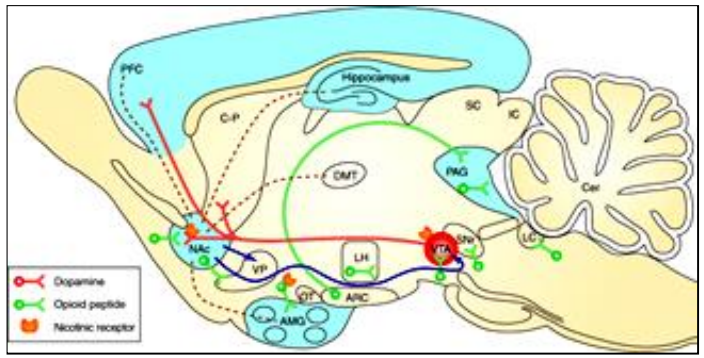
Epigenetics and Addiction

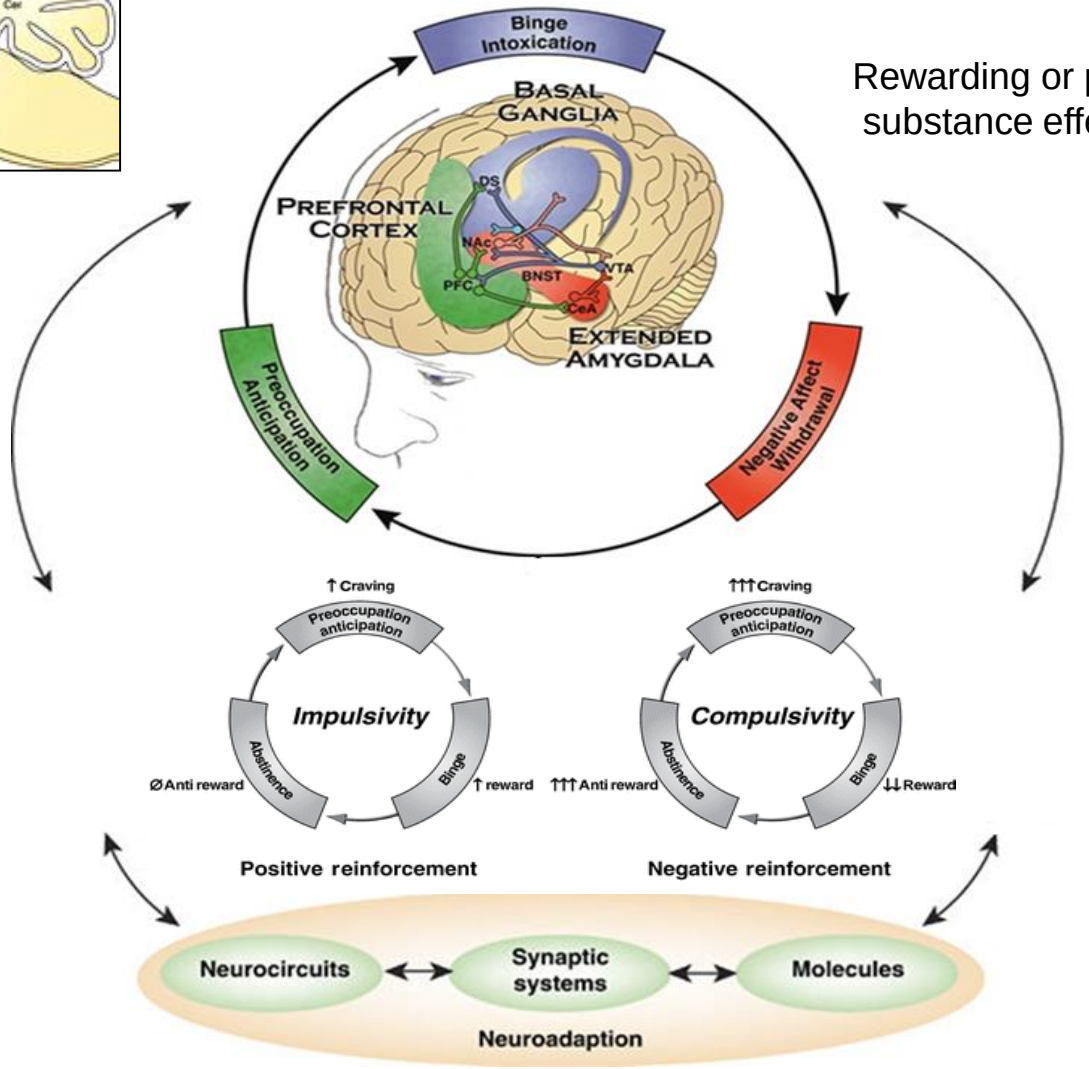


Drug addiction defined as a THREE-STAGE CYCLE

Rewarding or pleasurable substance effects

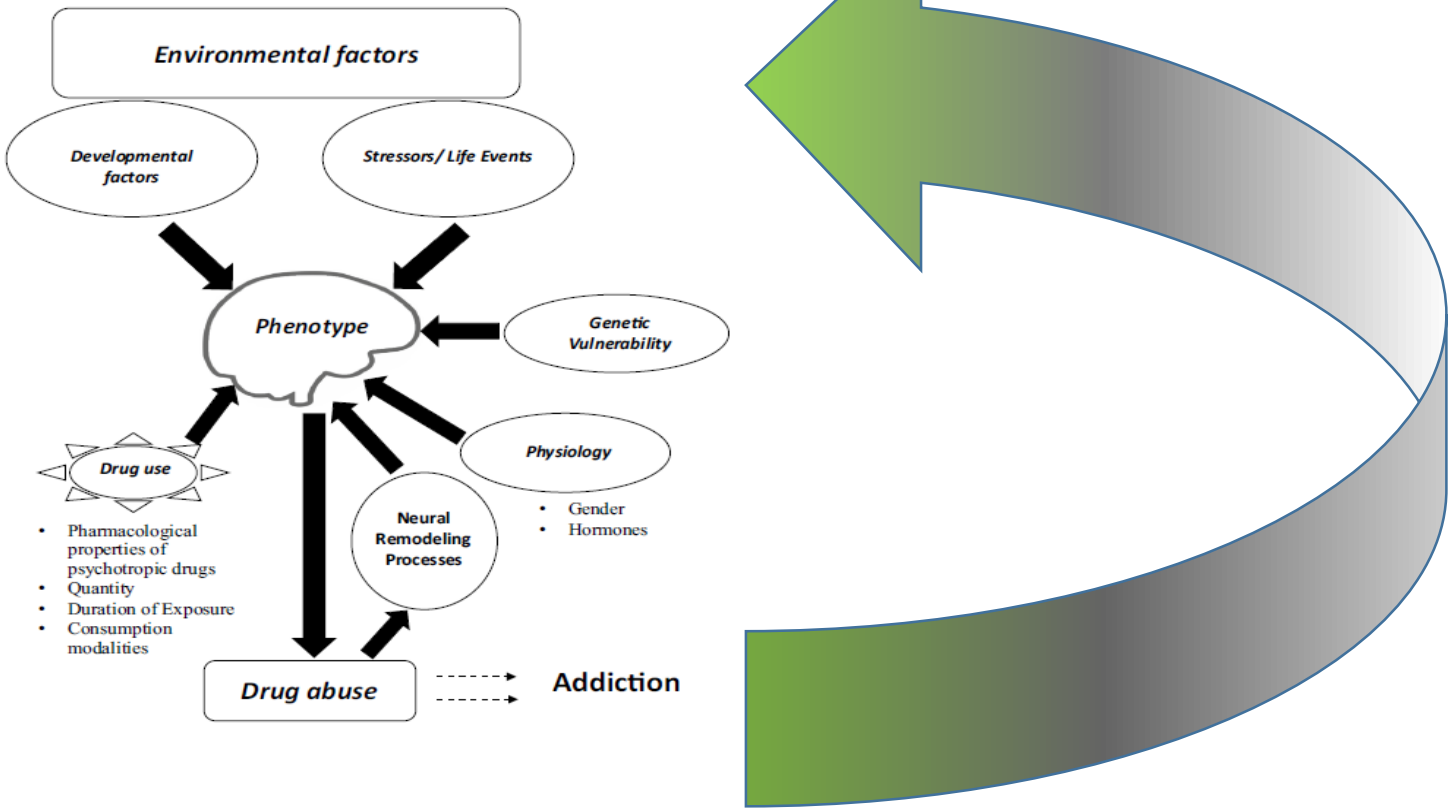
Substances seeking after a period of abstinence

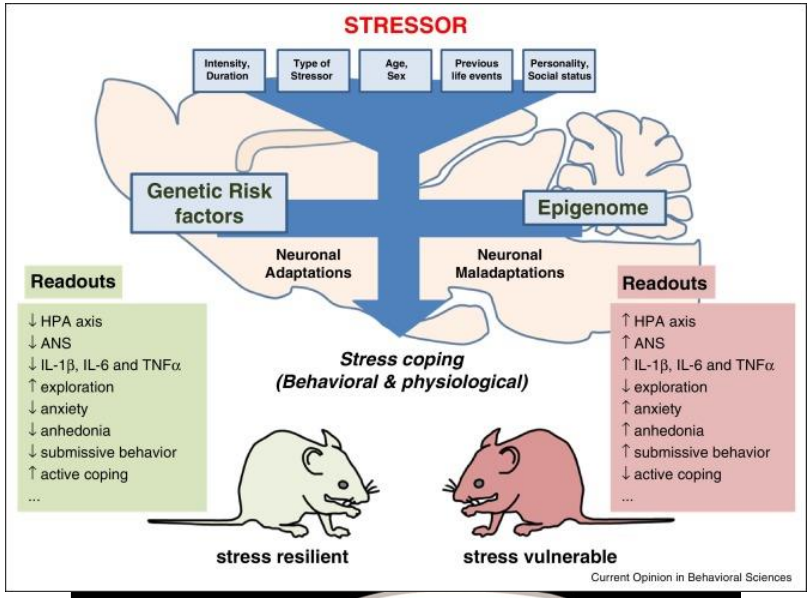
Negative emotional state in the absence of substances

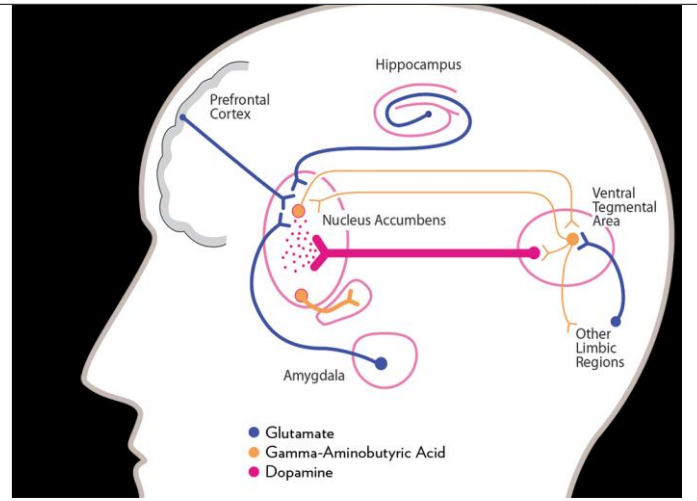


(Koob et al. 2017)

