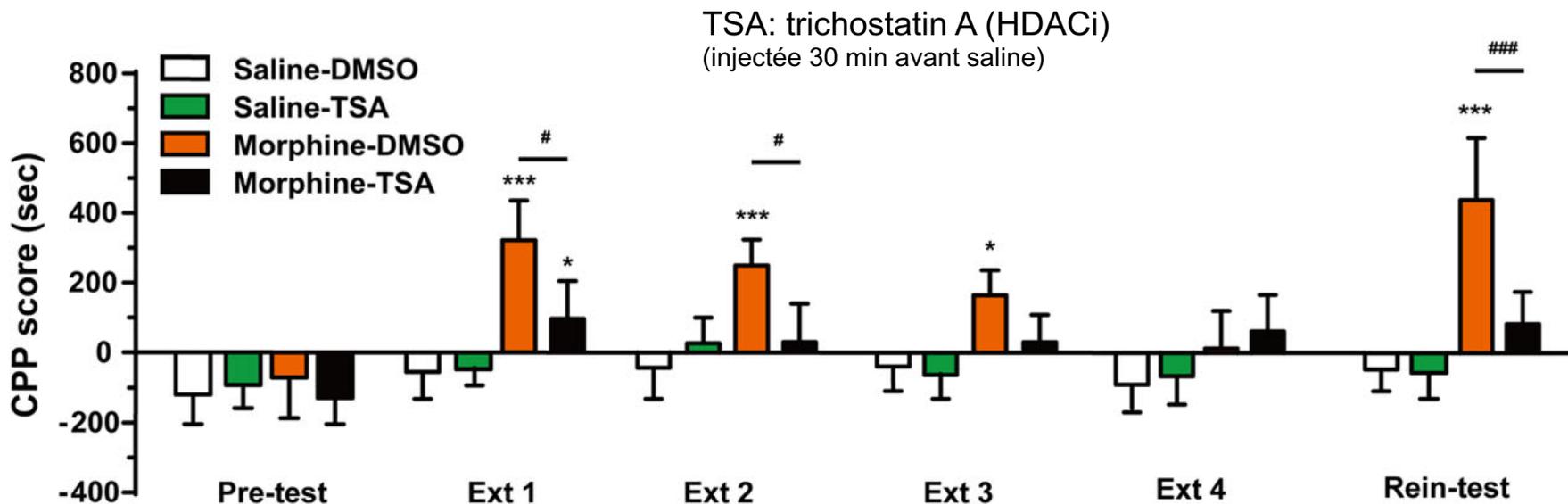
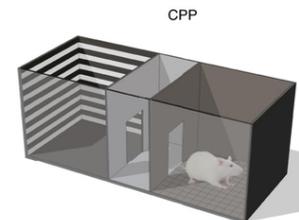
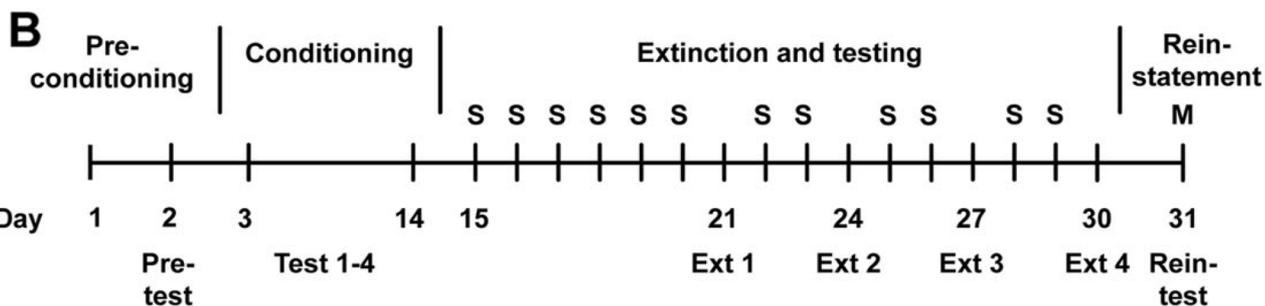
Wang et al. (2015) *J. Mol. Neuro.* 55: 269-278

Effet de la TSA sur l'acétylation des histones et la synthèse protéique



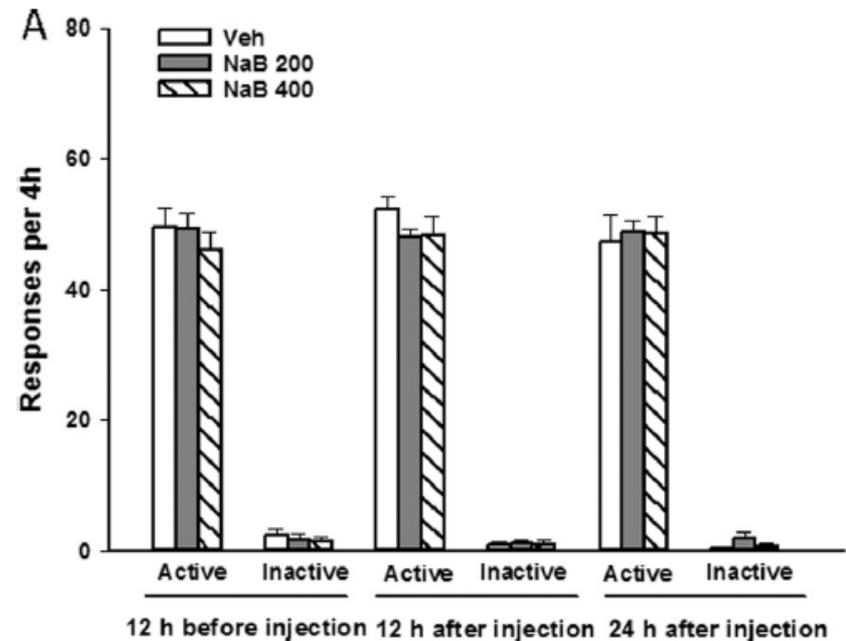
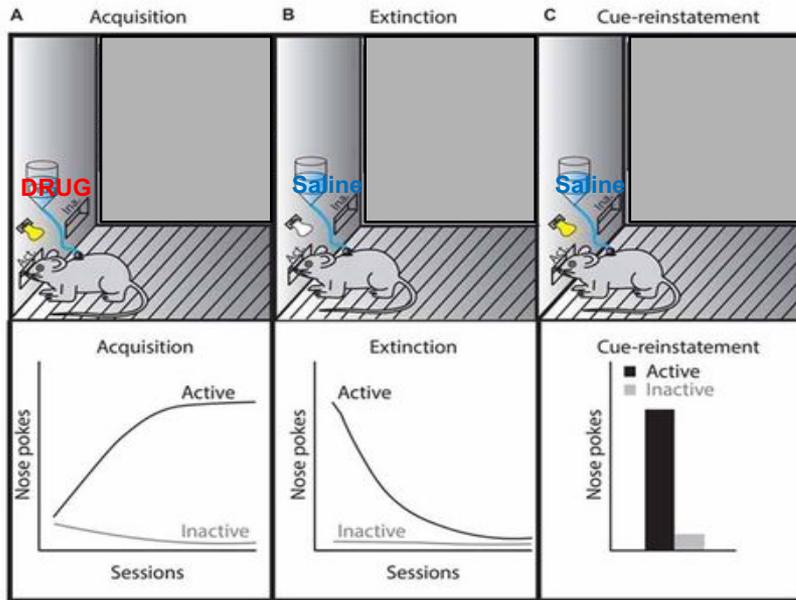
Wang et al. (2015) *J. Mol. Neuro.* 55: 269-278

Inhibiteur de HDAC dans l'amygdale basolatérale: effet sur l'extinction de la CPP et la réinstallation chez le rat



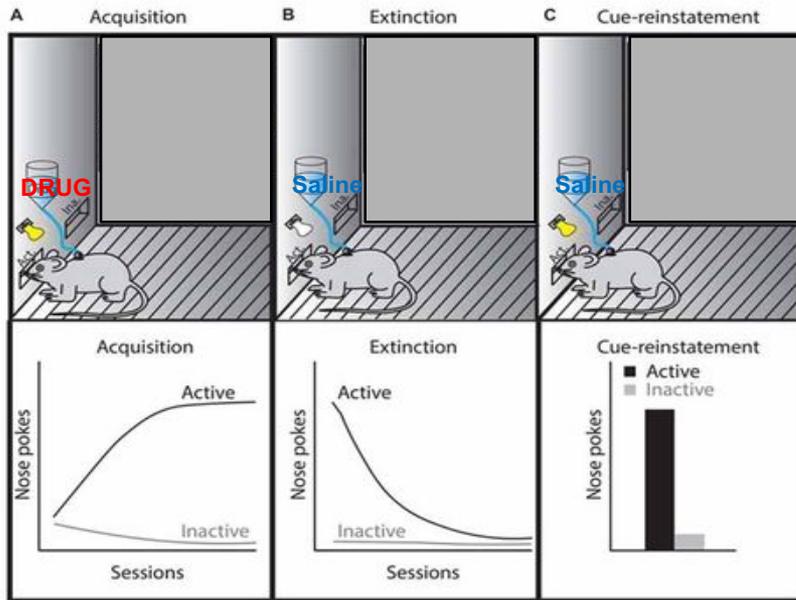
Wang et al. (2015) *J. Mol. Neuro.* 55: 269-278

Inhibiteur de HDAC (sodium butyrate) : effets sur l'acquisition et la réinstallation du comportement d'auto-administration d'héroïne chez le rat

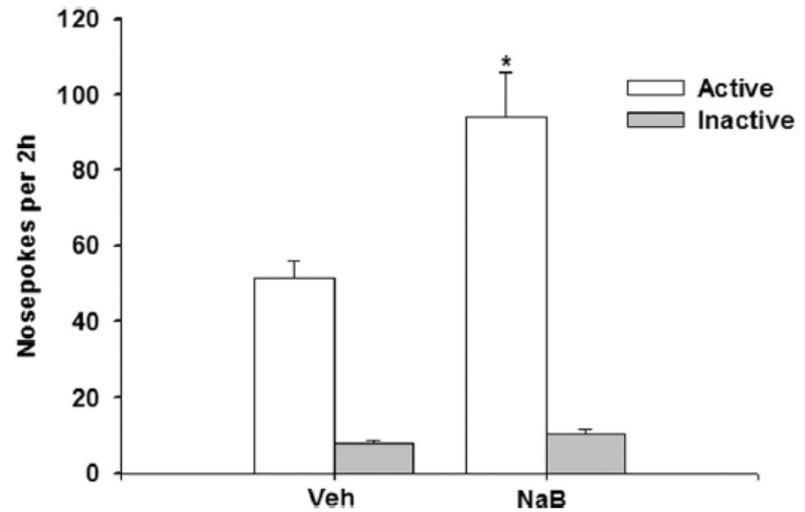


Chen et al. (2016) *Brain Res.* 1652:151-157

Inhibiteur de HDAC (sodium butyrate) : effets sur l'acquisition et la réinstallation du comportement d'auto-administration d'héroïne chez le rat



