Contributions of Gene Variants, Endorphins, and Stress Responsivity to Specific Addictions and Treatment

Mary Jeanne Kreek, M.D.
Patrick E. and Beatrice M. Haggerty Professor
Head of Laboratory
The Laboratory of the Biology of Addictive Diseases
The Rockefeller University
Senior Physician
The Rockefeller University Hospital

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Development of Methadone Maintenance Treatment – 1964 Onward

HYPOTHESIS: Heroin (opiate) addiction is a disease – a "metabolic disease" – of the brain with resultant behaviors of "drug hunger" and drug self-administration, despite negative consequences to self and others. Heroin addiction is not simply a criminal behavior or due alone to antisocial personality or some other personality disorder.

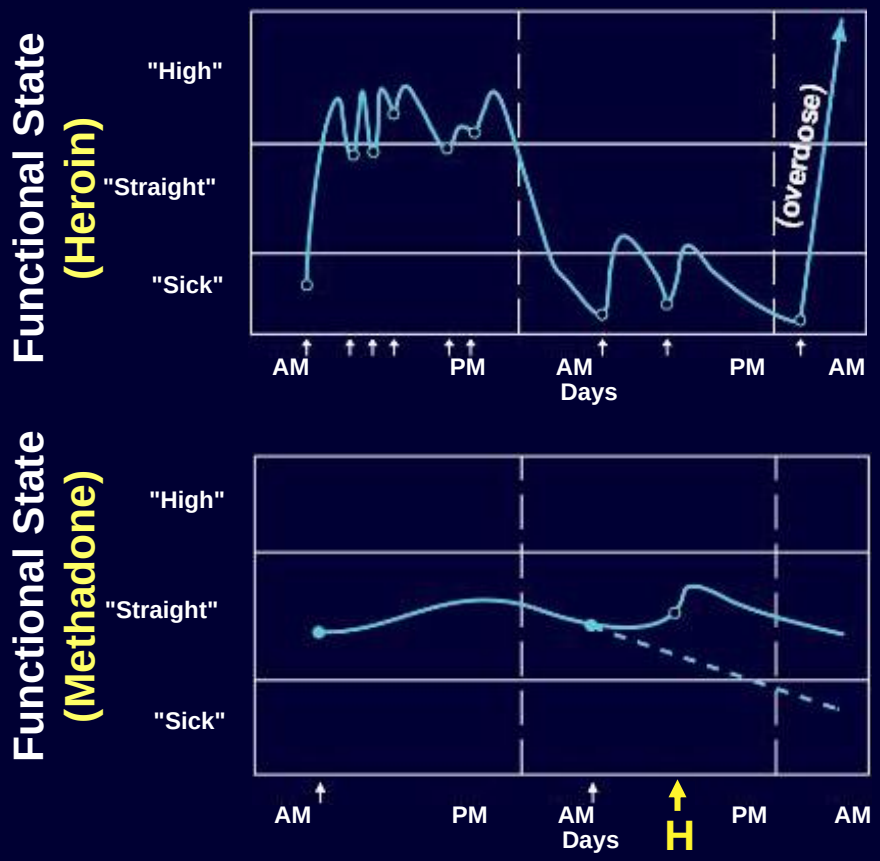
Vincent P. Dole, Jr., MD; Marie Nyswander, MD; and Mary Jeanne Kreek, MD

First publications describing methadone maintenance treatment research

1) **1964: Initial clinical research on development of treatment using methadone maintenance pharmacotherapy and on elucidating mechanisms of efficacy performed at The Rockefeller Hospital of The Rockefeller Institute for Medical Research:**
   Dole, V.P., Nyswander, M.E. and Kreek, M.J.: Narcotic blockade. Arch. Intern. Med., 118:304-309, 1966.
   (also recorded in the Association of American Physicians meeting transcription of discussion)
2) **1965: Translational applied clinical research performed at Manhattan General Hospital:**
   Dole, V.P. and Nyswander, M.E.: A medical treatment for diacetylmorphine (heroin) addiction. JAMA, 193:646-650, 1965.

Dole, Nyswander and Kreek, 1966, 2006

Impact of Short-Acting Heroin versus Long-Acting Methadone Administered on a Chronic Basis in Humans: "On-Off" *versus* "Steady-State" – Relationship Between Blood/Brain Levels of Drugs of Abuse and Addictions



Systemic Bioavailability After Oral Administration	Apparent Plasma Terminal Half-life ($t_{1/2}$ Beta)	Major Route of Biotrans-formation
Heroin		
Limited (<30%)	3min (30min for active 6-acetyl-morphine metabolite; 4-6h for morphine and active morphine-6-glucuronide metabolite)	Successive deacetylation and morphine glucuronidation
Methadone		
Essentially Complete (>70%)	24h (48h for active [R](l)-enantiomer)	N-demethylation

Rate of rise of blood (and presumable brain) levels of drugs of abuse are related positively to their reinforcing effects and rate of fall related to withdrawal and craving.