Environmental factor and Drug addiction

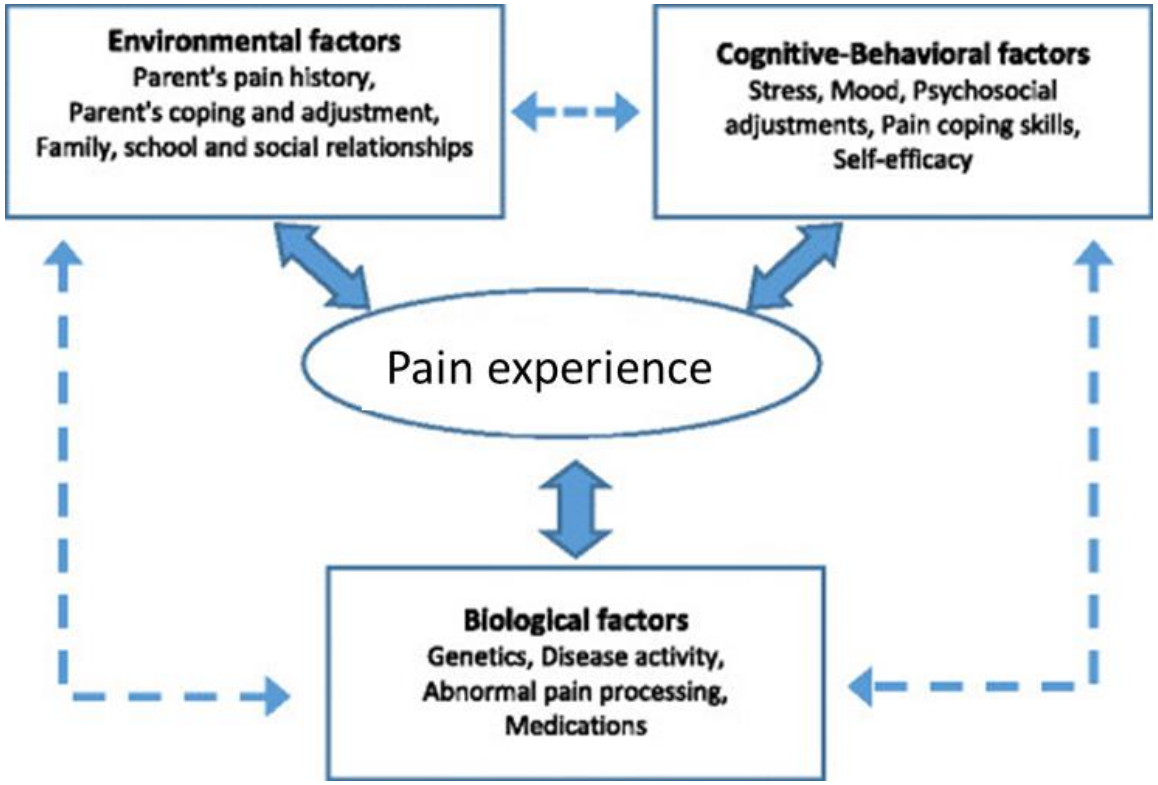


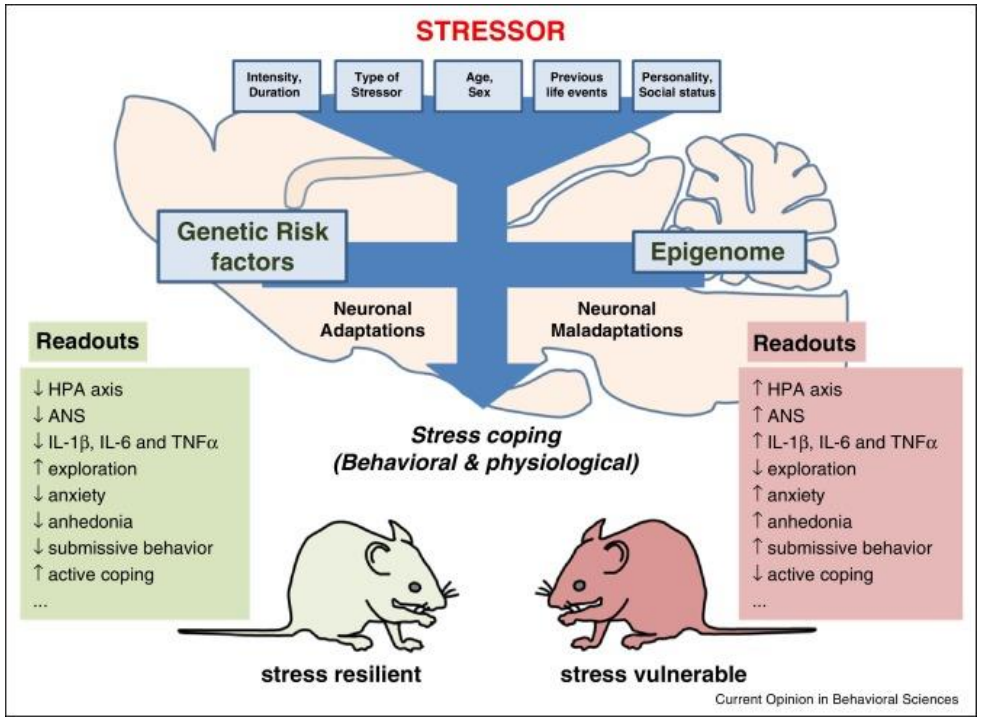




Environmental factor and neuropathic pain

❖**Genes, environment and brain circuitry <u>in neuropathic pain??</u>**



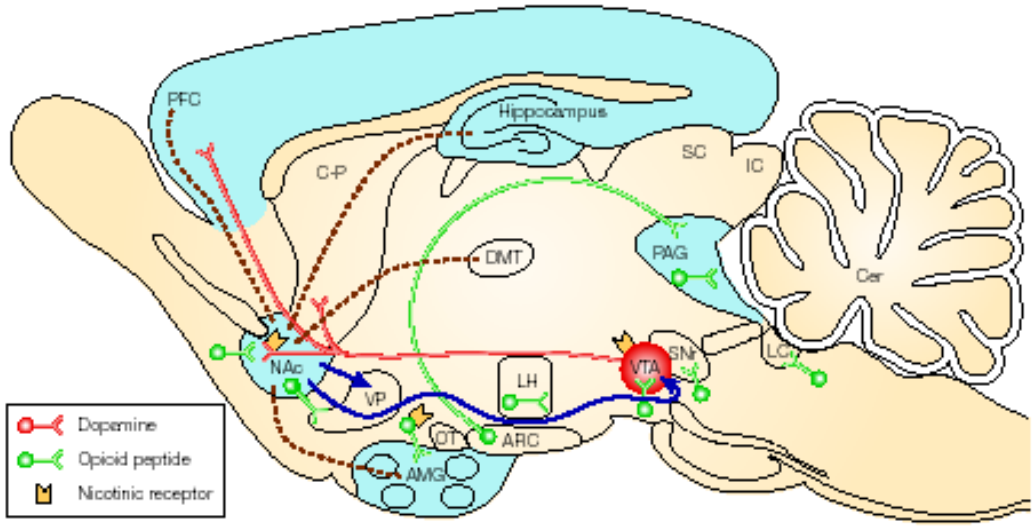


Dalla Tolleranza alla Dipendenza fisica…che si rivela con l'astinenza.

Dipendenza psichica (OUD)

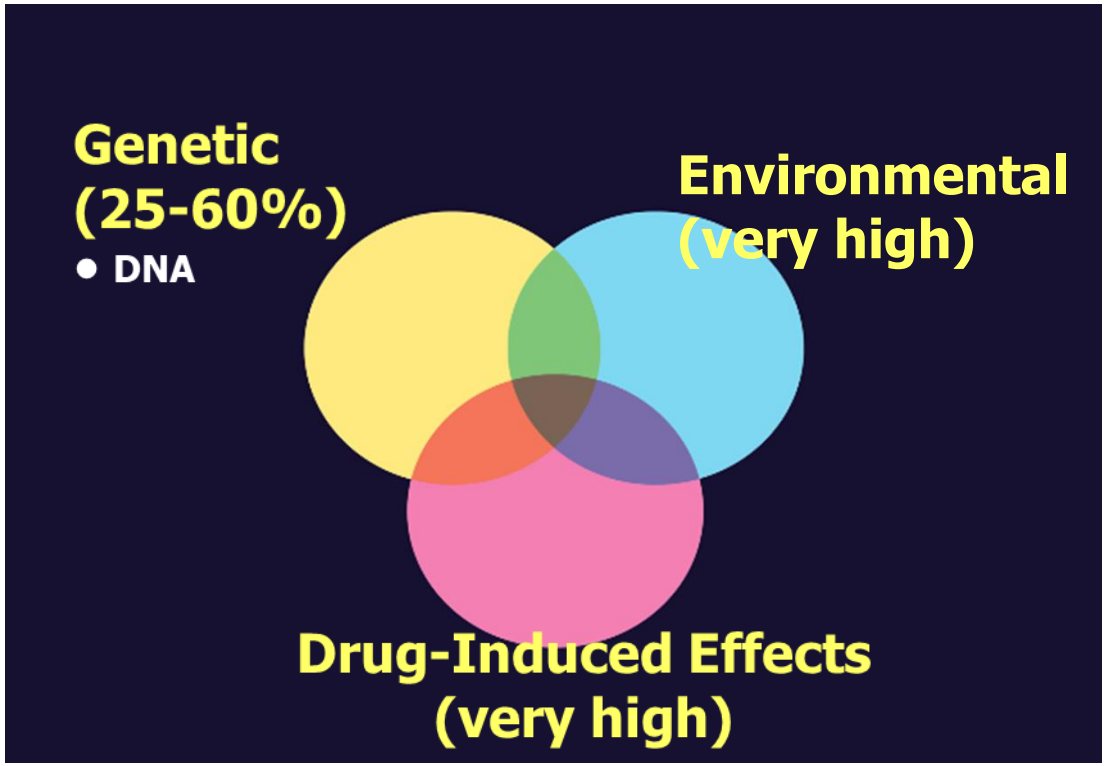
FATTORI NEUROBIOLOGICI (genetici, farmacodinamici)

FATTORI INDIVIDUALI (psico-emozionali, socio-culturali



da: Nestler EJ, Nature Rev. Neurosci., 2, 119-128, 2001

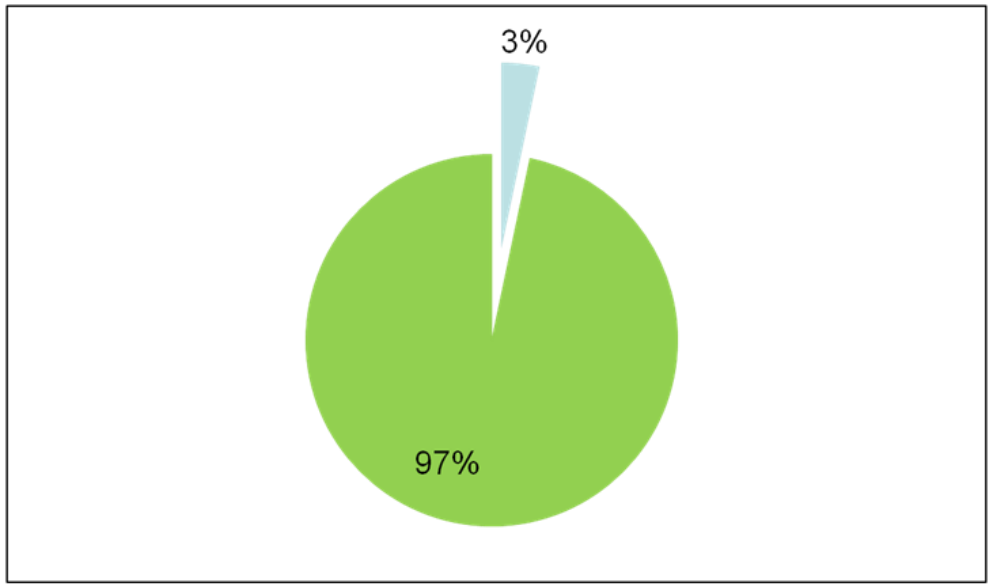
Factors Contributing to Vulnerability To Develop a Specific Addiction