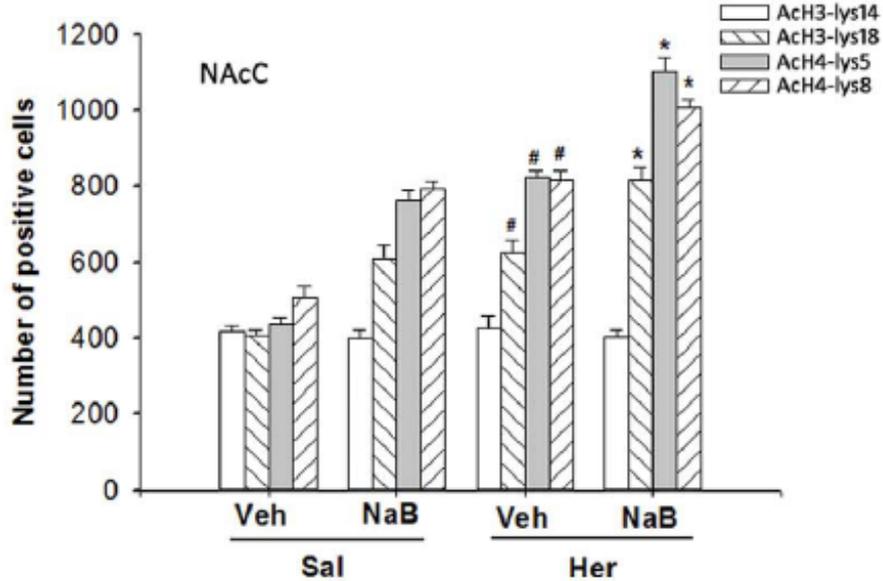
Réinstallation avec indices et héroïne (s.c.)



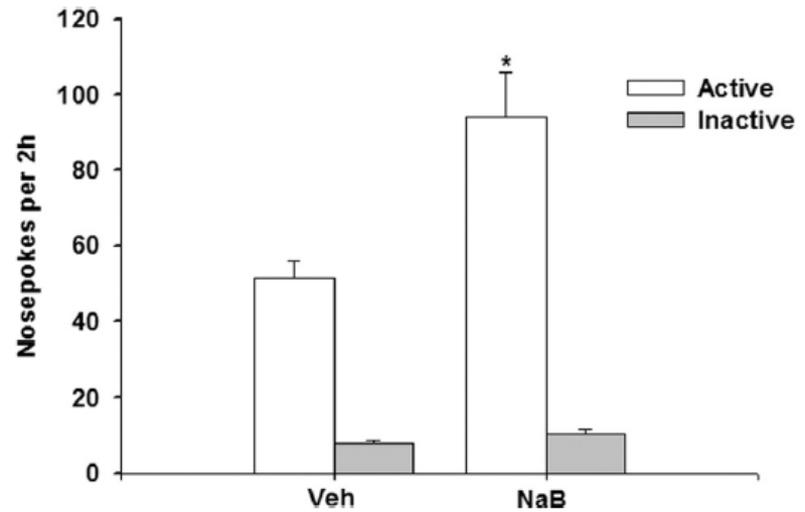
Chen et al. (2016) *Brain Res.* 1652:151-157

Inhibiteur de HDAC (sodium butyrate) : effets sur l'acquisition et la réinstallation du comportement d'auto-administration d'héroïne chez le rat

Acétylation des histones après réinstallation avec indices et héroïne (s.c.)



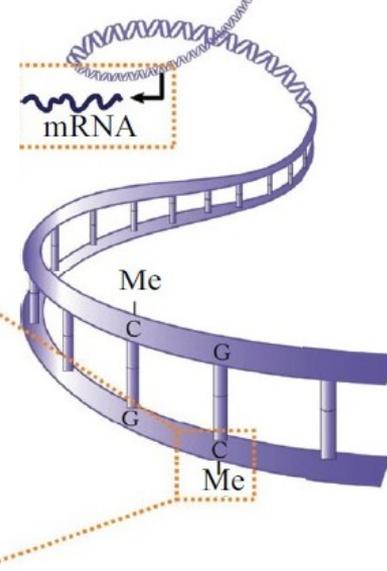
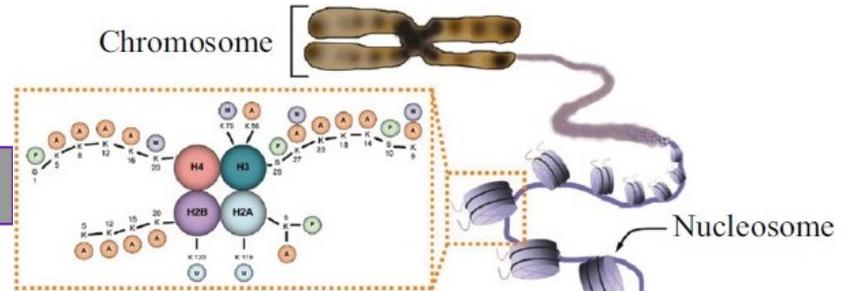
Réinstallation avec indices et héroïne (s.c.)



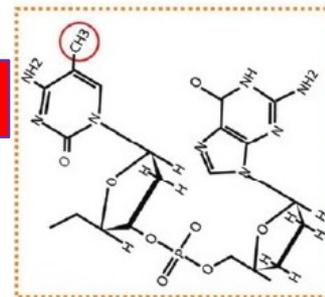
Chen et al. (2016) *Brain Res.* 1652:151-157

Régulations épigénétiques

Modification de l'histone

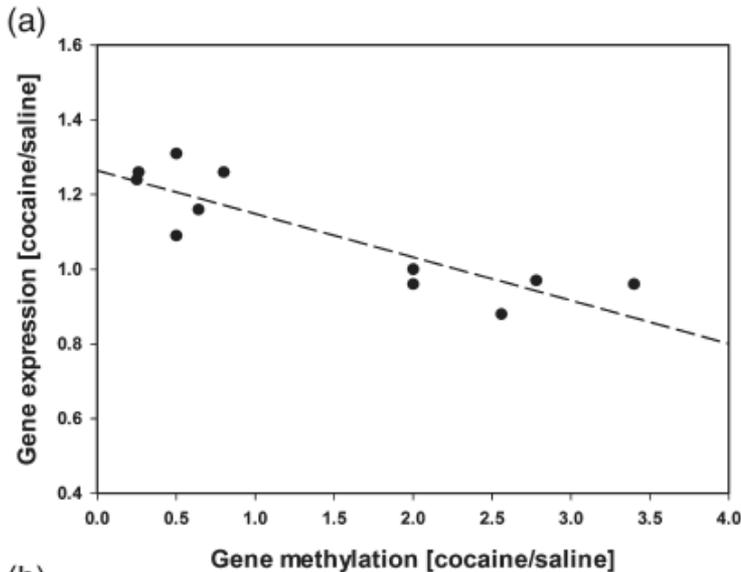
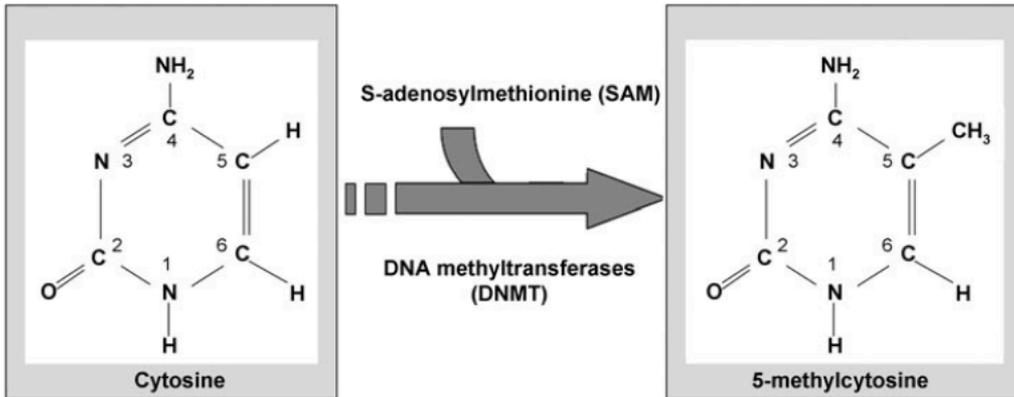


Méthylation de l'ADN



Méthylation et l'ADN et addiction aux opioïdes

Méthylation de l'ADN au niveaux des îlots CpG

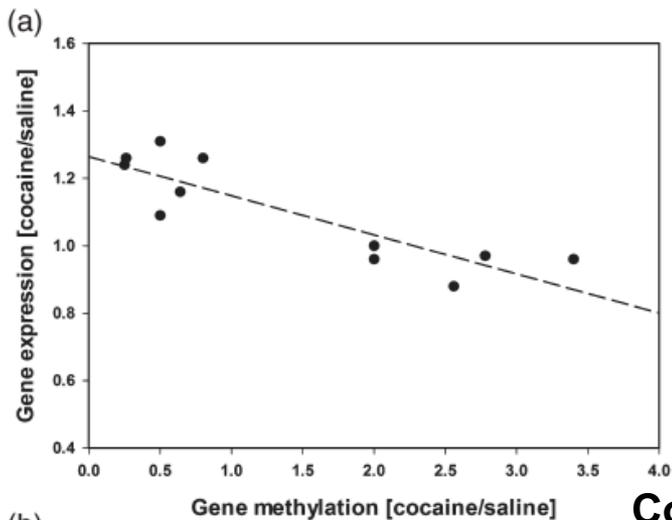
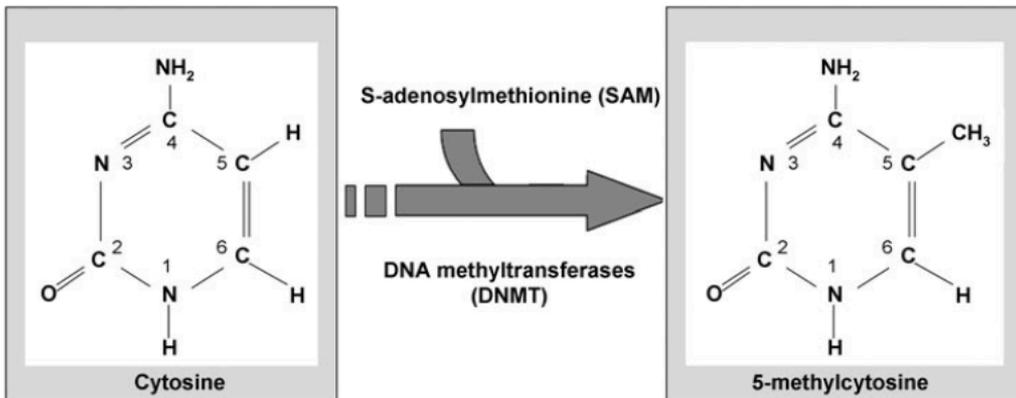


Fonteneau et al. (2017) *Gen. Brain Behav.* 16:313-327

Corrélation négative entre le taux de méthylation et le taux d'expression des gènes