Dole, Nyswander and Kreek, 1966; Kreek et al., 1973; 1976; 1977; 1979; 1982; Inturrisi et al, 1973; 1984

Methadone Maintenance Treatment for Opiate (Heroin) Addiction – 2010

Number of patients currently in treatment: **~ 1 million worldwide**

- **USA: ~ 260,000**
- **Europe: ~ 500,000**
- **Rest of world: ~250,000**

Efficacy in "good" methadone treatment programs using adequate doses (80 to 150mg/d):

Voluntary retention in treatment (1 year or more)	50 – 80%
Continuing use of illicit heroin	5 – 20%

Actions of methadone treatment:

- **Prevents withdrawal symptoms and "drug hunger"**
- **Blocks euphoric effects of short-acting narcotics**
- **Allows normalization of disrupted physiology**

Mechanism of action: **Long-acting narcotic provides steady levels of opioid at specific receptor sites.**

- **methadone found to be a full mu opioid receptor agonist which internalizes like endorphins (beta-endorphin and enkephalins)**
- **methadone also has modest NMDA receptor complex antagonism**

Kreek, 1972; 1973; 2011

Few Targeted Pharmacotherapies Available for Specific Addictive Diseases

I. Opiate Addiction (Heroin and Illicit Use of Opiate Medications)
   a. METHADONE (80 to 150 mg/d; 50-80%)**
   b. BUPRENORPHINE (+ NALOXONE) (40-50%)* (***)
   [c. NALTREXONE (<15%)**]
   [d. SUSTAINED RELEASE NALTREXONE (<15%)**]

II. Alcoholism
   a. NALTREXONE (30-40%)*
   b. ACAMPROSATE (low in USA)

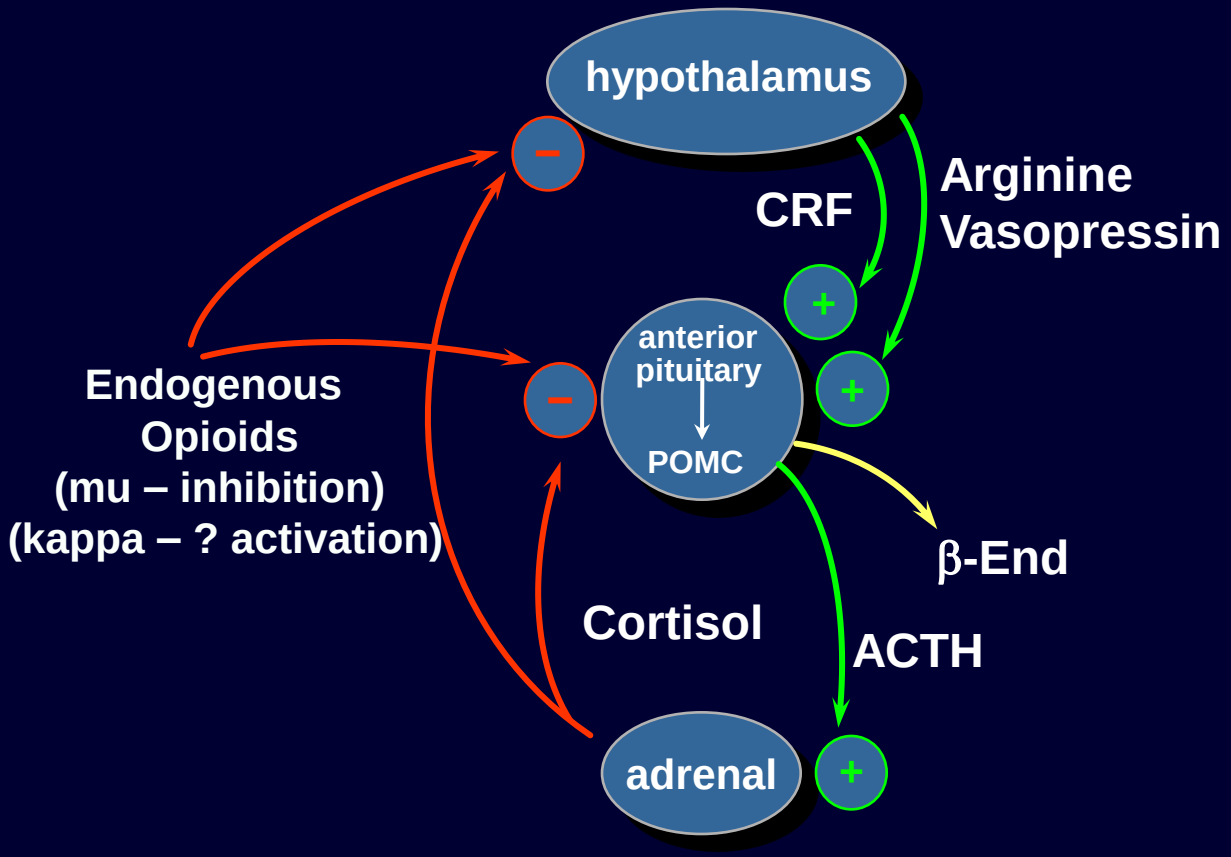
III. Cocaine, Amphetamines and Other Stimulants
   NONE

(%) is % of unselected persons with specific addictions who can be retained voluntarily in treatment for 3 months (*) or 12 months (**), with moderate to high success in eliminating specific drug use.