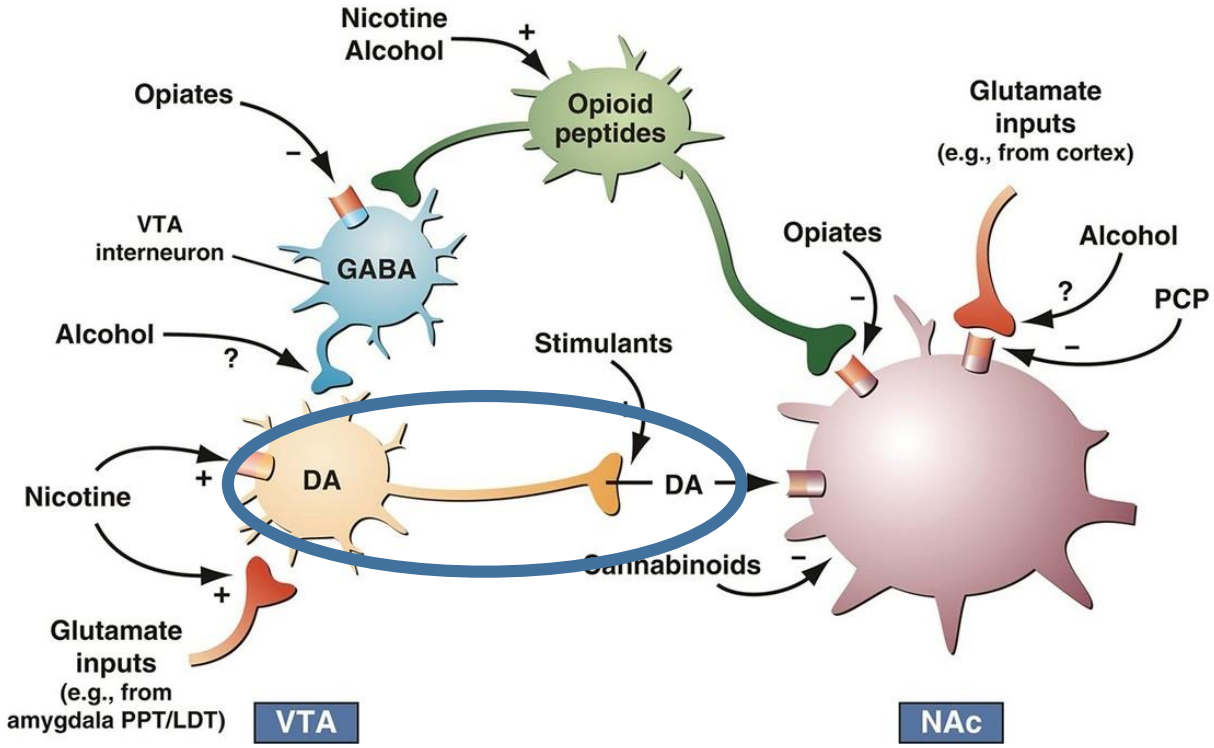


Kreek et al., 2005

The question is:

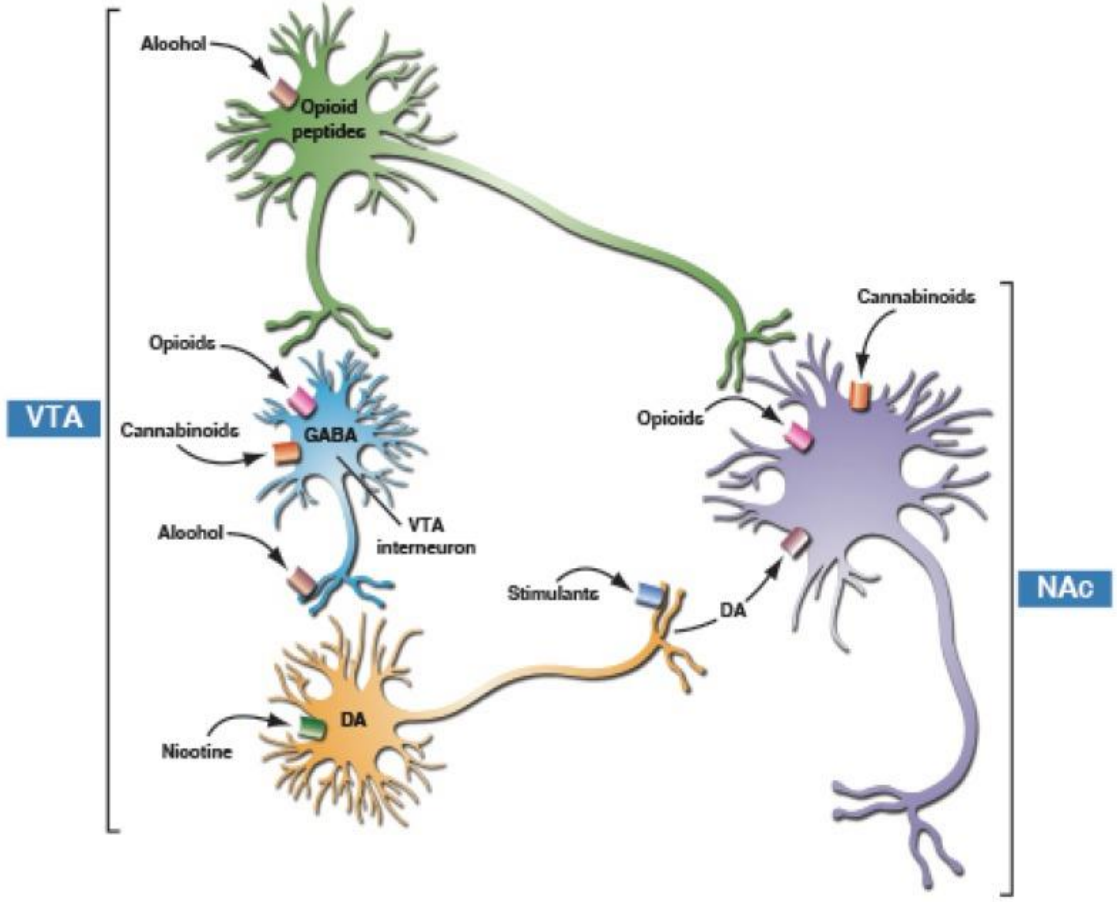
Are there neurobiological basis for which patients suffering from chronic or neuropathic pain under chronic opioid therapy do not develop an OUD?





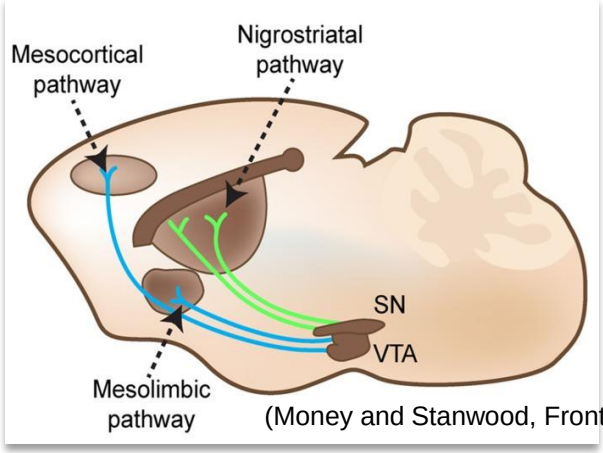
Patrizia Romualdi

Actions of Addictive Substances on the Brain



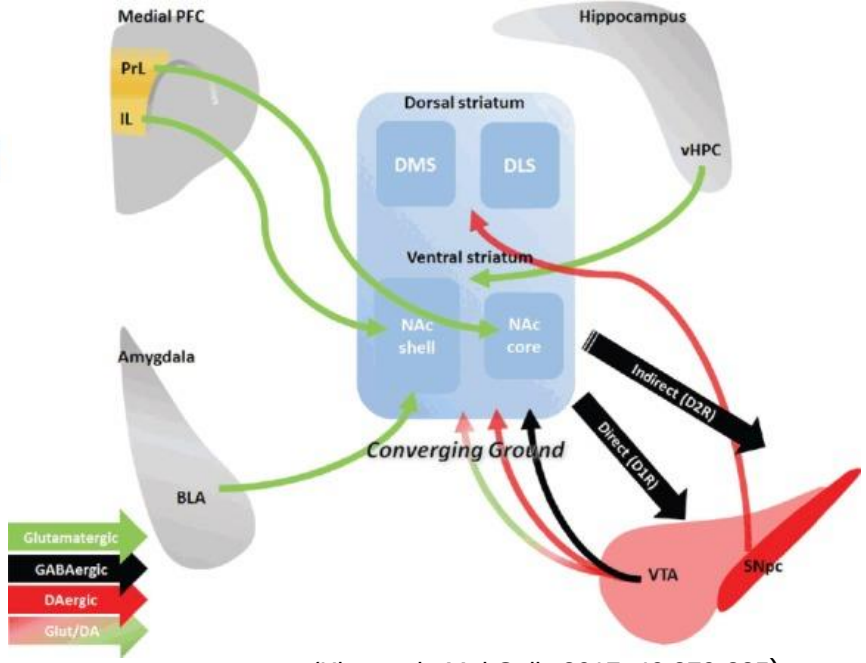
(Nestler EJ. Nature Neuroscience. 2005;8:1445–1449)

❖ **Genes, environment and brain circuitry <u>in drug addiction</u>**



(Money and Stanwood, Front Cell Neurosci 2013 ;7:260)

Diverse afferent and efferent connectivity in the striatum



(Kim et al., Mol Cells 2017; 40:379-385)

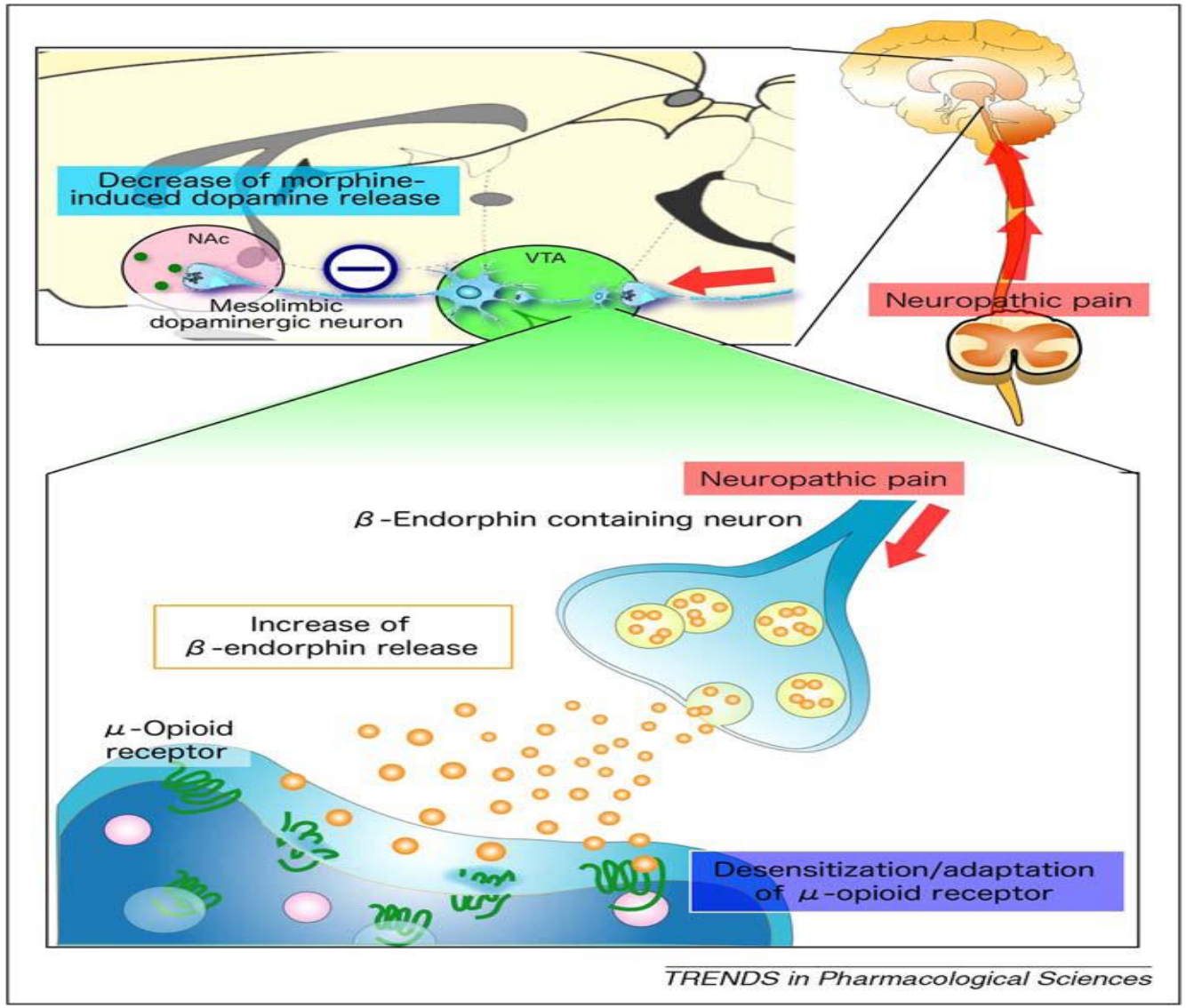


Figure 1. Model of the mechanism of suppression of m-agonist-induced reward in neuropathic pain. Peripheral nerve injury can cause sustained activation of the endogenous b-endorphinergic system in the brain. b-Endorphin released by chronic nociceptive stimuli can continuously activate m-opioid receptors in the VTA, thus leading to downregulation of m-opioid receptor function and resulting in a decrease in dopamine release in the NAc. This phenomenon could explain the mechanism that underlies the suppression of m-opioid reward under neuropathic pain-like states observed in animal models [28].