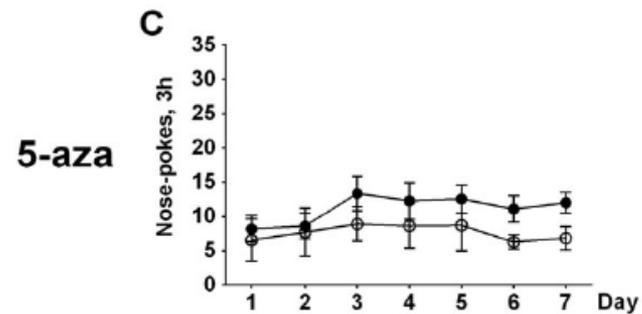
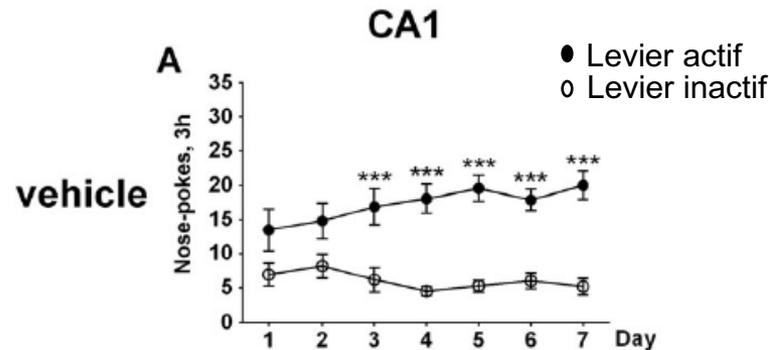
Méthylation et l'ADN et addiction à la cocaïne

Méthylation de l'ADN au niveaux des îlots CpG



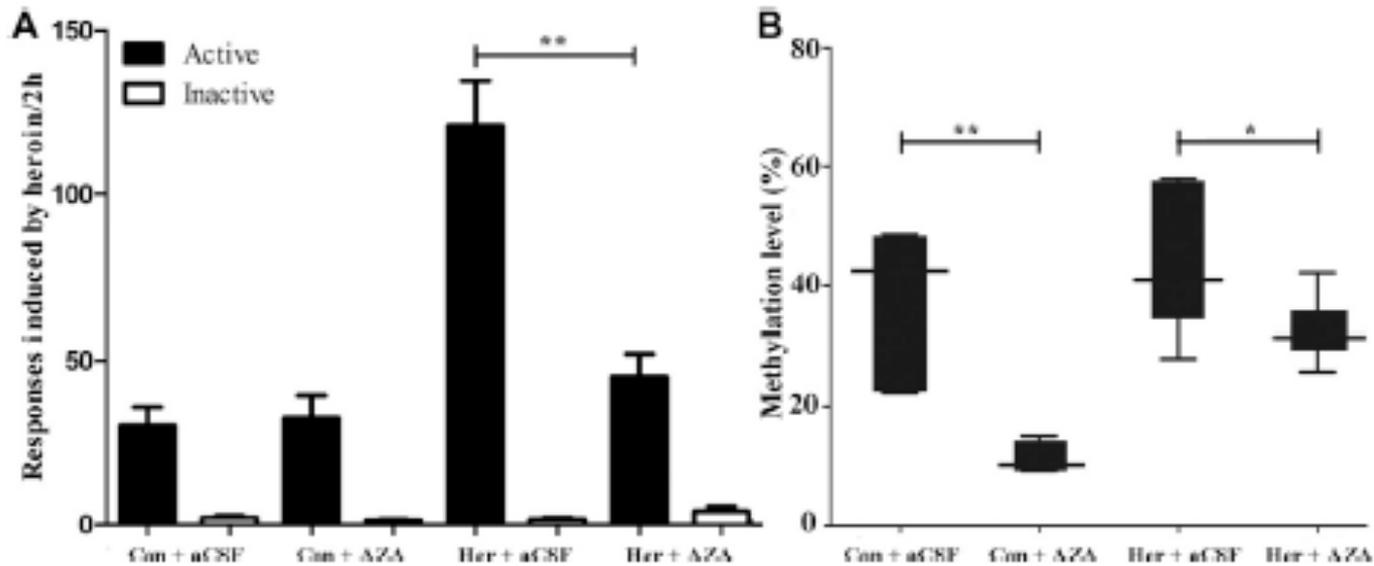
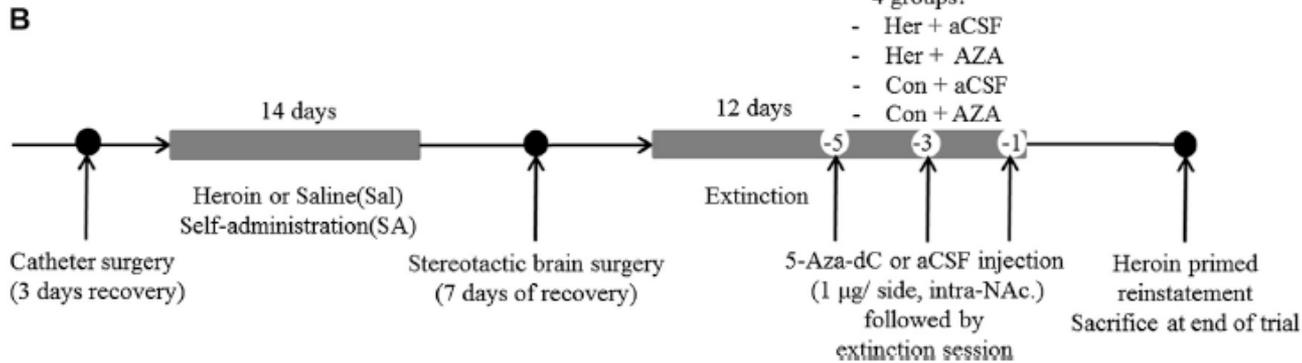
Corrélation négative entre le taux de méthylation et le taux d'expression des gènes

diminution de l'AA de morphine par des inhibiteurs de DNMT



Zhang et al. (2020) *Addict. Biol.* 25 (2):e12730

Diminution de la réinstallation de l'auto-administration d'héroïne chez le rat après administration d'inhibiteurs de DNMT dans le Nac