*** *Maximum effective dose, 24 or 32 sl, equivalent to 60 to 80 mg of methadone.*

Kreek, 2011

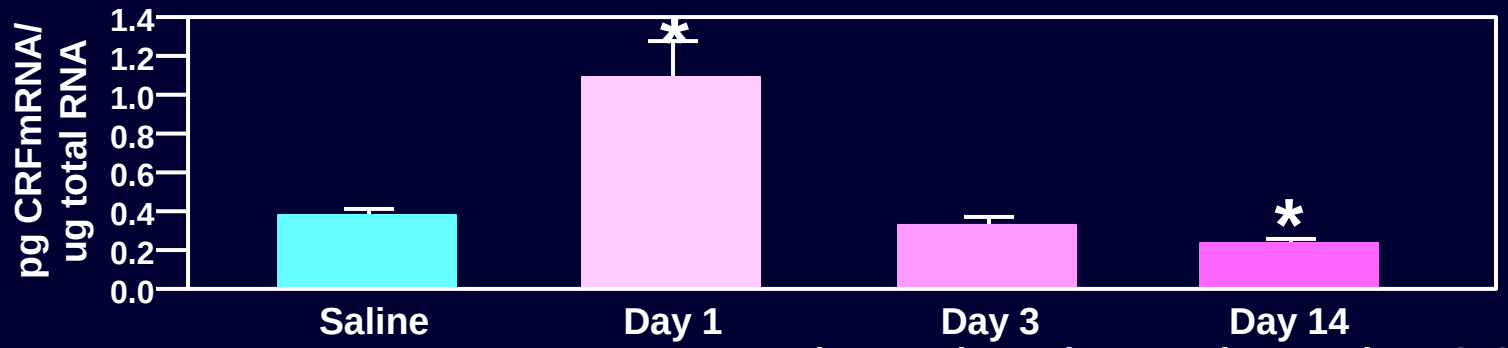
STRESS RESPONSIVITY:
Hypothesis – Atypical Responsivity to Stressors, A Possible Etiology of Addictions – HPA Axis



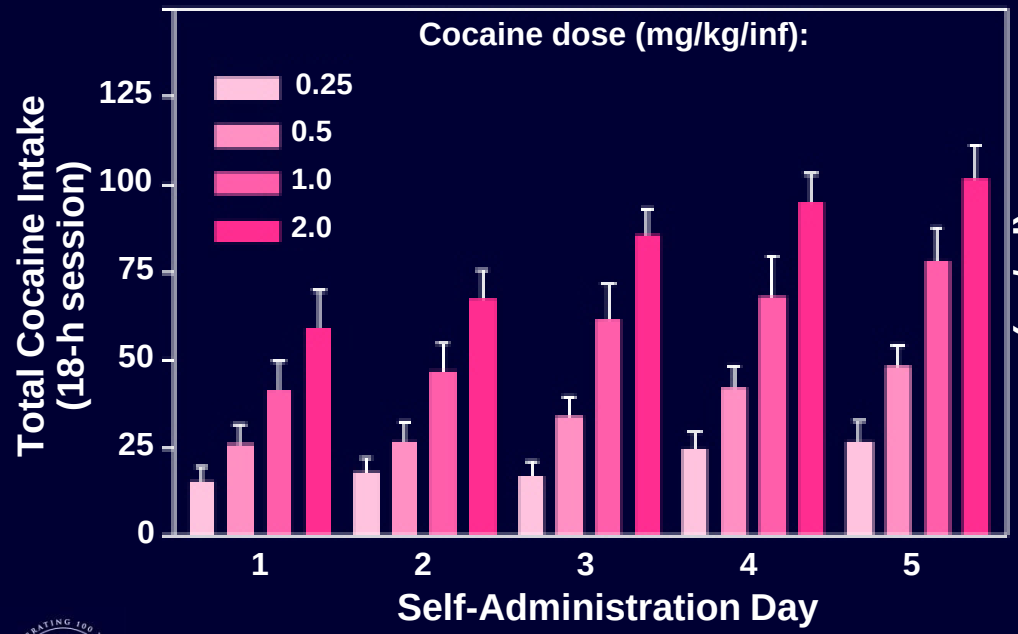
Atypical responsivity to stress and stressors may, in part, contribute to the persistence of, and relapse to self-administration of drugs of abuse and addictions. Such atypical stress responsivity in some individuals may exist prior to use of addictive drugs on a genetic or acquired basis, and lead to the acquisition of drug addiction.