Opinion

Cell

Neuropathic and chronic pain stimuli downregulate central μ -opioid and dopaminergic transmission

Keiichi Niikura[1,2], Minoru Narita[1], Eduardo R. Butelman[2], Mary Jeanne Kreek[2] and Tsutomu Suzuki[1]

[1]Department of Toxicology, Hoshi University School of Pharmacy and Pharmaceutical Sciences, 2-4-41 Ebara, Shinagawa-ku, Tokyo 142-8501, Japan
[2]The Laboratory of the Biology of Addictive Diseases, The Rockefeller University, 1230 York Avenue, New York, NY 10065, USA

Patrizia Romualdi

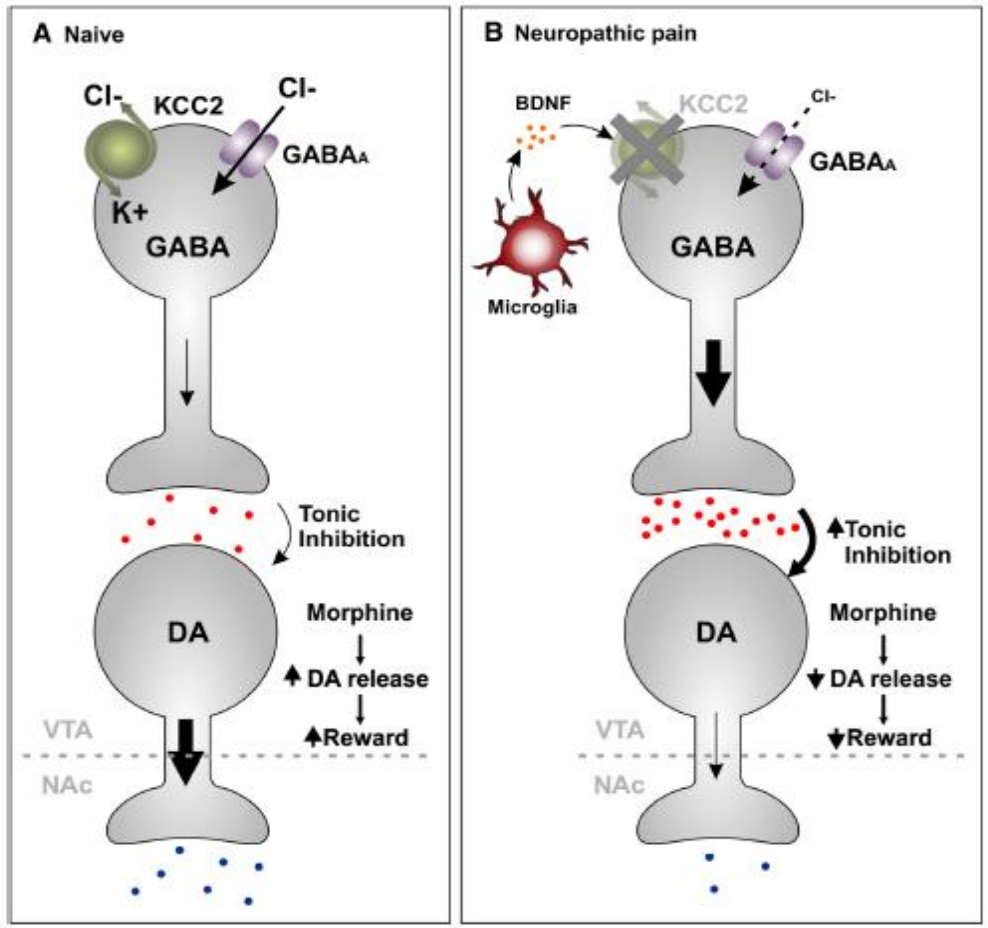


Figure 3. Model of microglial-mediated altered reward circuitry. As a consequence of chronic opioid exposure or chronic pain, microglial activation occurs in many areas of the CNS, including brain regions involved in reward such as the VTA. It is hypothesized that gliosis triggers a reorganization of reward circuitry such that microglia change E_{GABA} in GABAergic neurons within the VTA. ***A***, In the naive state, these neurons tonically inhibit mesolimbic dopaminergic neurons projecting to the NAc. ***B***, In chronic pain states, activated microglia release BDNF. This in turn causes disruption of E_{GABA} via downregulation of the K^+/Cl^- transporter KCC2 protein levels and activity. The loss of KCC2 causes a disruption of Cl^- homeostasis in GABAergic neurons, resulting in these neurons being more depolarized. Consequently, activation of $GABA_A$ receptors on these GABAergic neurons results in their depolarization rather than hyperpolarization because of a net inward anion (Cl^-/HCO_3^-) current (that is normally outward). An increase in the excitability of GABAergic neurons results in an increase in GABA release and augmentation of the inhibitory tone on dopaminergic neurons, leading to less dopamine release in the NAc. DA, Dopamine.

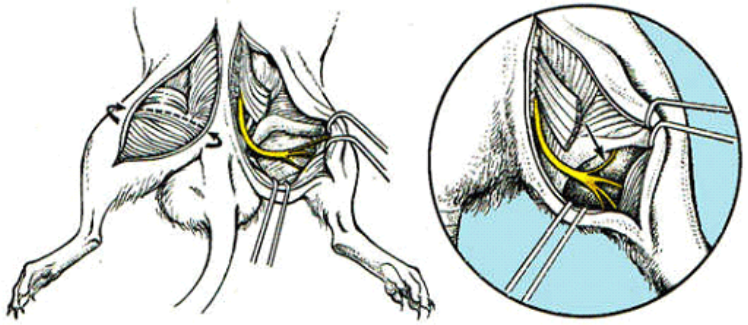
Mini-Symposium

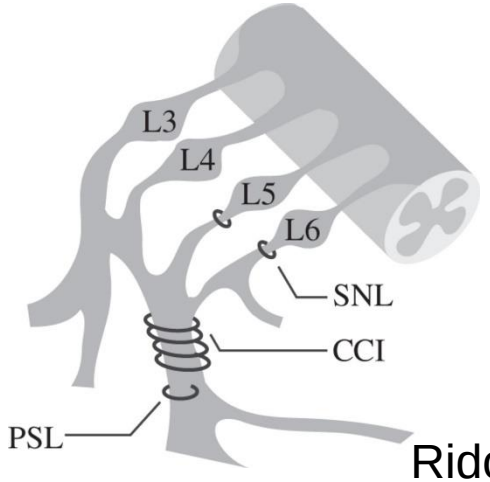
Pain and Poppies: The Good, the Bad, and the Ugly of Opioid Analgesics

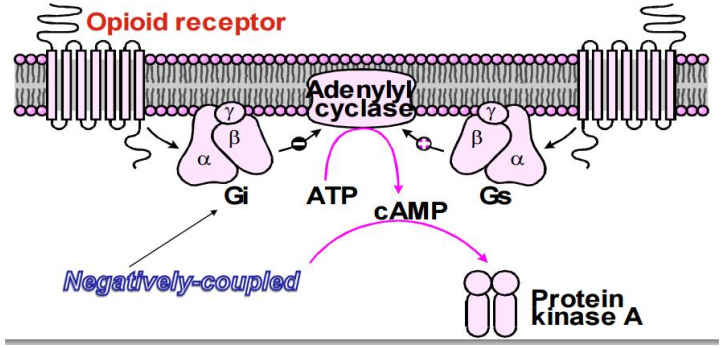
Tuan Trang,[1,2] Ream Al-Hasani,[3,4] Daniela Salvemini,[5] Michael W. Salter,[6] Howard Gutstein,[7] and Catherine M. Cahill[8]

Departments of [1]Comparative Biology and Experimental Medicine and [2]Physiology and Pharmacology, Hotchkiss Brain Institute, University of Calgary, Calgary, Alberta T2N 4N1, Canada, Departments of [3]Anesthesiology and [4]Anatomy–Neurobiology, Washington University School of Medicine, St. Louis, Missouri 63110, [5]Department of Pharmacological and Physiological Science, St. Louis University School of Medicine, St. Louis, Missouri 63104, [6]Neurosciences and Mental Health Program, Hospital for Sick Children, Toronto, Ontario M5G 0A4, Canada, [7]MD Anderson Cancer Center, Houston, Texas 77030, and [8]Department of Anaesthesiology and Perioperative Care, University of California Irvine, Irvine, California 92697

Un modello murino di dolore neuropatico: la legatura del nervo sciatico







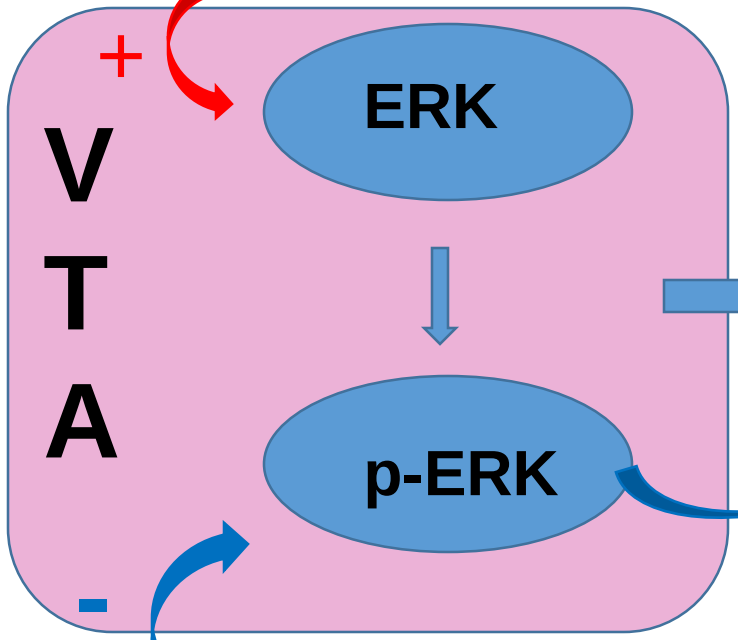
Ridotta funzione dei recettori oppioidi μ nel mesencefalo (VTA)

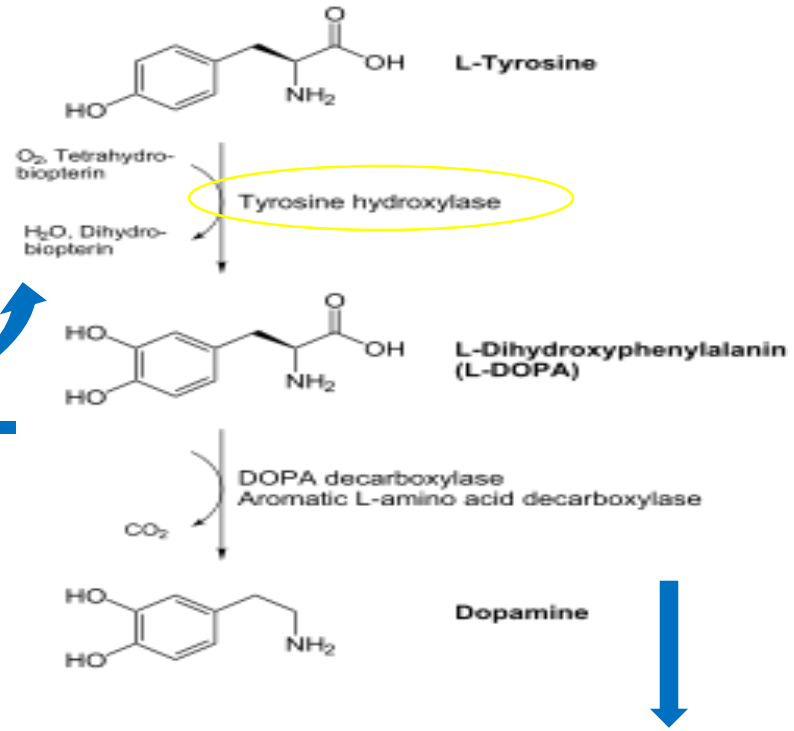
Sciatic nerve ligation suppresses the rewarding effects of morphine *in vivo* (but not antinociception)