Hong et al. (2021) *Front. Pharmacol.* 11:612200

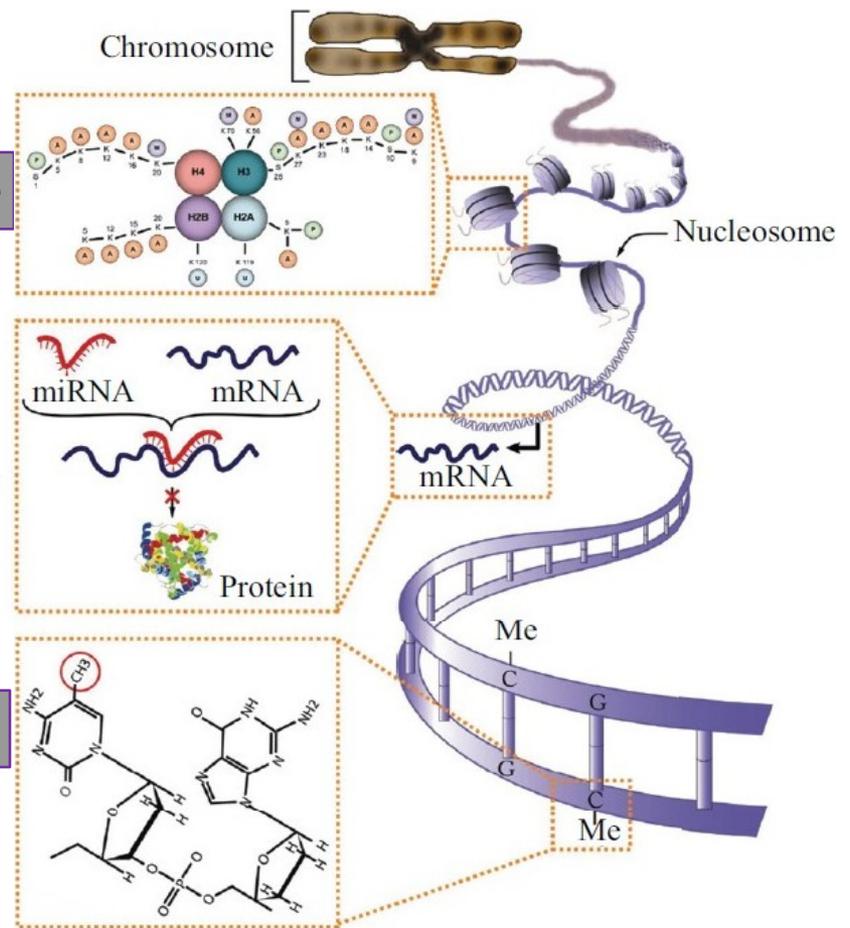
Régulations épigénétiques



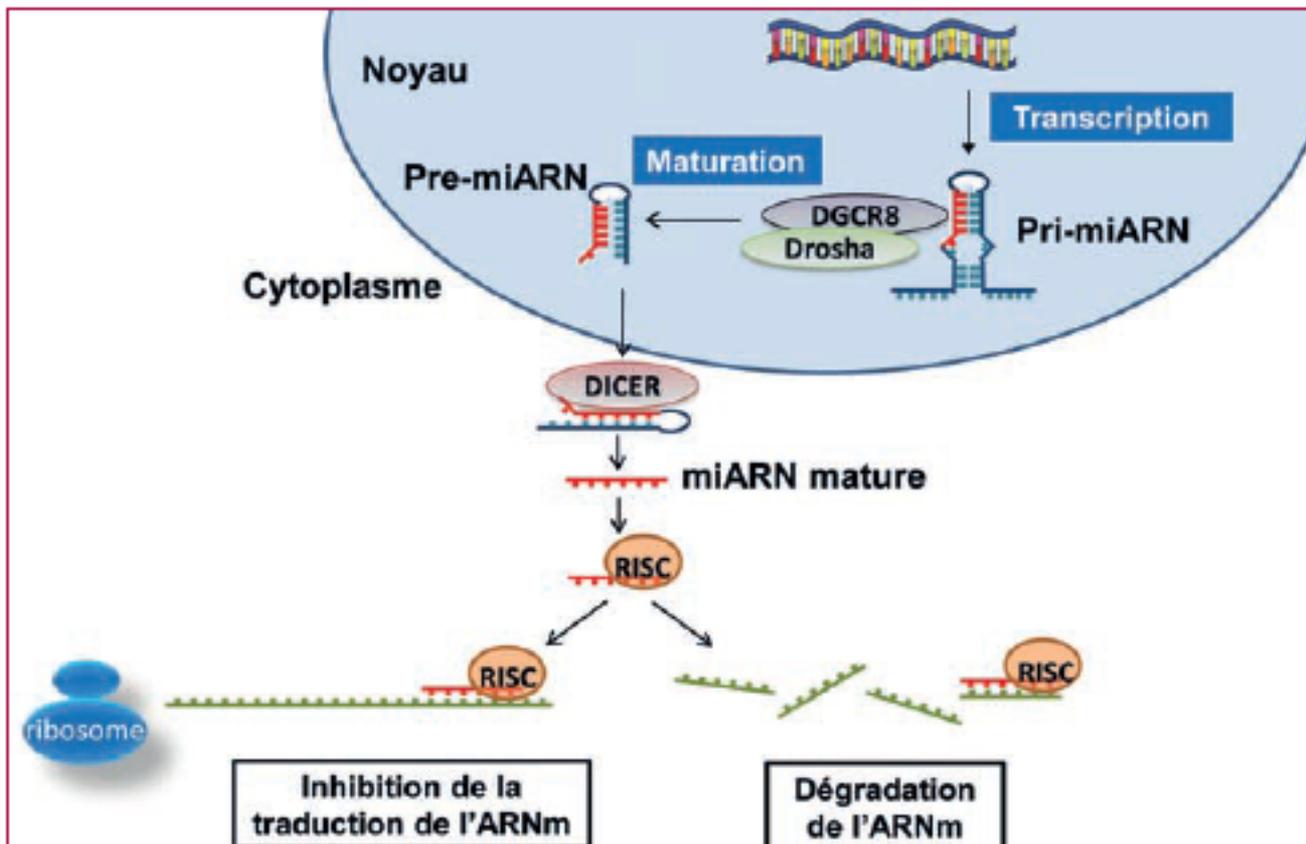
Modification de l'histone

ARN non codants

Méthylation de l'ADN



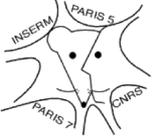
Voie de synthèse des micro ARNs



Jacovetti and Regazzi (2019) *Pour la Science* 13(5):427-432.

Figure 1. Voie canonique de synthèse des microARNs.

Dans le noyau, un transcrite primaire (pri-miARN) est produit à partir de l'ADN et est ensuite clivé par le complexe enzymatique Drosha-DGCR8 pour générer le précurseur du microARN (pre-miARN). Après son exportation dans le cytoplasme, le précurseur est incorporé dans le complexe enzymatique Dicer pour générer le microARN mature (environ 22 nucléotides). Celui-ci s'associe à RISC-Ago2 et guide ce complexe sur le 3'UTR des ARN messagers cibles conduisant à la dégradation des ARN messagers ou à l'inhibition de leur traduction.



De nombreux miR régulés par les drogues

Table 1. MiRNAs involved in addiction

Drug	miRNAs involved	Mechanisms affected	References
Cocaine	miR-212	Decreases activity of CREB and TORC1, and controls expression of gene encoding BDNF via interaction with MeCP2	[4,15]
	miR-181a	Upregulated by cocaine; affects expression of genes encoding BDNF, DAT, CREB, Homer1 and Drd3	[10,11]
	let-7d	Downregulated by cocaine; targets semaphorins, BDNF, neuropilins and mGluR5	[10,11]
	miR-124	Downregulated by cocaine; targets Drd3, DAT and FosB	[10,11]
	miR-324-5p	Induced by Arg2 in Drd2 neurons; regulates cdk5r1, FosB and Mef2d	[5,9]
	miR-369-3p	Induced by Arg2 in Drd2 neurons; regulates cdk5r1, FosB and Mef2d	[5,9]
Nicotine	miR-140	Induced by nicotine; regulates dynamin-1, regulates synaptic endocytosis	[12,16]
	miR-504	Upregulates gene encoding Drd1	[12,16]
Opiates	miR-23b	Induces mOR	[19]
	miR-190	Induced by mOR by upregulation of talin2	[13,19]
	miR-15b, miR-181b	Induced by morphine	[20,21]
	miR-133b	Induced by morphine	[6]
Antidepressants, alcohol and CYP3A4	miR-133b	Specifically expressed in dopaminergic neurons; downregulates expression of gene encoding Pitx3; Pitx3 induces production of TH and DAT and regulates maturation of midbrain dopaminergic neurons	[6,21]
	miR-16	Induced by SSRI in serotonergic neurons; reduces expression of gene encoding SERT	[23]
	miR-9	Induced by alcohol; downregulates BK channels	[4]
	miR-212	Induced by alcohol; decreases ZO-1, affects cell permeability	[22]
	miR-27b	Downregulates CYP3A4	[17]
	miR-298	Downregulates CYP3A4	[17,24]

Arg2, arginase, type II; cdk5r1, cyclin-dependent kinase 5, regulatory subunit 1; CREB, cAMP-responsive element binding protein; BDNF, brain-derived neurotrophic factor; CYP3A4, cytochrome P₄₅₀ 3A4; DAT, dopamine transporter; Drd1, dopamine receptor D1R; Drd2, dopamine receptor D2R; Drd3, dopamine receptor D3R; FosB, FBJ murine osteosarcoma viral oncogene homolog B; MeCP2, methyl CpG binding protein 2; Mef2d, myocyte enhancer factor 2D; mGluR5, metabotropic glutamate receptor 5; SSRI, selective serotonin reuptake inhibitor; TH, tyrosine hydroxylase; TORC1, transducer of regulated CREB activity 1; ZO-1, tight junction protein.

Dreyer (2010) *Genome Med.* 2(12):92

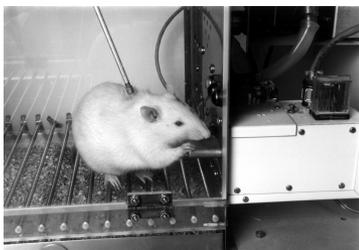
Auto-administration de cocaïne ou héroïne pendant 20 jours: étude comportementale et expression des miRNA dans le striatum dorsale

Auto-administration intraveineuse de drogue

Insertion du cathéter dans la veine jugulaire



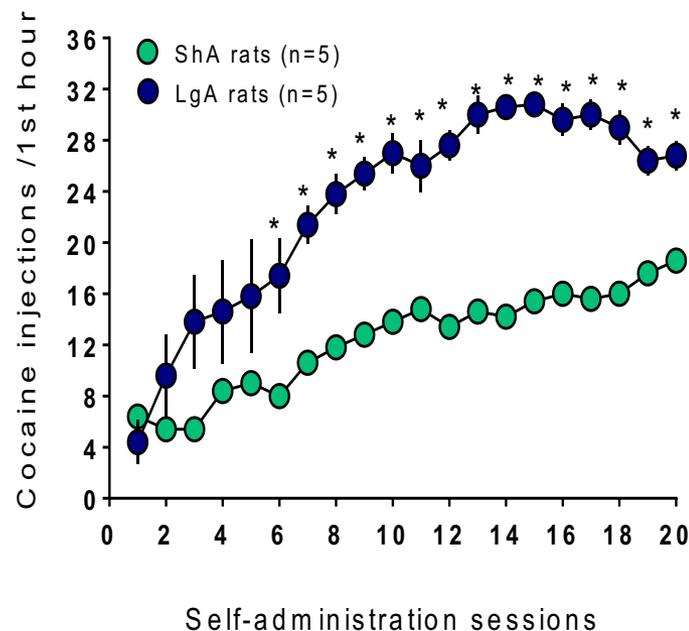
Cage opérante



Protocole ShA (1h) et LgA (6h)

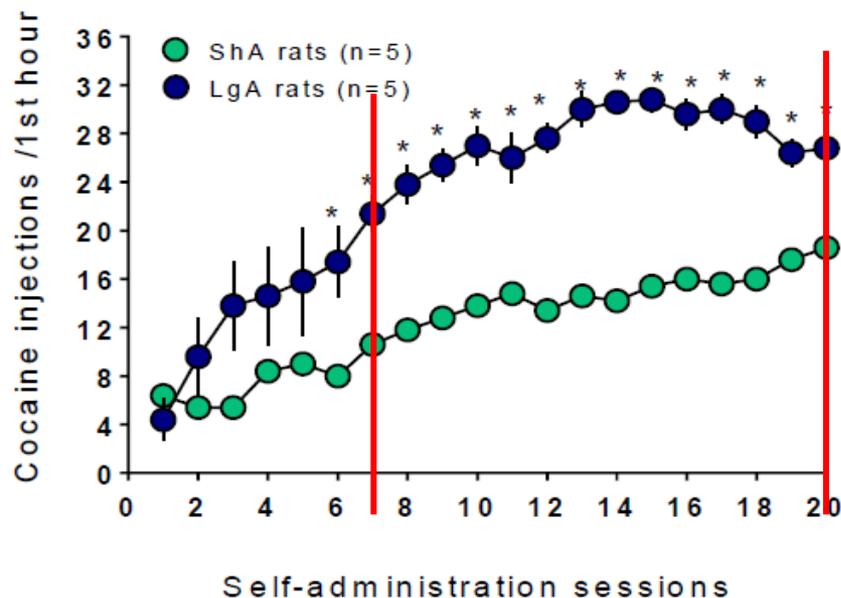
Lenoir et al. (en préparation)

Consommation de cocaïne



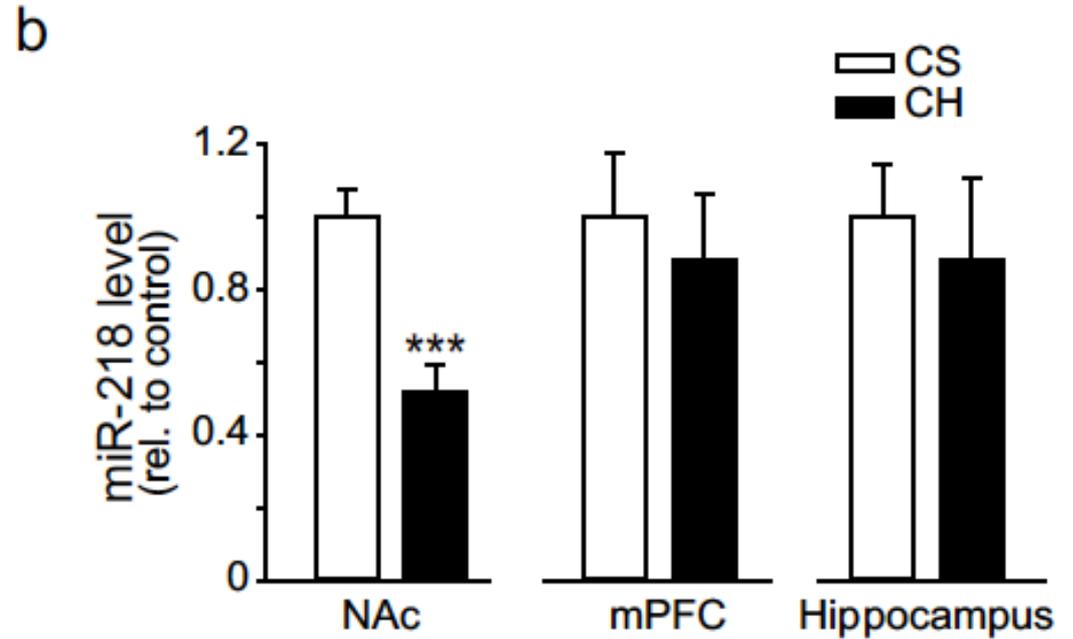
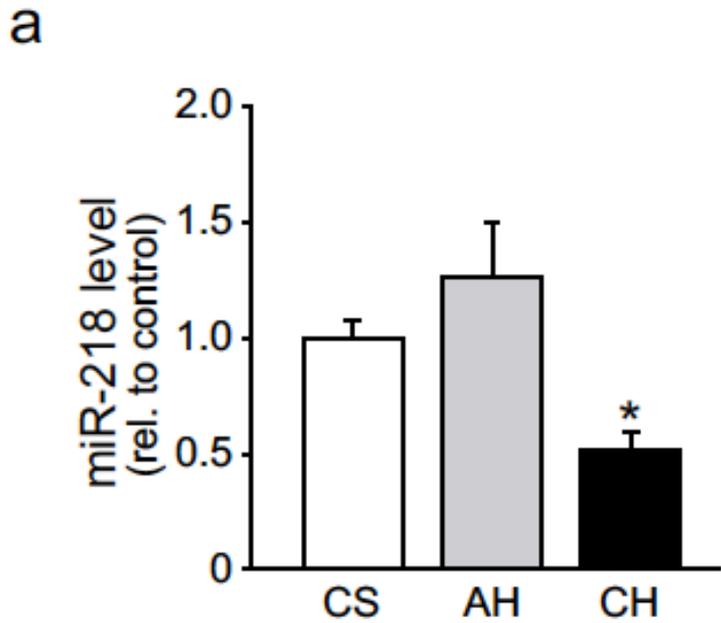
Escalade de la consommation lors de la première heure chez les rats LgA