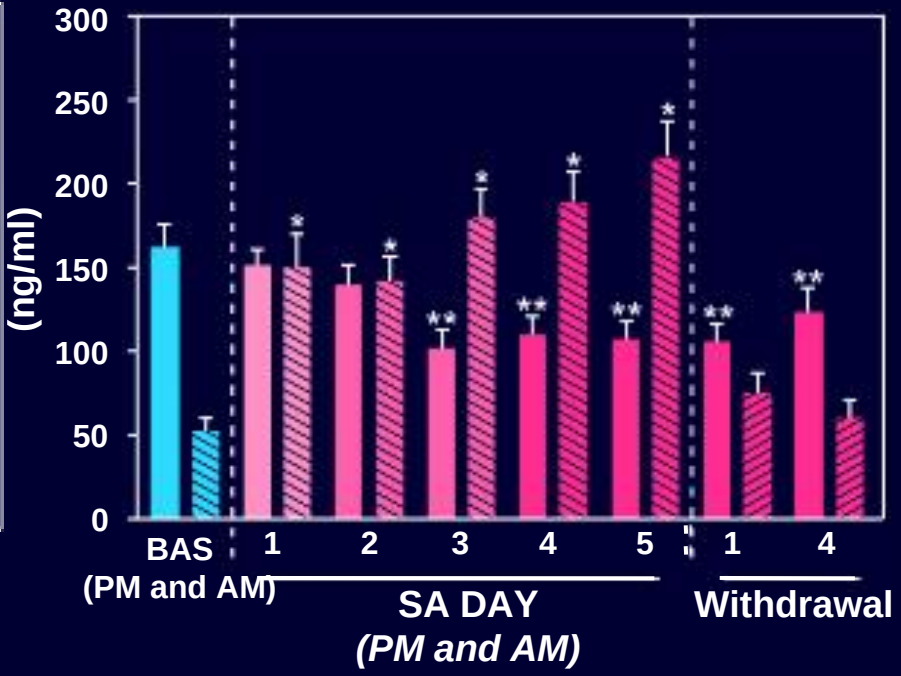
Kreek, 1972; 1981; 1982; 1984 … 2011

TOLERANCE/ADAPTATION OF STRESS RESPONSIVITY EFFECTS OF COCAINE– Cocaine Self-Administration by Rats Under Extended Access Conditions (18h): Effects on Plasma Corticosterone Levels



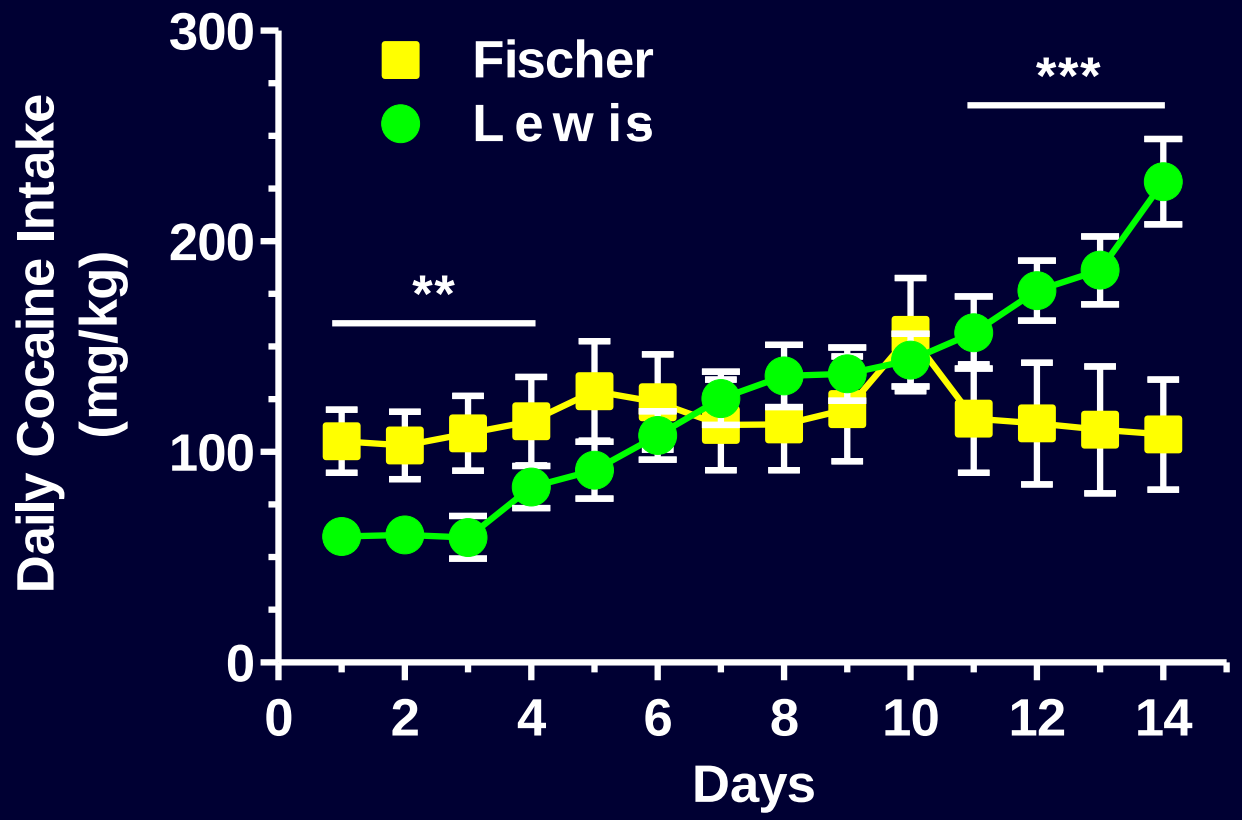
Zhou et al., J. Pharmacol. Exp. Ther., 279:351, 1996