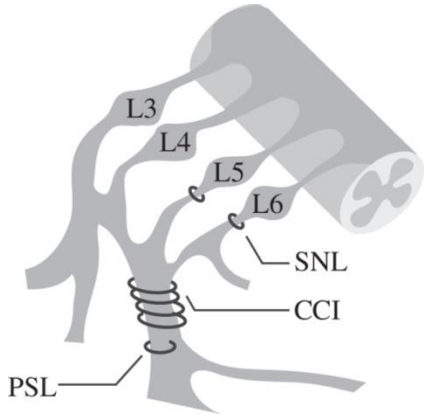
Riduzione della ricompensa e del potenziale d'abuso indotti da morfina

Ozaki S. et al., *Neuroscience* 2003

Morfina
(somministrazione cronica)





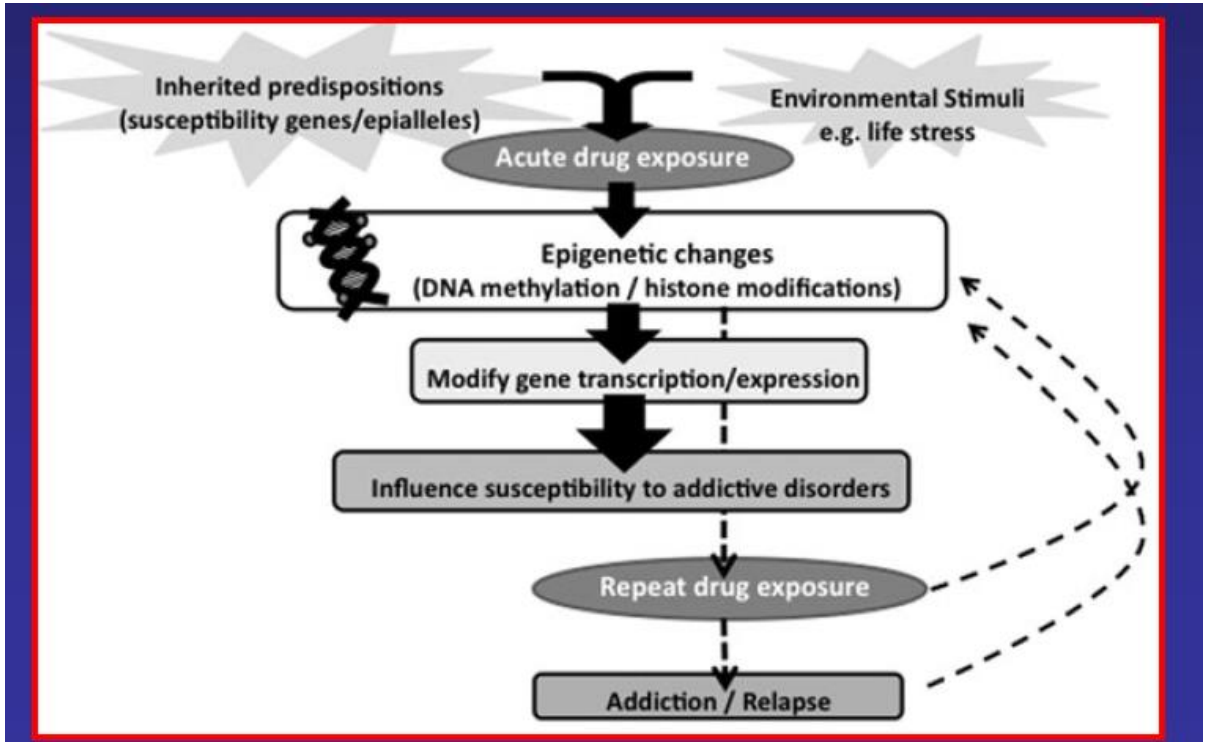


In modelli di dolore neuropatico la ridotta attività di p-ERK in VTA causa a sua volta una riduzione dell'attività di TH con conseguente riduzione del tono dopaminergico

Epigenetics and addictive drugs

Epigenetic alterations interact with genetic predisposition, environmental factors, exposure to addictive drugs.

All together induce long-lasting gene expression alterations influencing the typical behavior of addiction



EPIGENETICS and Addictive drugs

Cocaine epigenetic effects

1 single cocaine administration ↑ in 30 min histone H4 acetylation (H4ac) e histone H3 phospho-acetylated (H3pac) levels in rat NAc

Self-administration of cocaine in the rat ↑ **H3ac and H4ac levels in NA** but only H3ac levels are related to motivation of the cocaine intake.

On the contrary, repeated exposure to cocaine ↓ **H3 dimethylation in Lysine in position 9 (H3K9me2), a repressive modification**.

EPIGENETICS and Addictive drugs

Combining the two modifications (increase of acetylation and decrease of methylation on H3 determines the final effect: repeated exposure to cocaine induce a global increase of DNA transcription.

Therefore the chronic exposure to cocaine induce in the Nac neurons a global modification of chromatin that is favorable to transcription by means of increase of acetylation (H3ac, H4ac) and phosphoacetylation (H3pac) and a decrease of methylation (H3K9me2)

Progress in Neuro-Psychopharmacology & Biological Psychiatry 49 (2014) 36–46

Contents lists available at ScienceDirect

Progress in Neuro-Psychopharmacology & Biological Psychiatry

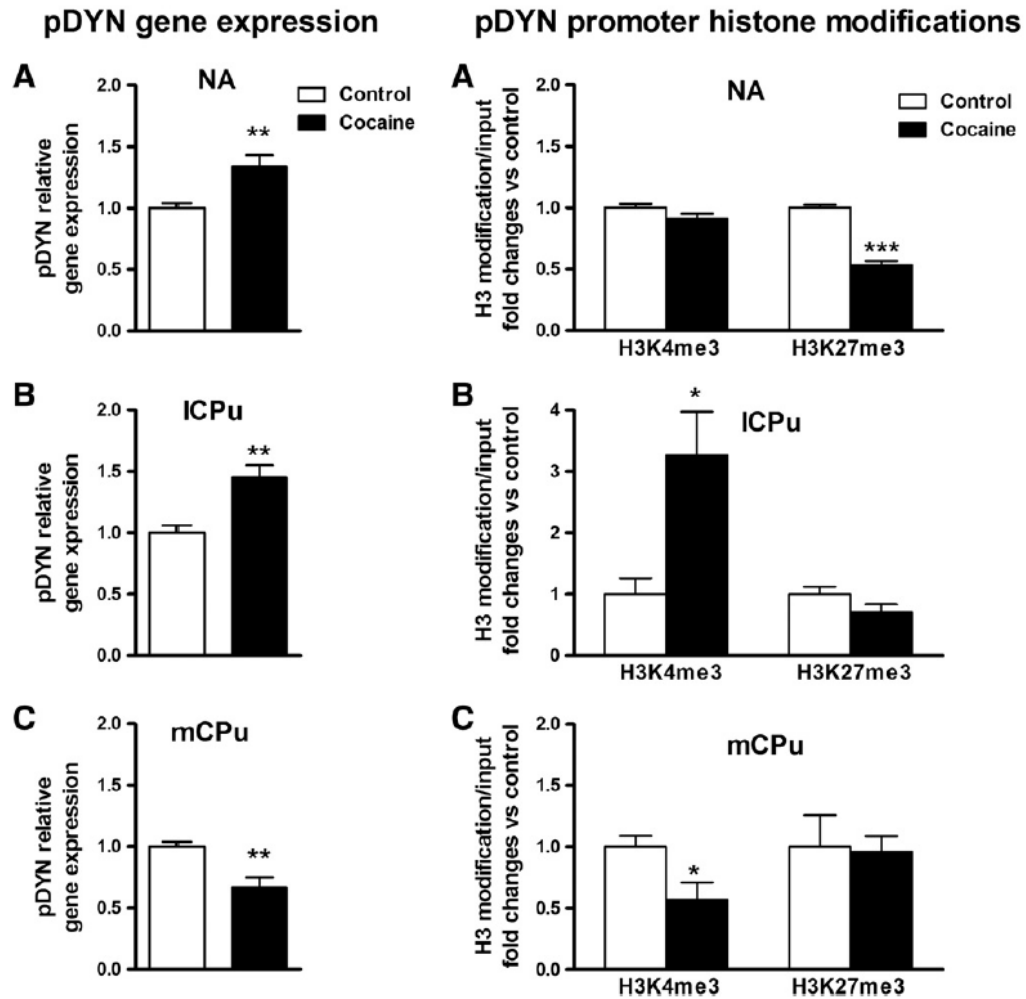
journal homepage: www.elsevier.com/locate/pnp

Dynorphin/KOP and nociceptin/NOP gene expression and epigenetic changes by cocaine in rat striatum and nucleus accumbens

Francesca Felicia Caputi [1], Manuela Di Benedetto [1], Donatella Carretta, Sussy Bastias del Carmen Candia, Claudio D'Addario, Chiara Cavina, Sanzio Candeletti, Patrizia Romualdi *

Department of Pharmacy and Biotechnologies, Alma Mater Studiorum, University of Bologna, Irnerio 48, 40126 Bologna, Italy

Cocaine induces neurochemical changes of endogenous prodynorphin-kappa opioid receptor (pDYN-KOP) and pronociceptin/orphaninFQ-nociceptin receptor (pN/OFQ-NOP) systems. Both systems play an important role in rewarding mechanisms and addictive stimulus processing by modulating drug-induced dopaminergic activation in the mesocortico-limbic brain areas. They are also involved in regulating stress mechanisms related to addiction. The aim of this study was to investigate possible changes of gene expression of the dynorphinergic and nociceptinergic system components in the nucleus accumbens (NA) and in medial and lateral caudate putamen (mCPu and lCPu, respectively) of rats, following chronic subcutaneous infusion of cocaine. In addition, the epigenetic histone modifications H3K4me3 and H3K27me3 (an activating and a repressive marker, respectively) at the promoter level of the pDYN, KOP, pN/OFQ and NOP genes were investigated. Results showed that cocaine induced pDYN gene expression up-regulation in the NA and lCPu, and its down-regulation in the mCPu, whereas KOP mRNA levels were unchanged. Moreover, cocaine exposure decreased pN/OFQ gene expression in the NA and lCPu, while NOP mRNA levels appeared significantly increased in the NA and decreased in the lCPu.
Specific changes of the H3K4me3 and H3K27me3 levels were found at pDYN, pN/OFQ, and NOP gene promoter, consistent with the observed gene expression alterations.
The present findings contribute to better define the role of endogenous pDYN-KOP and pN/OFQ-NOP systems in neuroplasticity mechanisms following chronic cocaine treatment. The epigenetic histone modifications underlying the gene expression changes likely mediate the effects of cocaine on transcriptional regulation of specific gene promoters that result in long-lasting drug-induced plasticity.



g. 2. Left panel — pDYN mRNA levels in the striatum. pDYN gene expression was measured in rat nucleus accumbens (A), lateral (B) and medial (C) caudate-putamen. Animals (n = 6) ere chronically infused s.c. with cocaine (total daily dose: 50 mg/kg) for 7 days and compared to saline-treated rats (control group, n = 6). Bars represent 2^{-DDCt} value calculated by lta–Delta Ct (DDCt) method. Gene expression was normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH); data are expressed as means ± SE of 6 animals for each oup. **p < 0.01 versus control group. Right panel — pDYN promoter histone modifications. RT-qPCR analyses of H3K4me3 and H3K27me3 immuno-precipitated DNA fragments at YN promoter in rat nucleus accumbens (A), lateral (B) and medial (C) caudate-putamen. ChIP histogram shows the levels of specific histone modifications, normalized to total input IA, in rats chronically infused with cocaine for 7 days. Data are expressed as means ± SE of 6 animals for each group. ***p < 0.001 and *p < 0.05 versus control.