Epigenetic regulation during intravenous self-administration of heroin or cocaine in rats : importance of the kinetics



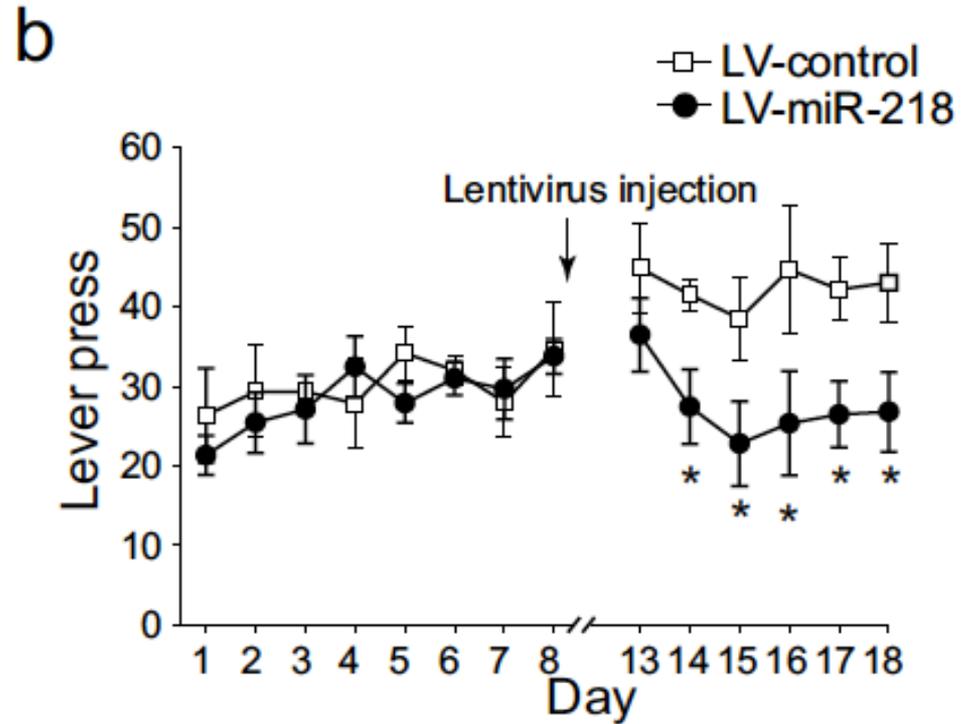
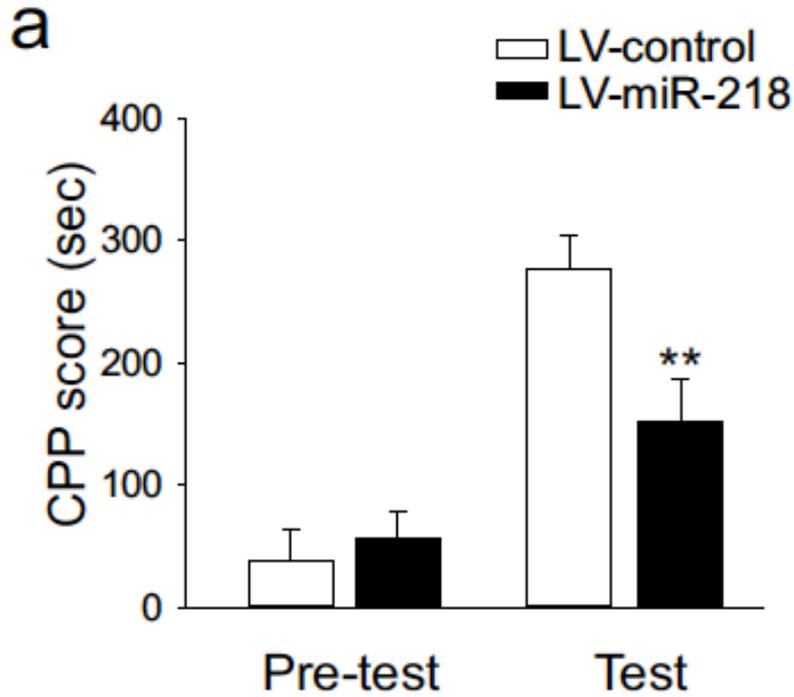
	Early phase (D7)	Late phase (D20)
miR-212 (coke)	↑	0
miR-APRICOH (Hero)	0	↑

Régulation du miR218 après un traitement chronique à l'héroïne chez le rat



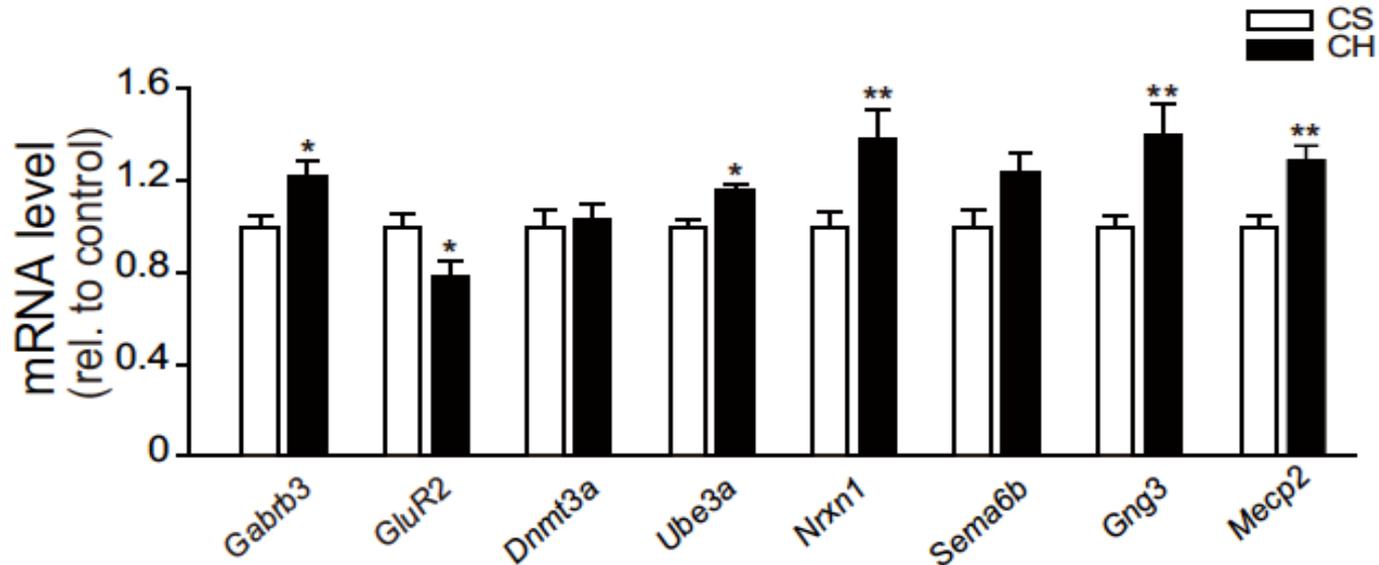
Yan et al. (2017) *Scientific Rep.* 7:40413

Le miR218 inhibe le comportement de recherche d'héroïne



Yan et al. (2017) *Scientific Rep.* 7:40413

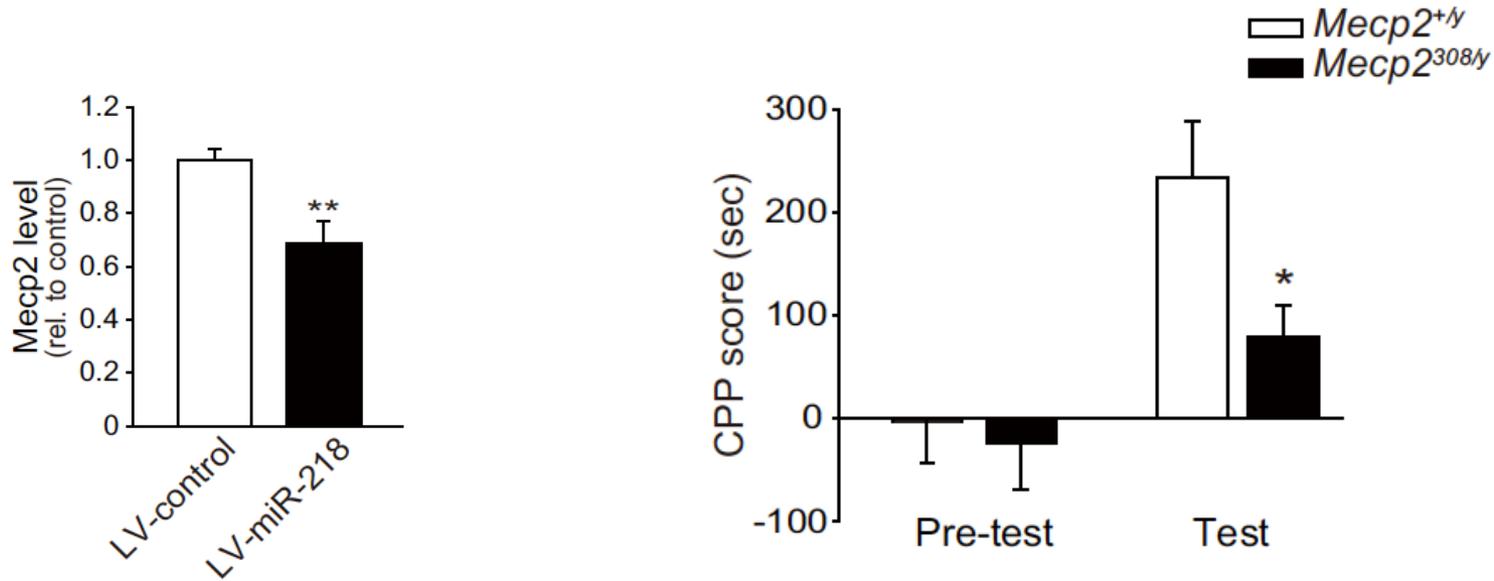
Le miR218 régule l'expression de plusieurs gènes