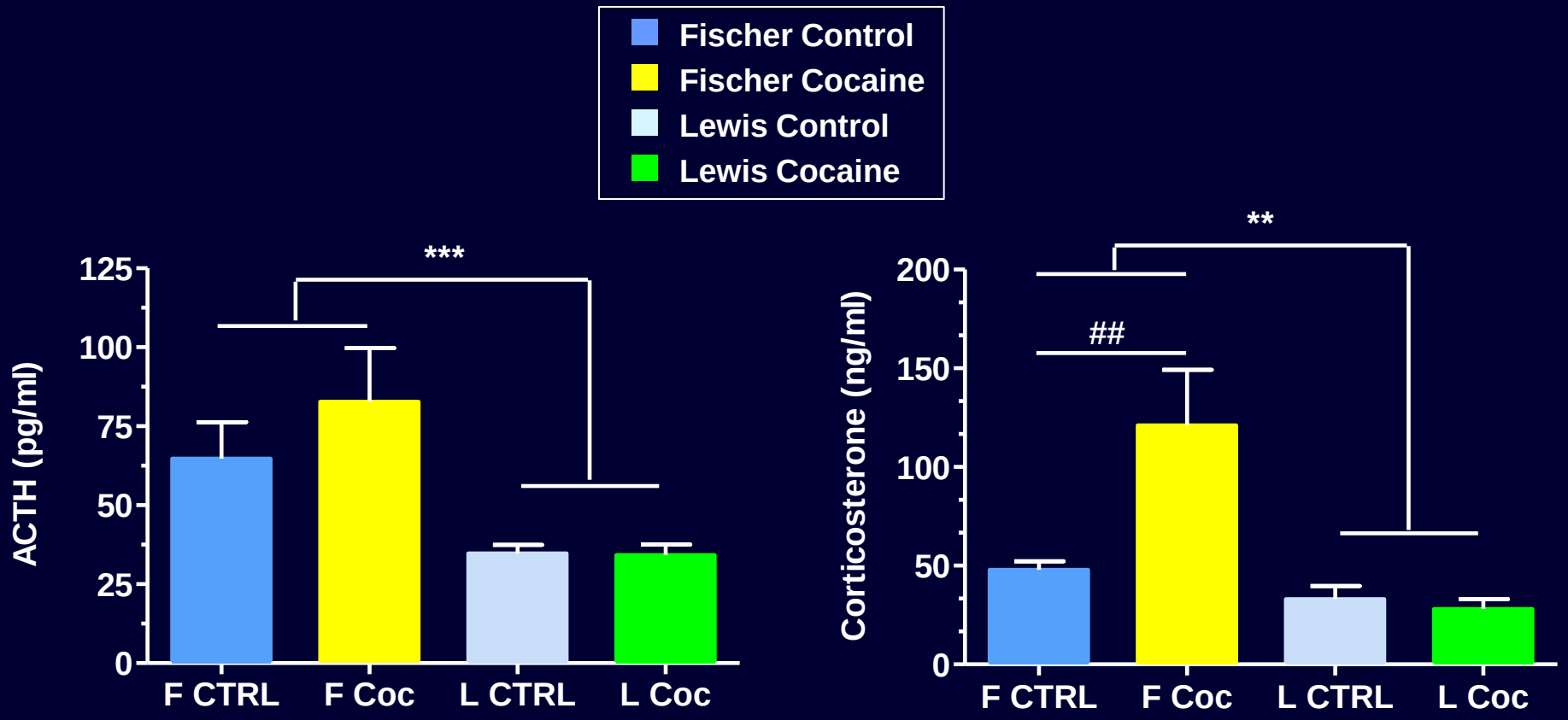
Mantsch et al., JPET, 294:239, 2000

Daily Intake of Cocaine During Extended Sessions



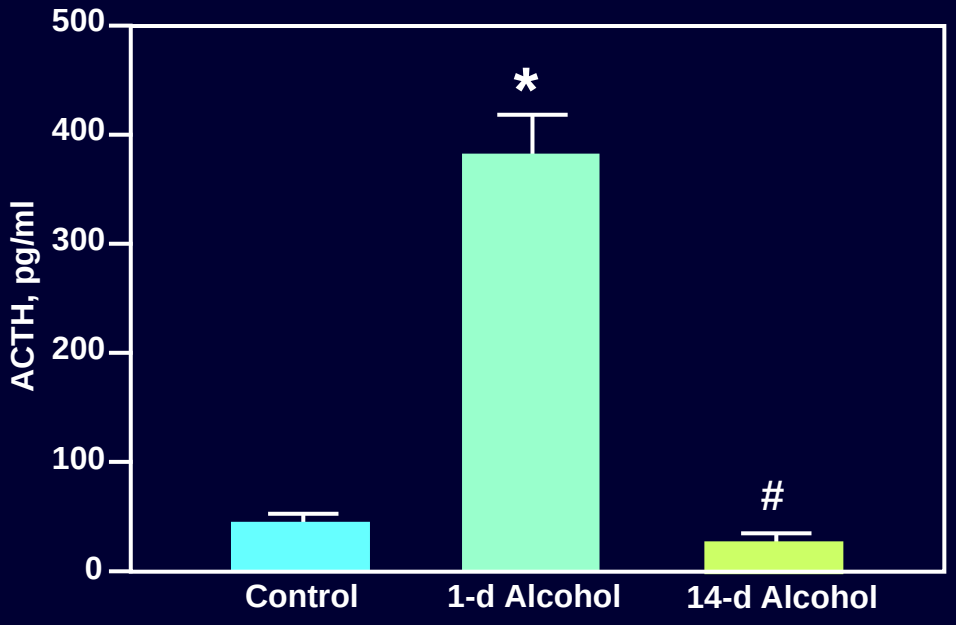
Picetti et al., Psychopharmacology, 211:313, 2010