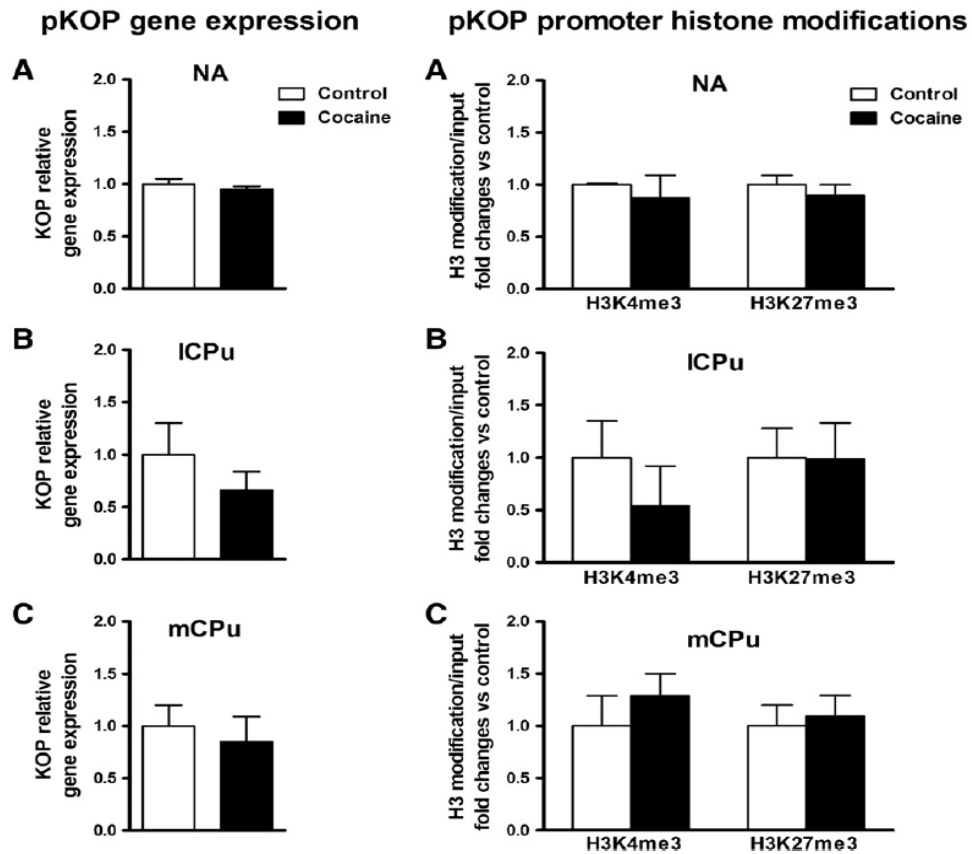
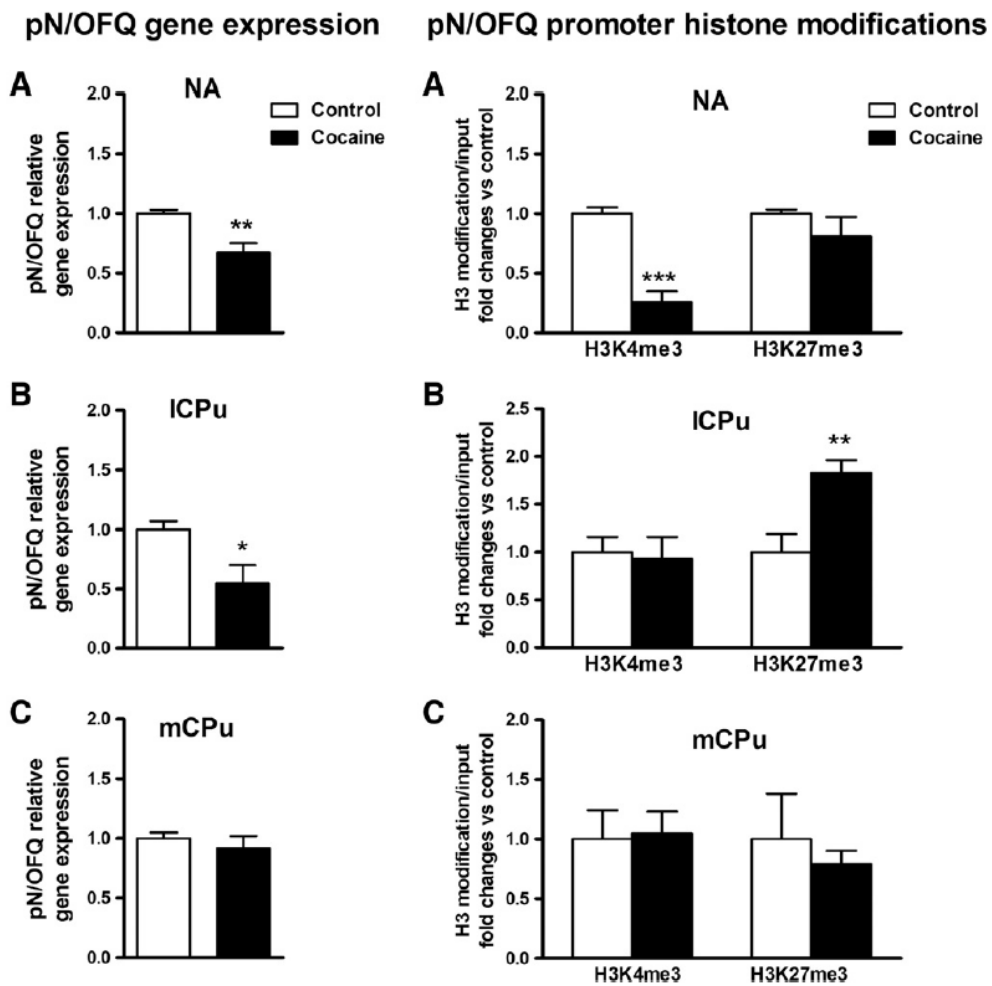


Fig. 3. Left panel — KOP mRNA levels in the striatum. KOP gene expression was measured in rat nucleus accumbens (A), lateral (B) and medial (C) caudate-putamen. Animals were chronically infused with cocaine (total daily dose: 50 mg/kg) for 7 days and compared to saline-treated rats (control group). Bars represent 2^{-DDCt} value calculated by Delta–Delta Ct (DDCt) method ($n = 6$). Gene expression was normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH); data are expressed as means ± SE of 6 animals for each group. Right panel — KOP promoter histone modifications. RT-qPCR analyses of H3K4me3 and H3K27me3 immuno-precipitated DNA fragments at KOP promoter were performed in rat nucleus accumbens (A), lateral (B) and medial (C) caudate-putamen. ChIP histogram shows the levels of specific histone modification, normalized to total input DNA, in rats chronically infused with cocaine for 7 days. Data are expressed as means ± SE of 6 animals for each group.


pN/OFQ gene expression
pN/OFQ promoter histone modifications
A
NA
Control
Cocaine
pN/OFQ relative gene expression
2.0
1.5
1.0
0.5
0.0
**
B
lCPu
*
C
mCPu
H3 modification/input fold changes vs control
***
H3K4me3
H3K27me3
2.5
**

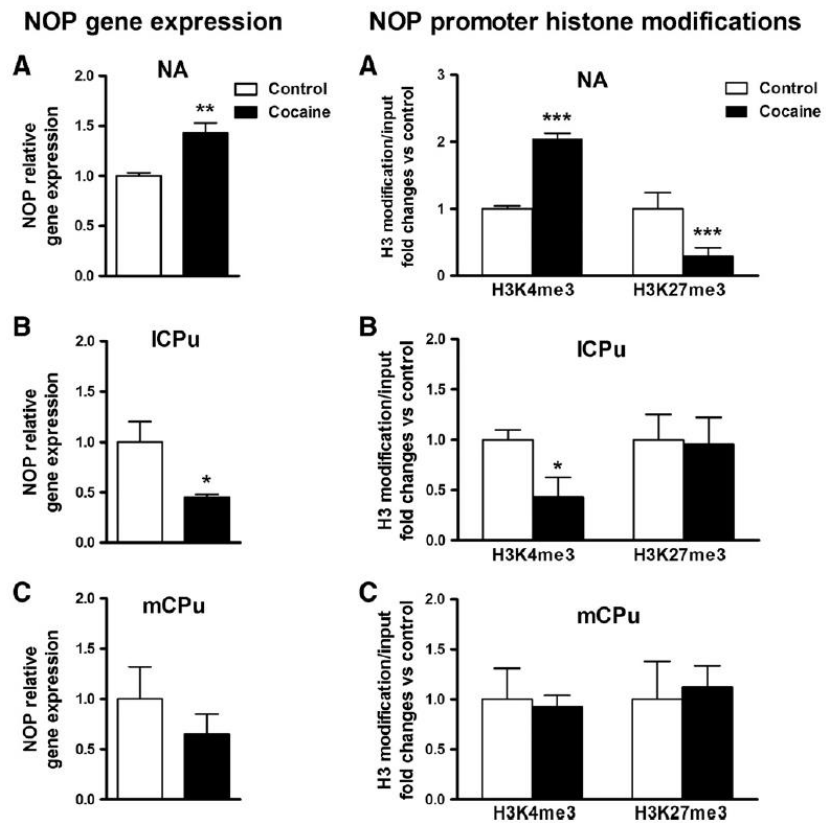


Fig. 5. Left panel — Levels of NOP mRNA in the striatum. The NOP gene expression was measured in rat nucleus accumbens (A), lateral (B) and medial (C) caudate-putamen. Animals were chronically infused with cocaine (total daily dose: 50 mg/kg) for 7 days and compared to saline-treated rats (control group). Bars represent 2^{-DDCt} value calculated by Delta–Delta Ct (DDCt) method ($n = 6$). Gene expression was normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and data are expressed as means ± SE of 6 animals for each group. $^{**}p < 0.01$ and $^{*}p < 0.05$ versus control group. Right panel — NOP promoter histone modifications. RT-qPCR analyses of H3K4me3 and H3K27me3 immuno-precipitated DNA fragments at NOP promoter were performed in rat nucleus accumbens (A), lateral (B) and medial (C) caudate-putamen. ChIP histogram shows the levels of specific histone modification normalized to total input DNA in rats chronically infused with cocaine for 7 days. Data are expressed as means ± SE of 6 animals for each group. $^{***}p < 0.001$ and $^{*}p < 0.05$ versus control.

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Original article

Opioid gene expression changes and post-translational histone modifications at promoter regions in the rat nucleus accumbens after acute and repeated 3,4-methylenedioxy-methamphetamine (MDMA) exposure

Francesca Felicia Caputi, Martina Palmisano, Lucia Carboni, Sanzio Candeletti [1], Patrizia Romualdi [*,1]

Department of Pharmacy and Biotechnology, Alma Mater Studiorum – University of Bologna, Irnerio 48, 40126 Bologna, Italy

The recreational drug of abuse 3,4-methylenedioxymethamphetamine (MDMA) has been shown to produce neurotoxic damage and long-lasting changes in several brain areas. In addition to the involvement of serotoninergic and dopaminergic systems, little information exists about the contribution of nociceptin/orphaninFQ (N/OFQ)-NOP and dynorphin (DYN)-KOP systems in neuronal adaptations evoked by MDMA. Here we investigated the behavioral and molecular effects induced by acute (8 mg/kg) or repeated (8 mg/kg twice daily for seven days) MDMA exposure.

MDMA exposure affected body weight gain and induced hyperlocomotion; this latter effect progressively decreased after repeated administration. Gene expression analysis indicated a down-regulation of the N/OFQ system and an up-regulation of the DYN system in the nucleus accumbens (NAc), highlighting an opposite systems regulation in response to MDMA exposure.

Since histone modifications have been strongly associated to the addiction-related maladaptive changes, we examined two permissive (acH3K9 and me3H3K4) and two repressive transcription marks (me3H3K27 and me2H3K9) at the pertinent opioid gene promoter regions. Chromatin immunoprecipitation assays revealed that acute MDMA increased me3H3K4 at the pN/OFQ, pDYN and NOP promoters. Following acute and repeated treatment a significant decrease of acH3K9 at the pN/OFQ promoter was observed, which correlated with gene expression results. Acute treatment caused an acH3K9 increase and a me2H3K9 decrease at the pDYN promoter which matched its mRNA up-regulation.