- Gabrb3, Mecp2: plasticité synaptique
- Nrnx1, Gng3, Ube3a: guidance axonale et morphogénèse dendritique

Yan et al. (2017) *Scientific Rep.* 7:40413

MeCP2 (Methyl CpG-binding protein 2) : une cible protéique régulée par le miR218

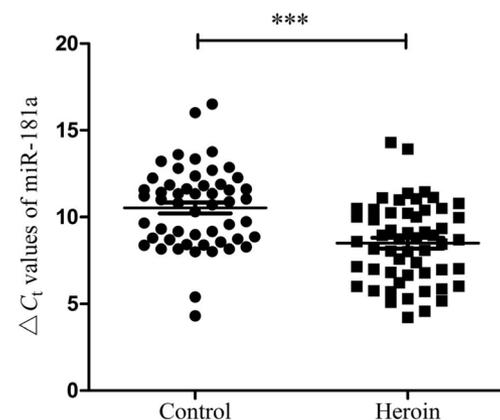


Yan et al. (2017) *Scientific Rep.* 7:40413

Les miR chez les patients dépendants aux opioïdes

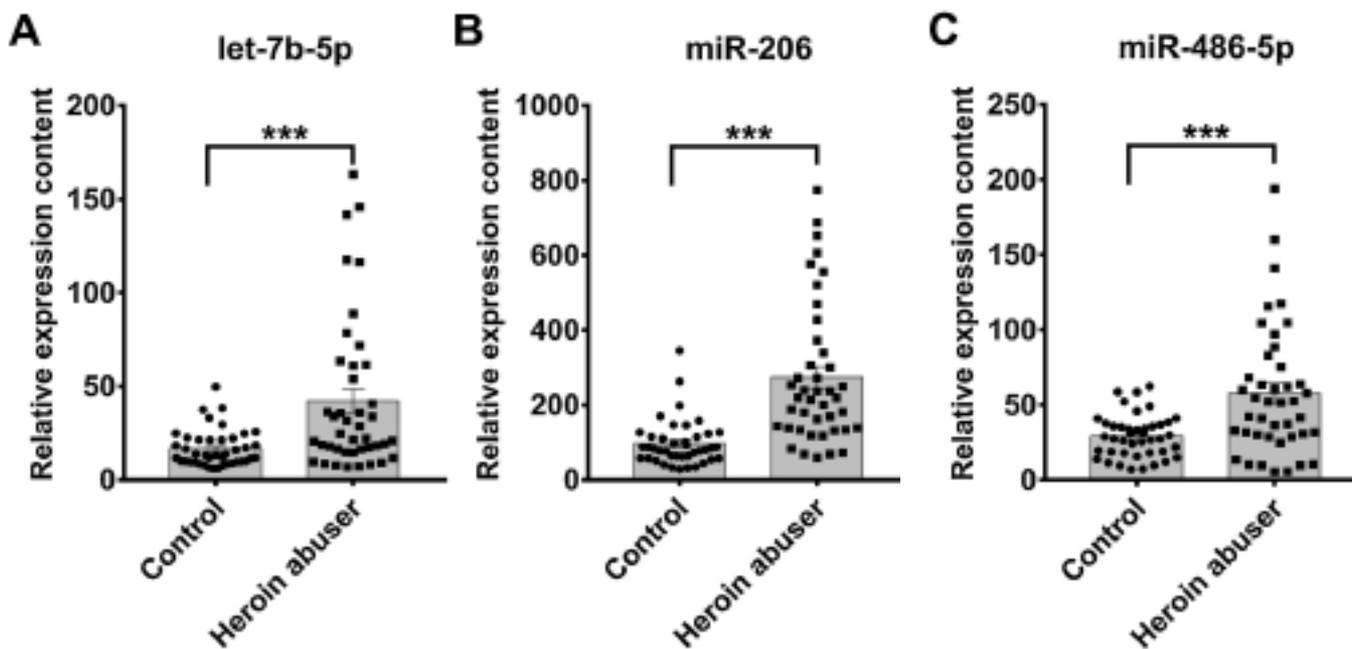
TABLE 2 Drug use of heroin addiction patients

Variables	Cases	Proportion (%)
Duration of use (mo)		
<12	3	5.66
12-24	4	7.55
24-48	12	22.64
48-96	13	24.53
≥96	21	39.62
Daily dose (g)		
<0.5	9	16.98
0.5-1	14	26.42
>1	30	56.6
Usage		
Snorting	26	49.06
Intravenous injection	25	47.17
Else	2	3.77
Frequency of use		
<1 times/d	5	9.43
1-3 times/d	7	13.21
>3 times/d	41	77.36
Frequency of detoxification		
<2	21	39.62
2-4	15	28.3
4-6	3	5.66
>6	14	26.42

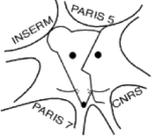


Xu et al. (2020) *J. Clin. Lab. Anal.* 34(11): e23486

Les miR chez les patients dépendants aux opioïdes

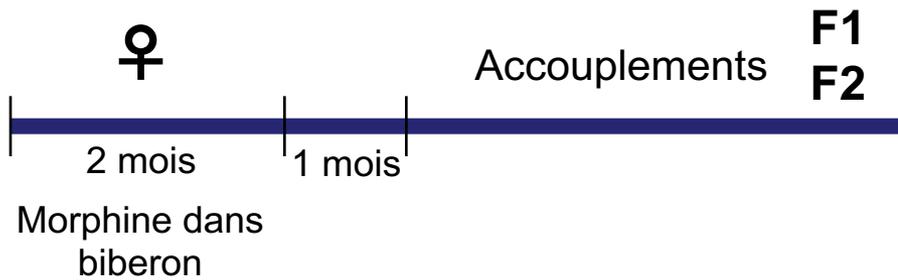


Gu et al. (2020) *Biomed. Pharmacother* 125: 109918

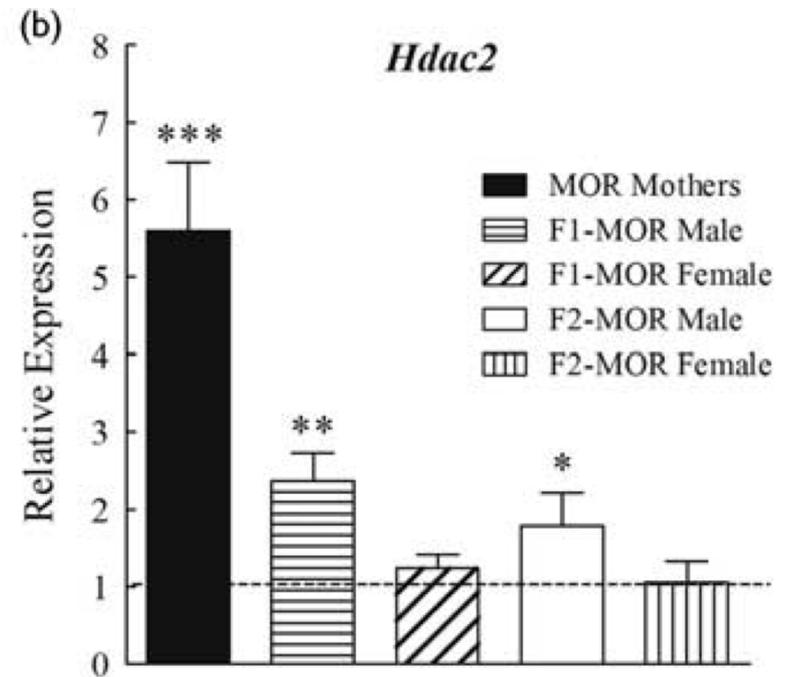
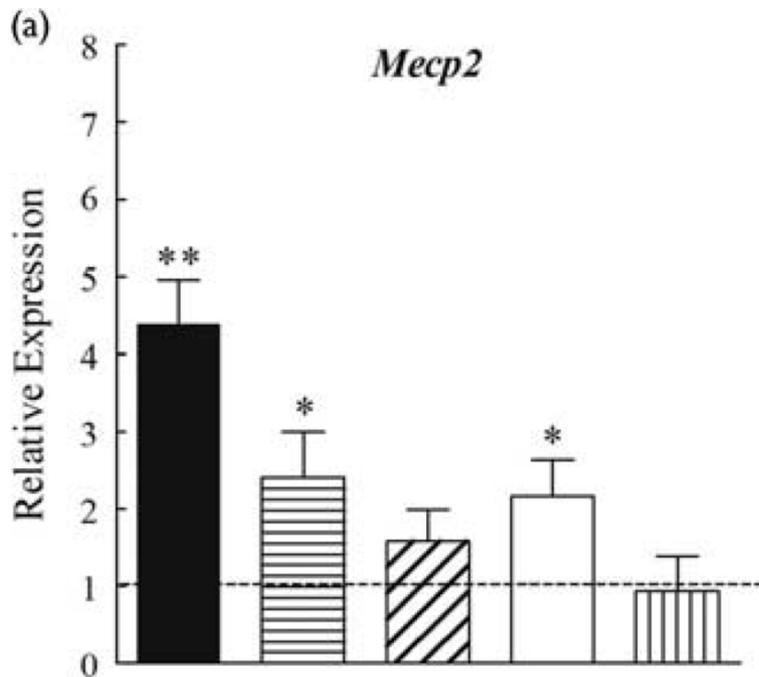


Transmission transgénérationnelle ?