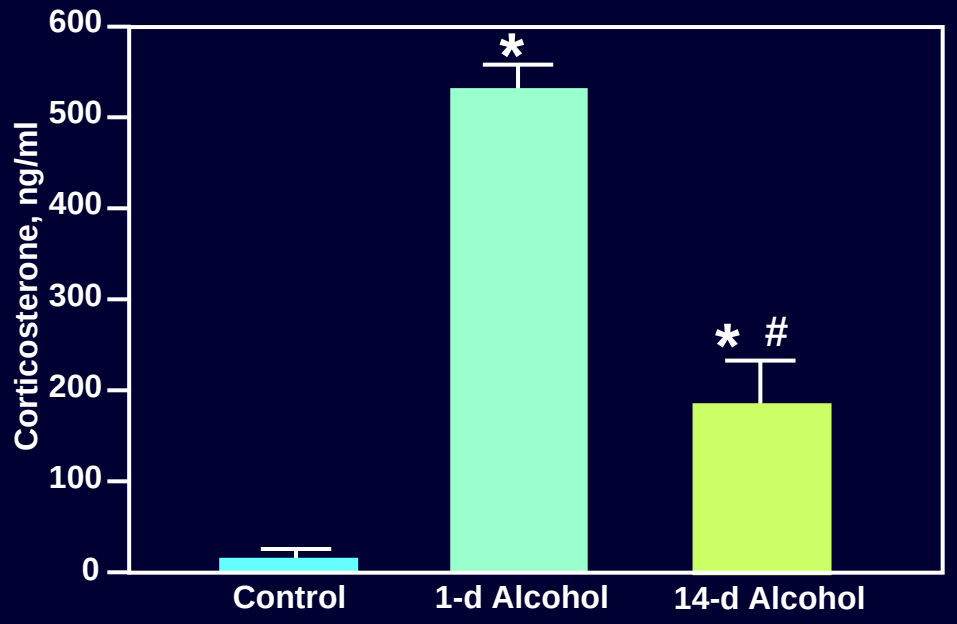
ACTH and CORT Levels 24 h After the Last Extended Self-Administration Session



Picetti et al., Psychopharmacology, 211:313, *2010*

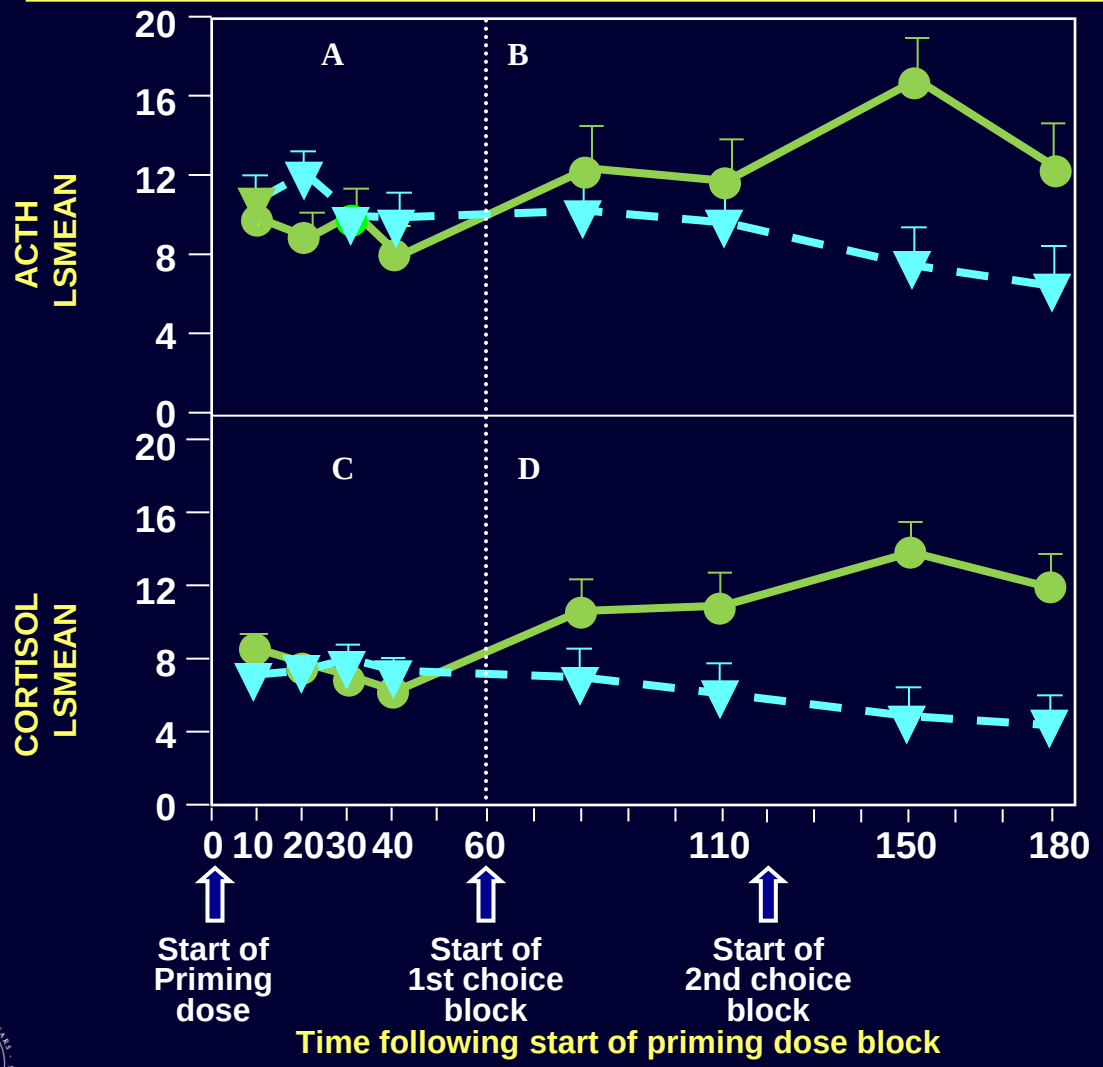
TOLERANCE/ADAPTATION OF STRESS RESPONSIVITY EFFECTS OF ALCOHOL – Plasma ACTH and Corticosterone Levels After Binge Pattern Alcohol Administration (po, 1.5g/kg/h x 3)