Our data indicate that the activation of the DYNergic stress system together with the inactivation of the N/OFQergic anti-stress system contribute to the neuroadaptive actions of MDMA and offer novel epigenetic information associated with MDMA abuse.

Table 1
Primers used in quantitative PCR for gene expression.

	Forward (5′–3′)	Reverse (5′–3′)	Product size
pN/OFQ	TGCAGCACCTGAAGAGAATG	CAACTTCCGGGCTGACTTC	170
NOP	AGCTTCTGAAGAGGCTGTGT	GACCTCCCAGTATGGAGCAG	101
pDYN	CCTGTCCTTGTGTTCCCTGT	AGAGGCAGTCAGGGTGAGAA	157
KOP	TTGGCTACTGGCATCATCTG	ACACTCTTCAAGCGCAGGAT	177
GAPDH	AGACAGCCGCATCTTCTTGT	CTTGCCGTGGGTAGAGTCAT	207

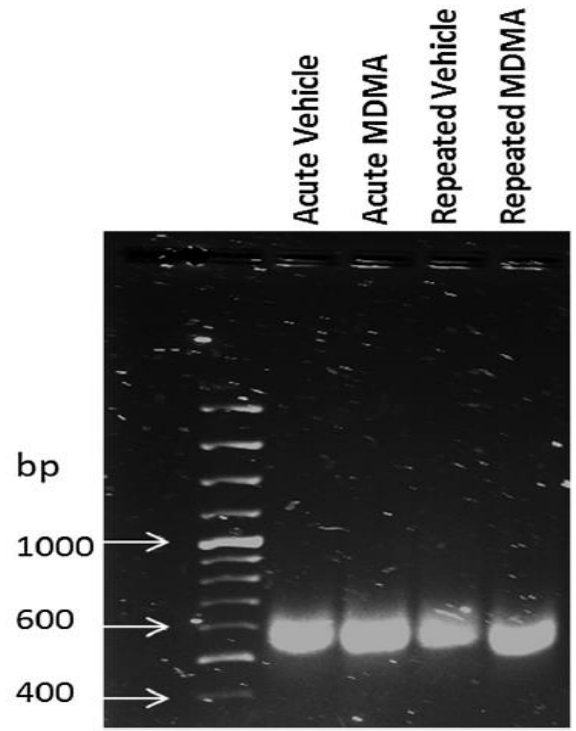
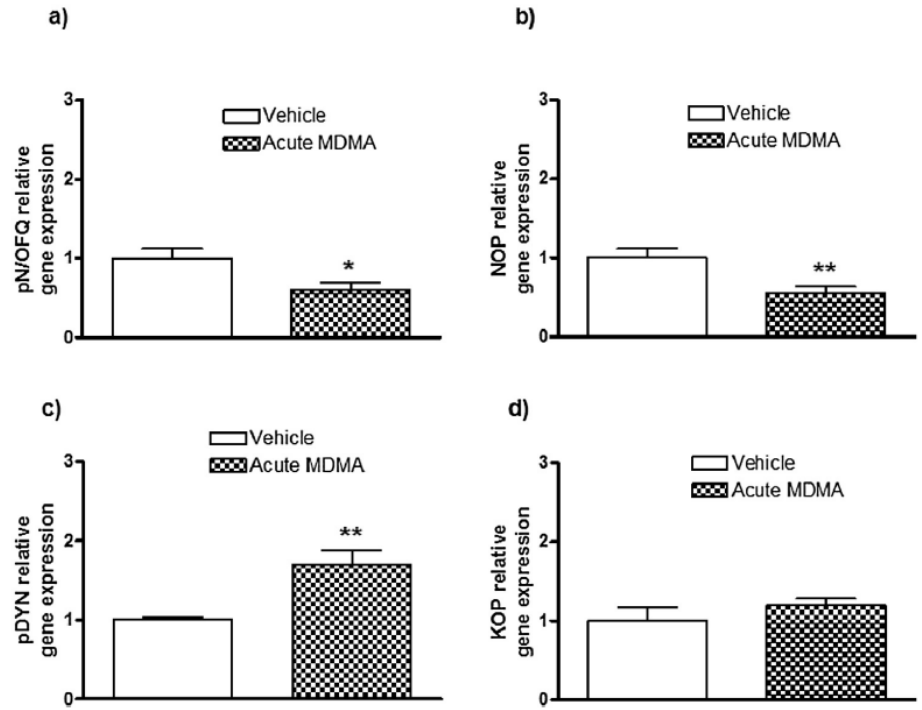


Fig. 1. Sheared chromatin fragments. DNA fragment size was determined by 1.5% agarose gel electrophoresis. Sonication produced fragments ranging from 400 to 600 bp.


a)
b)
c)
d)
Vehicle
Acute MDMA
pN/OFQ relative gene expression
NOP relative gene expression
pDYN relative gene expression
KOP relative gene expression
0
1
2
3
*
**

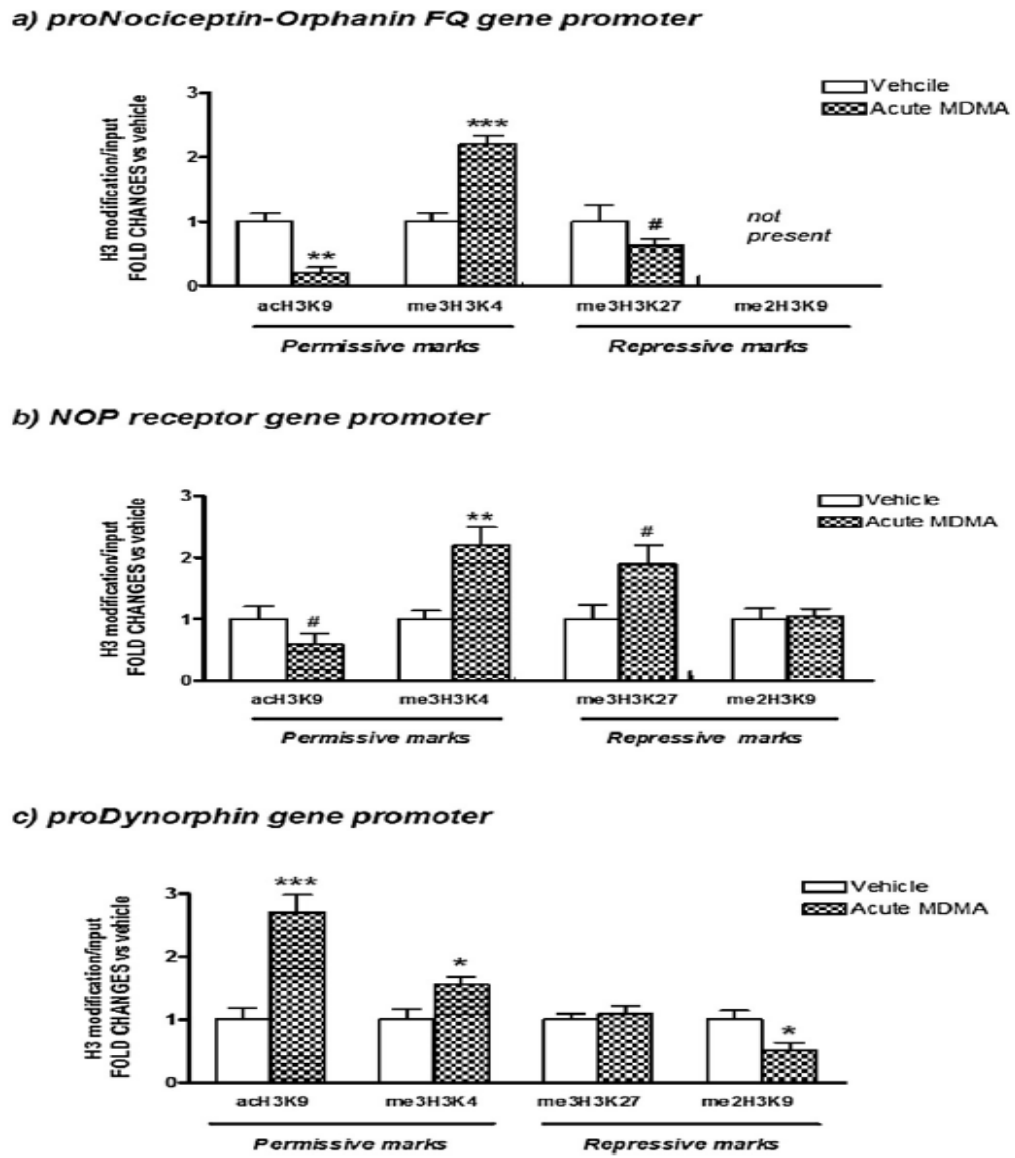
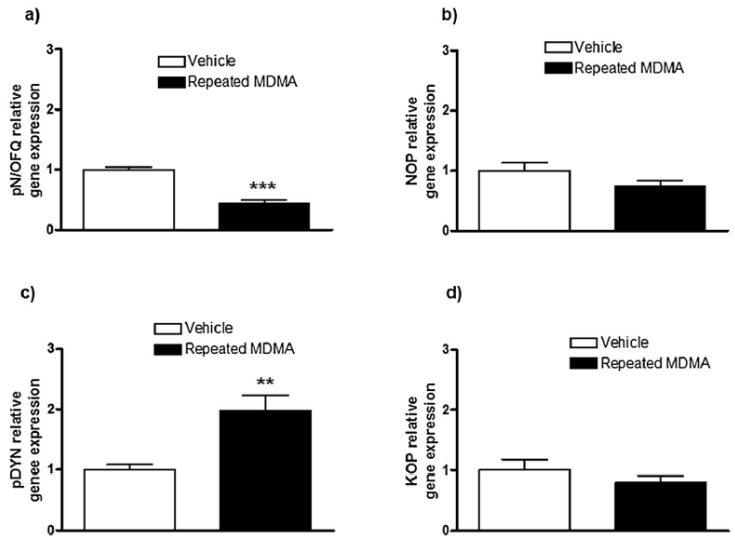
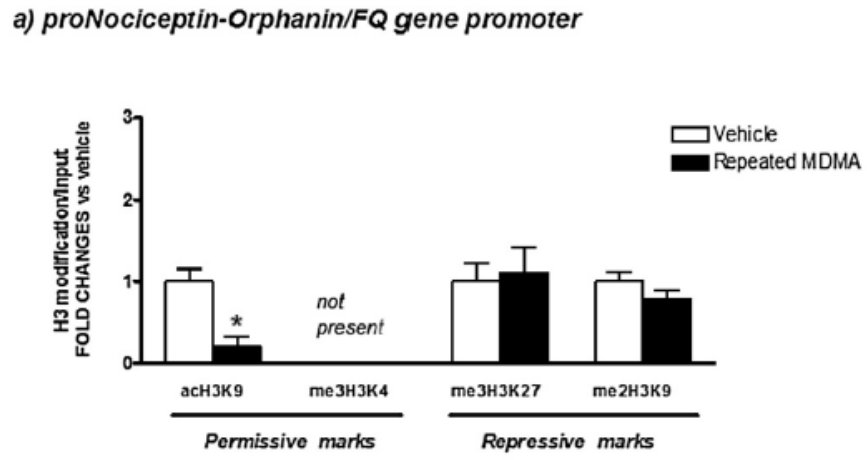


Fig. 5. Post-translational histone modifications after acute MDMA exposure. qPCR analyses of immunoprecipitated DNA fragments at pN/OFQ (Fig. 5a), NOP (Fig. 5b) and pDYN (Fig. 5c) promoters. The levels of permissive or repressive marks following a single 8 mg/kg MDMA injection were assessed by ChIP analysis. Data are expressed as means ± SEM of six rats/group (each sample was run in triplicate) (# $0.051 < p < 0.09$; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ in Student's *t*-tests).