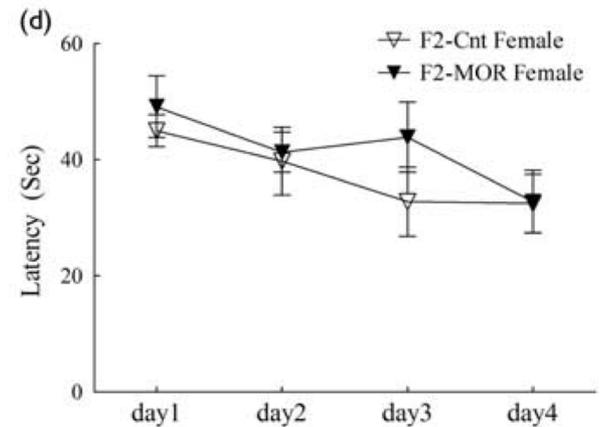
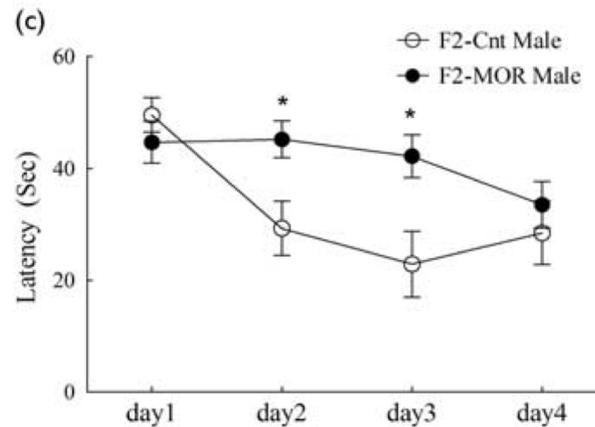
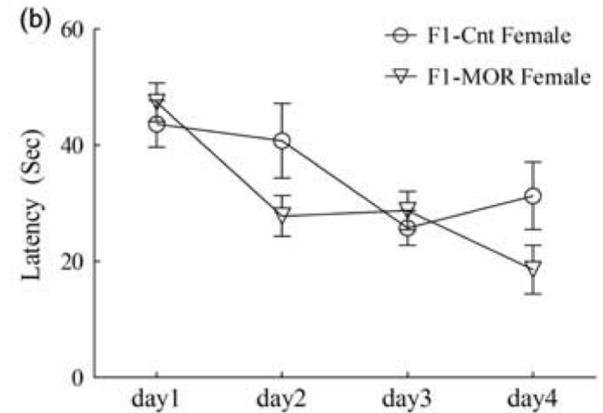
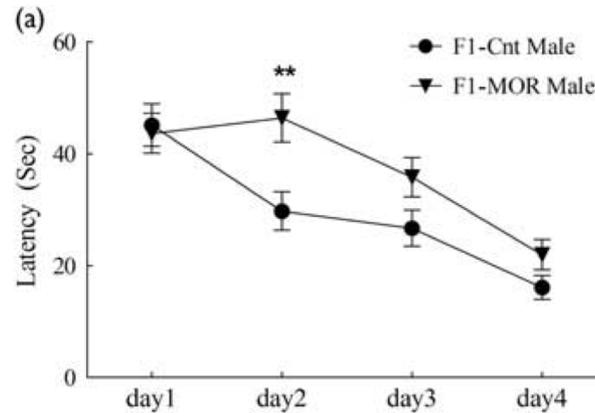
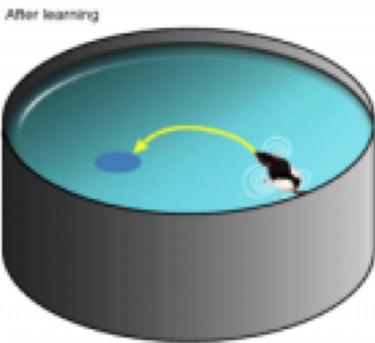
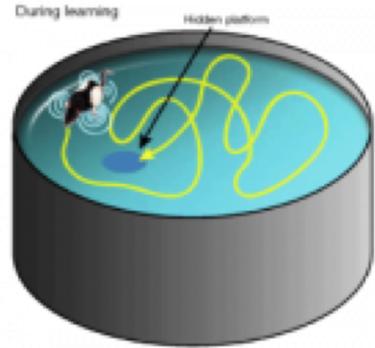
Impact sur la descendance d'une exposition à la morphine: régulation de MeCP2 et HDAC dans l'hippocampe



Moulaei et al. (2018) *NeuroReport* 29:739-744

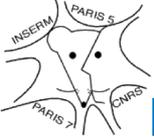


Impact sur la descendance d'une exposition à la morphine: Altération mnésique chez les mâles, pas chez les femelles (F1 et F2)



Mémoire spatiale altérée chez les mâles (F1 et F2)

Moulaei et al. (2018) *NeuroReport* 29:739-744



Exposition aux opioïdes des rats mâles et impact sur la descendance

TABLE 3. Multigenerational and transgenerational effects of paternal exposure to opioids on behavioral and neural function.

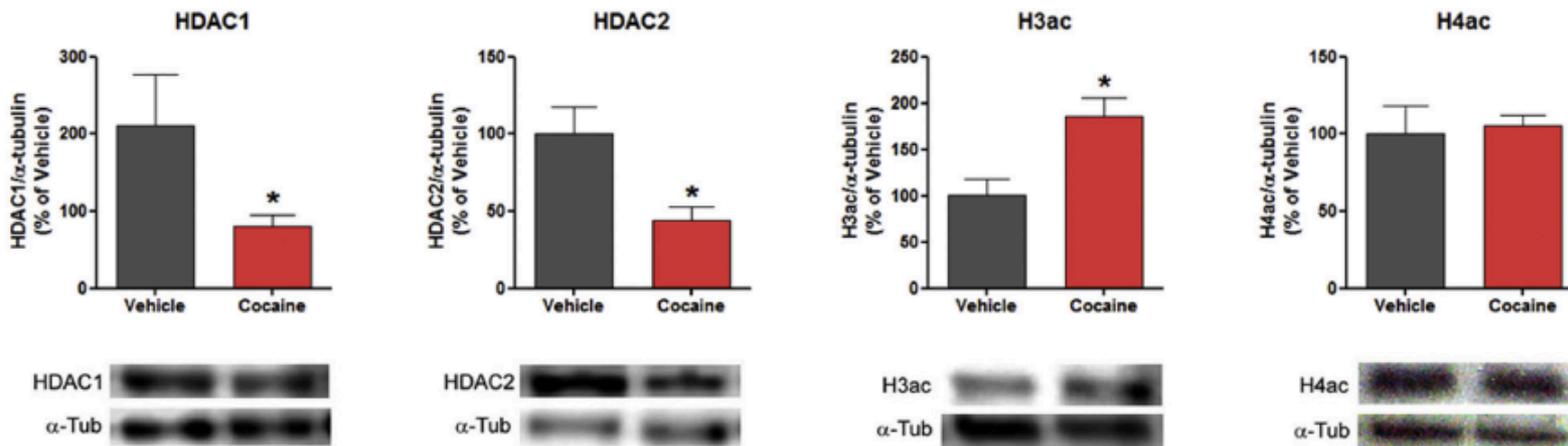
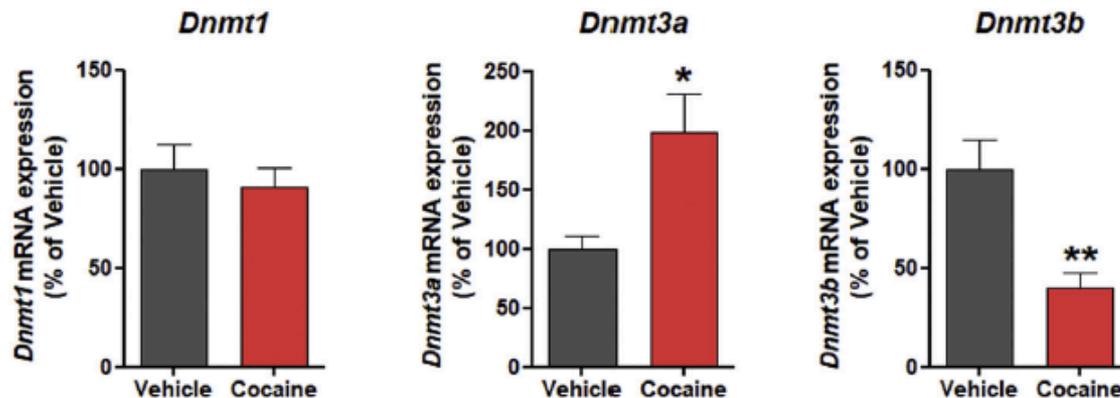
F0 exposure	F0 exposure age	Species	Generation tested	Sex tested	Phenotype (in offspring)	Effect
Chronic: Sustained-released morphine pellets (3–4 weeks)	4–8 weeks	Sprague Dawley Rats	F1	Males & Females	Hypothalamic beta-endorphin levels	Male: No effect, Female: Increased
Acute: morphine (35 mg/kg, i.p.)	9 weeks	Sprague Dawley Rats	F1	Males & Females	Antinociceptive activity of morphine: hot plate	Male: Enhanced sensitivity, Female: No effect
Chronic: morphine (32 mg/kg, 2x daily, i.p., 5 days)	16–20 weeks	Wistar Rats	F1	Males & Females	Hippocampal LTP	Reduced LTP
Chronic: morphine (increasing dosing regimen, 38 days, i.p.)	6–12 weeks	Wistar Rats	F1, F2	Males & Females	Antinociceptive activity of morphine: Tail-withdrawal latency	F1 male: Enhanced sensitivity, F1 female: No effect; F2 male: No effect, F2 female: Enhanced sensitivity
Chronic: morphine (increasing dosing regimen, 10 days, i.p.)	8–10 weeks	Sprague Dawley Rats	F1	Males & Females	Elevated Plus Maze Open field Morris Water Maze Hippocampal Dendritic Morphology	Increased anxiety-like behaviors Increased anxiety-like behaviors; no effect on overall locomotor activity No effect Decreased dendritic, length, dendritic branching, and dendritic spine number
Chronic: heroin (increasing dosing regimen i.p.)	6–8 weeks	Sprague Dawley Rats	F1, F2, F3	Males	Western Blotting (IGF-2) Open field Elevated Plus Maze Resident-Intruder	Decreased levels F1, F2: Increased anxiety-like behaviors; F3: no effect F1, F2: Increased anxiety-like behaviors; no effect on overall locomotor activity; F3: no effect F1, F2: Increased aggressive behavior; F3: no effect
Chronic: morphine (10 mg/kg, 2x daily, s.c., 14 days)	Adult (age not specified)	Wistar Rats	F1	Males & Females	Elevated Plus Maze Sucrose Preference Test Morphine Two-bottle Choice	No effect No effect No effect
Chronic: morphine (increasing dosing regimen, 10 days, i.p.)	4–6 weeks	Wistar Rats	F1	Males	Formalin-induced Pain Test Morphine-induced Antinociception (2.5 mg/kg)	Decreased duration of interphase period (quiescent phase with little to no pain behaviors) No effect

Goldberg and Gould (2019) *Eur. J. Neurosci.* 50(3):2453-2466

Transmission transgénérationnelle ?