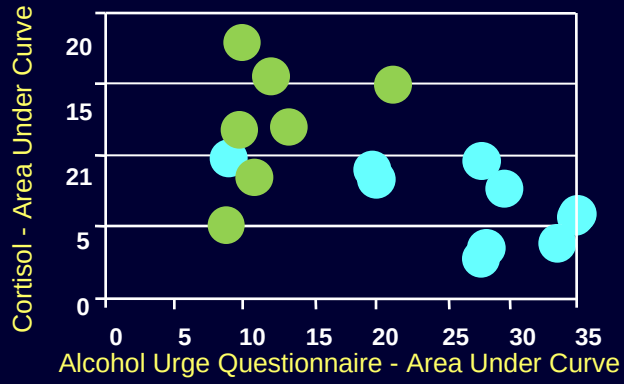
Zhou, Franck et al, Alcohol. Clin. Exp. Res. 24:1575, 2000

TOLERANCE/ADAPTATION OF STRESS RESPONSIVITY EFFECTS OF ALCOHOL – Effects of Naltrexone vs. Placebo in Alcoholics: Greater Alcohol-Induced HPA Activation Following Naltrexone Disinhibition of Mu Opioid Receptor Inhibition



Naltrexone, n = 7 (1.9 +/- 0.72 drinks/ 2h choice)

Placebo, n = 9 (4.6 +/- 0.85 drinks/ 2h choice)



O'Malley… Sinha, and Kreek, Psychopharmacology 160:19, 2002

STRESS RESPONSIVITY – Heroin, Cocaine, and Alcohol Profoundly Alter Stress Responsive Hypothalamic-Pituitary-Adrenal (HPA) Axis: Normalization During Methadone Treatment

- Acute effects of opiates
- Chronic effects of short-acting opiates (e.g., heroin addiction)

} Suppression of HPA Axis (decrease levels of HPA hormones)

- Opiate withdrawal effects *
- Opioid antagonist effects
- Cocaine effects *
- Alcohol effects

} Activation of HPA Axis (increase levels of HPA Hormones)

- Chronic effects of long-acting opiate (e.g. methadone in maintenance treatment)

Normalization of HPA Axis

* Our challenge studies have shown that a relative and functional "endorphin deficiency" develops.

Kreek, 1972; 1973; 1987; 1992 … 2010

Role of Mu Opioid Receptor and Related Endorphin Systems in Normal Physiological Functions*

- Endogenous Response to Pain
- Neuroendocrine Functions
  - Stress responsive systems including hypothalamic-pituitary-adrenal axis
  - Reproductive function including hypothalamiac-pituitary-gonadal axis
- Immunological Function
- Gastrointestinal Function
- Cardiovascular Function
- Pulmonary Function
- ? Mood, Affect; Cognition