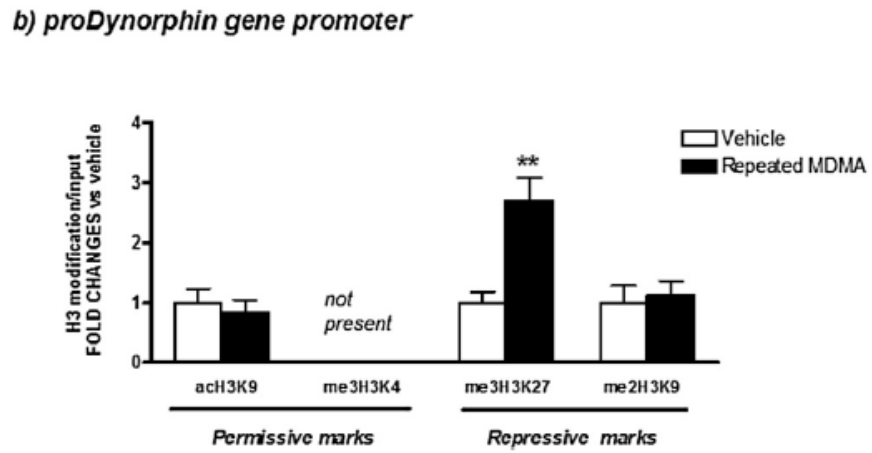


Fig. 7. Post-translational histone modifications after repeated MDMA exposure. qPCR analyses of immunoprecipitated DNA fragments at pN/OFQ (Fig. 7a) and pDYN (Fig. 7b) promoters. The levels of permissive or repressive marks following twice daily injections for seven days of 8 mg/kg MDMA were assessed by ChIP analysis. Data are expressed as means ± SEM of six rats/group (each sample was run in triplicate) (* $p < 0.05$; ** $p < 0.01$ in Student's *t*-tests).

Addiction Biology

PRECLINICAL STUDY

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Different alcohol exposures induce selective alterations on the expression of dynorphin and nociceptin systems related genes in rat brain

Claudio D'Addario[1,2], Francesca F. Caputi[1], Roberto Rimondini[1], Ottavio Gandolfi[1], Elia Del Borrello[3], Sanzio Candeletti[1] & Patrizia Romualdi[1]

Department of Pharmacology, University of Bologna, Bologna, Italy[1], Department of Biomedical Sciences, University of Teramo, Teramo, Italy[2] and Department of Medicine and Public Health, University of Bologna, Bologna, Italy[3]

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Ethanol Induces Epigenetic Modulation of Prodynorphin and Pronociceptin Gene Expression in the Rat Amygdala Complex

Claudio D'Addario · Francesca F. Caputi · Tomas J. Ekström · Manuela Di Benedetto · Mauro Maccarrone · Patrizia Romualdi · Sanzio Candeletti

Fig. 1 Sequences of *Rattus norvegicus* PDYN and PNOC promoter regions. The transcriptional start site (+1) is indicated. The TATA box on PDYN promoter and the CRE sequence on PNOC promoter are also indicated and highlighted. Primer sequences are *underlined* indicating also the starting positions

a) Rattus norvegicus prodynorphin gene promoter region (NM_019374.3)

-320

Actttctttctgcctttcgattggactctctacgtctctctatctctctct***ctgtctcctcccatctctgc***ctttctcctacttt

tgtctctgttttcctgtgagactctcctggaaatgctcaaagtggccgaatttgaagtgacaaacagcgctacaccgagaatcca

ccccggactgcgcgagagcatcctcctccacatcacatcacctggagcagctattgtgtttcactcaggcccagggagttgagt

Ggaaggacccgggggtctgagctctgactgccactcccccattggctcctggcagcctgtgctctgtgagcgagcgctgagcggg

TATA box +1

ctgagcgacagtgggggggagactctcttc**tataaa**aggggagggagtaaggctcacagaGAGTAAGGCTCACAGAACTGCCATAG

GGGGATTTGGTAGCCTTCAAGGCTTCCTCTGTGGCACTTCTCTGAGCTAGCGTCAGGGCTCCTTCTGAATCTTGGATCGGCCATC

CTATCACCTGATCAGCCAGAAGCCTGCCAGCGACAAAGCAGCACGCAG

b) Rattus norvegicus pronociceptin gene promoter region (NW_047454.2)

CRE -645

Aaacattcaaactatggcagcaactccattctgaca**tgactga*cagacagggaggacatggatgcttcctaggagggagagttga***

Gggacagcactcccagccaggccagagggacttgagaggctgagaggaagcactttagttgttcattcacctgtctctccctccc

Gagaaatgagatgagagatggataccaccactcggtgacttgtctgcactttgcagtccttacagctctcacttcagcttccaaa

tgaaagtacatggctacaAa.....c

aaactcttccttcccaggtgaacctcagacctgggaatcacctcagggaggaatggtgcttggcctttgtgttgtggtcctcac

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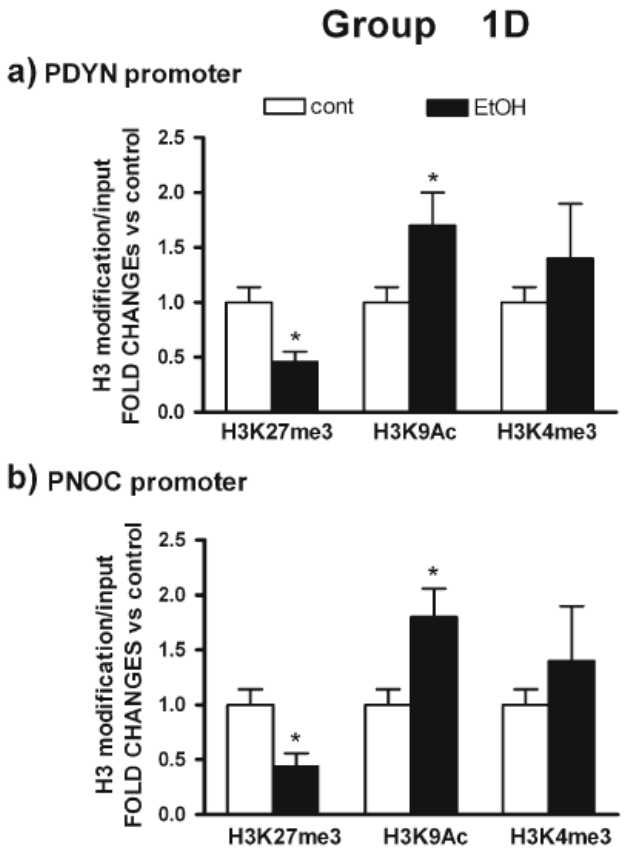

Fig. 2 RT-qPCR analysis of H3K27me3-, H3K9Ac-, and H3K4me3-immunoprecipitated DNA fragments at **a** PDYN and **b** PNOC promoters. Histogram shows specific histone modification levels, normalized to total input DNA, in rats treated with EtOH intragastrically (total daily dose, 4.5 gkg^{-1} day^{-1}; EtOH, n=7) or vehicle (cont, n=7) for 1 day (group 1D). Data are expressed as means±SE of triplicate independent samples. *P< 0.05 vs. control group

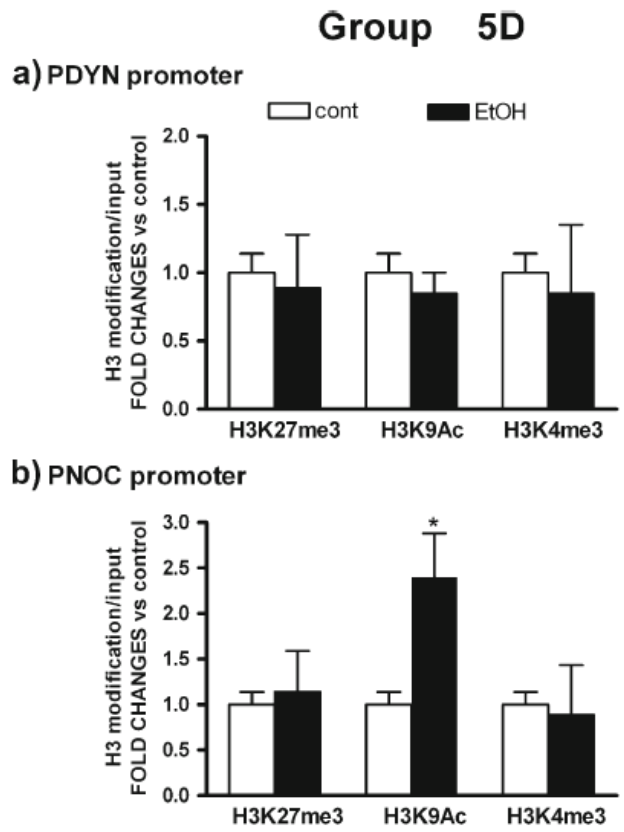

Fig. 3 RT-qPCR analysis of H3K27me3-, H3K9Ac-, and H3K4me3-immunoprecipitated DNA fragments at **a** PDYN and **b** PNOC promoters. Histogram shows specific histone modification levels, normalized to total input DNA, in rats treated with EtOH intragastrically (total daily dose, 4.5 gkg day; EtOH, n=7) or vehicle (cont, n=7) for 5 days (group 5D). Data are expressed as means±SE of triplicate independent samples. *P< 0.05 vs. control group

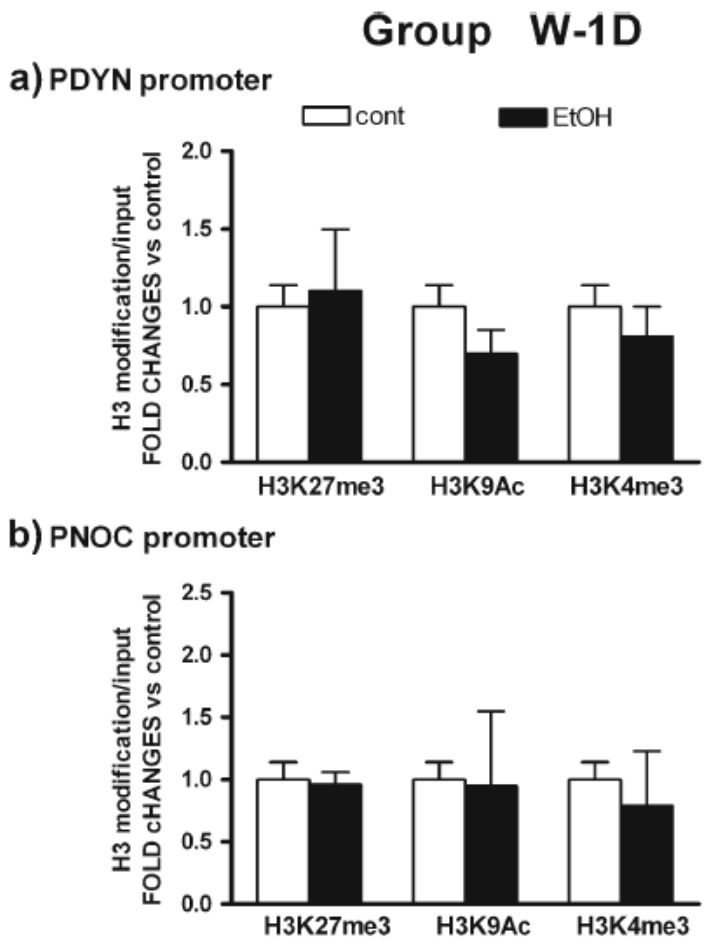

Fig. 4 RT-qPCR analysis of H3K27me3-, H3K9Ac-, and H3K4me3-immunoprecipitated DNA fragments at **a** PDYN and **b** PNOC promoters. Histogram shows the level of specific histone modifications, normalized to total input DNA, in rats treated with EtOH intragastrically (total daily dose, 4.5 gkg^{-1} day^{-1}; EtOH, $n=7$) or vehicle (cont, $n=7$) for 5 days, assessed after 1 day of withdrawal (group W-1D). Data are expressed as means±SE of triplicate independent samples

DNA methylation

Table 1 Percent of DNA methylation in promoter regions of PDYN and PNOC in rats treated with vehicle or EtOH intragastrically (total day dose, 4.5 gkg^{-1} day^{-1}) for 1 (1D group) or for 5 days (5D and W-1D groups)

	Control	EtOH
1D		
PDYN	46±4	44±5
PNOC	56±9	49±9
5D		
PDYN	41±12	48±18
PNOC	58±6	64±15
W-1D		
PDYN	39±12	30±14
PNOC	58±22	63±36