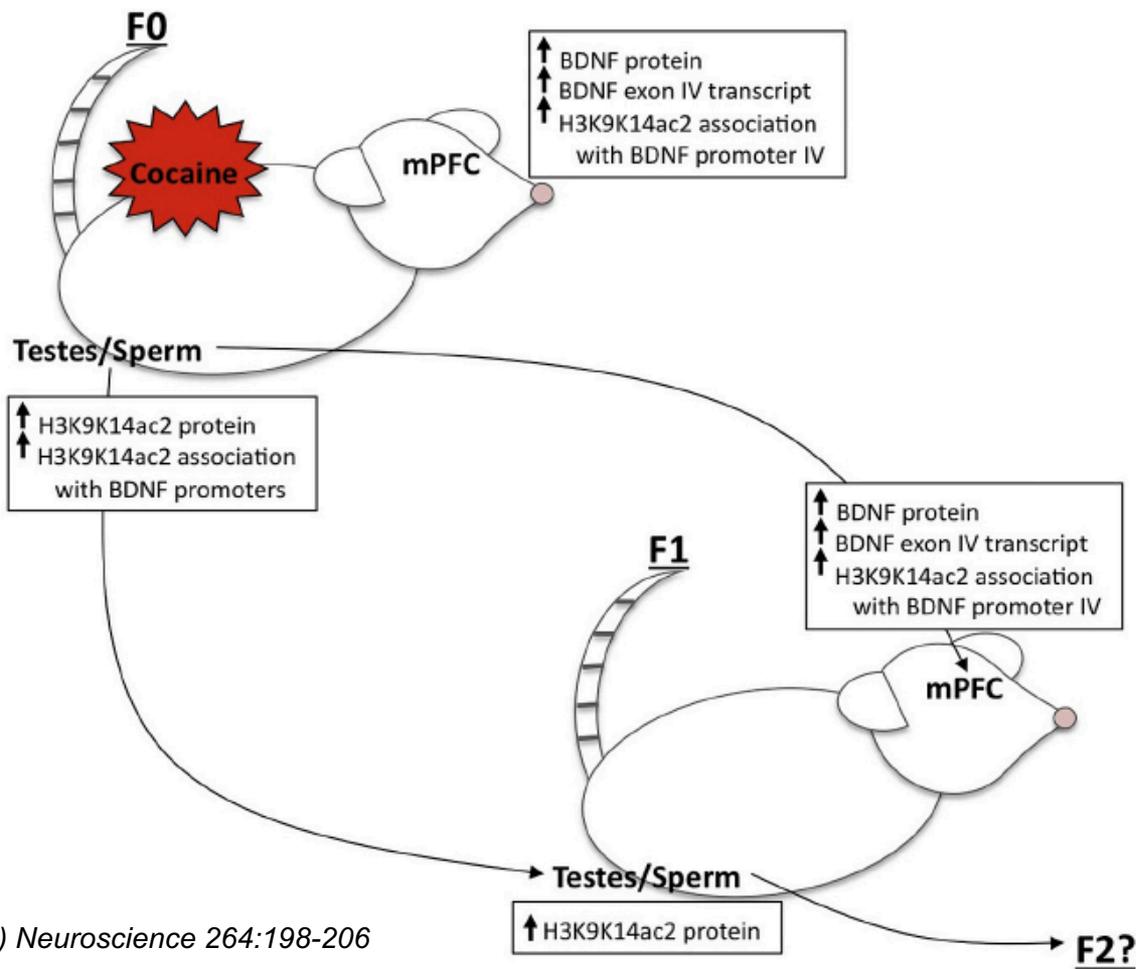
Traitement à la cocaïne,
souris mâles (14 jours)

Analyse dans cellules
germinales



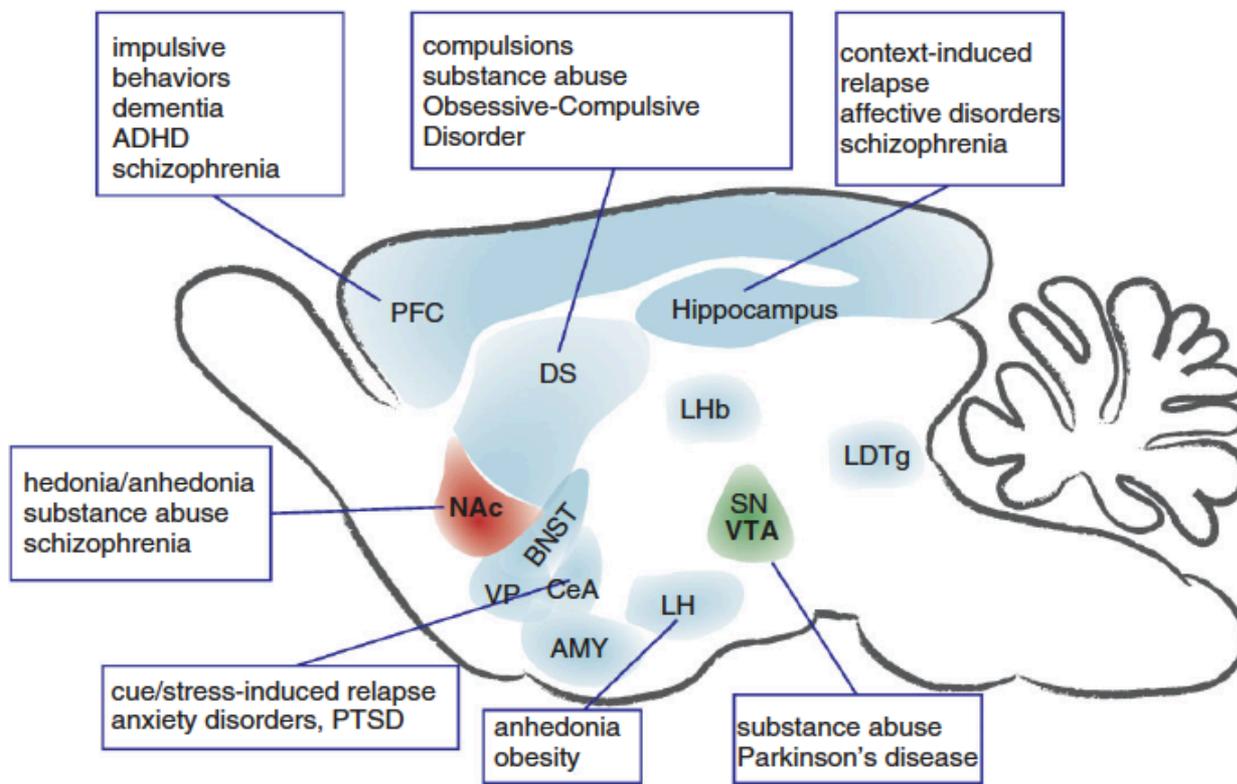
Gonzalez et al. (2018) *Reprod Biomed Online* 37(3):269-278

Transmission transgénérationnelle ?



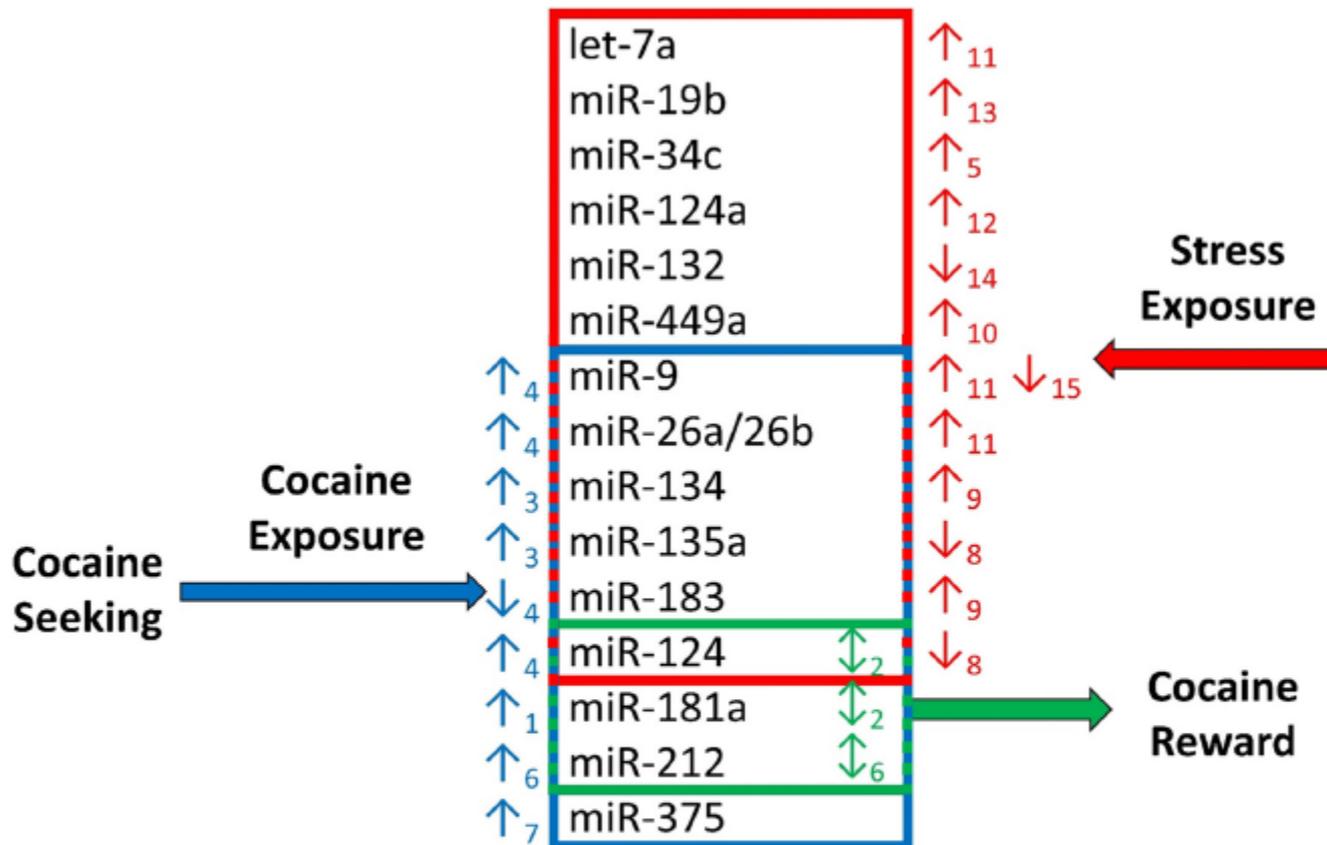
Vassoler and Sadri-Vakili (2014) *Neuroscience* 264:198-206

Schéma simplifié illustrant les régions cérébrales pouvant être impliquées dans des comorbidités avec des comportements addictifs



Joffe et al. (2014) *WIREs Cogn. Sci.* 5:151-171

Régulation des miRNA par la cocaïne et le stress



Doura and Unterwald (2016)

Pour résumer

- ✓ **Les plasticités neurobiologiques induites par opioïdes peuvent venir de régulations épigénétiques.**
- ✓ **Ces régulations sont multiples, dynamiques dans le temps (différentes étapes dans l'addiction)**
- ✓ **Elles peuvent être régio-sélectives, drogues-spécifiques**
- ✓ **Elles sont multiples**
- ✓ **Certaines modifications épigénétiques sont transmissibles à la descendance**
- ✓ **Les régulations épigénétiques peuvent être « partagées » entre différentes pathologies** (explication pour la vulnérabilité aux comorbidités)
- ✓ **Biomarqueurs** (e.g., diagnostic, efficacité des thérapies)? (ex. chez l'homme: héroïne: Xu et al. (2020) J Clin Lab Anal 34(11): e23486; met-amphétamine: Zhao et al. (2016) J Neuroimmune Pharmacol 11(3):542-8)
- ✓ **Stratégies thérapeutiques?**