* *All disrupted by chronic abuse of the short acting opiate, heroin*

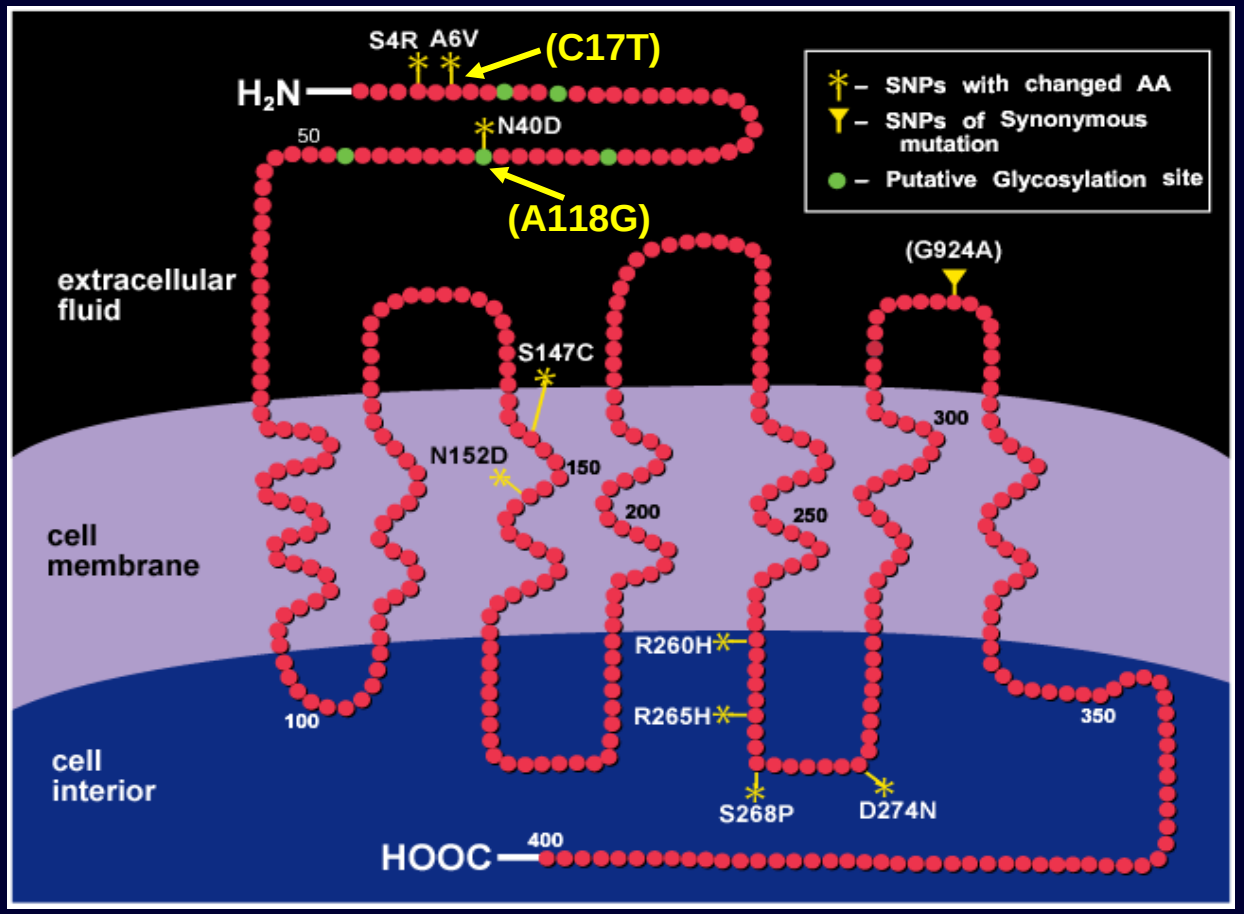
Kreek, 1978; 2010

Genetic Variants of the Human Mu Opioid Receptor: Single Nucleotide Polymorphisms in the Coding Region Including the Functional A118G (N40D) Variant

HYPOTHESIS

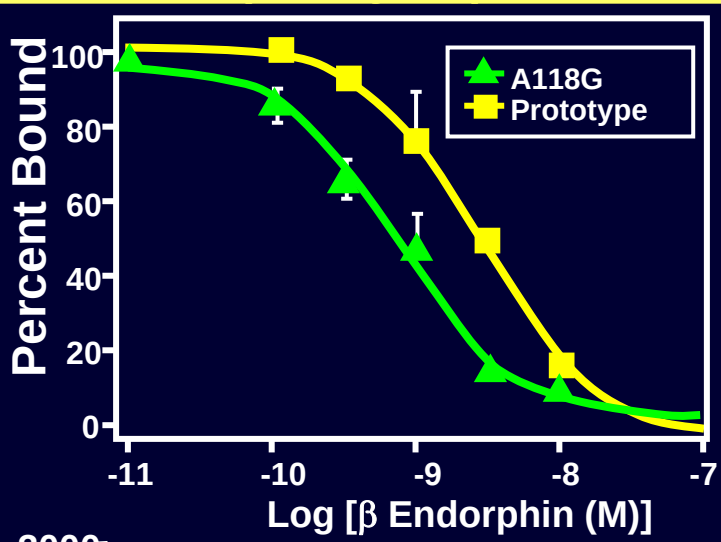
Gene variants:

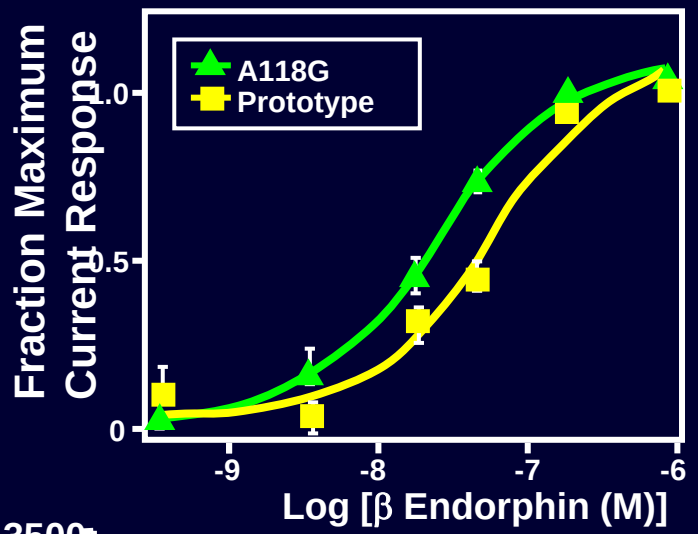
- **Alter physiology "PHYSIOGENETICS"**
- **Alter response to medications "PHARMACOGENETICS"**
- **Are associated with specific addictions**

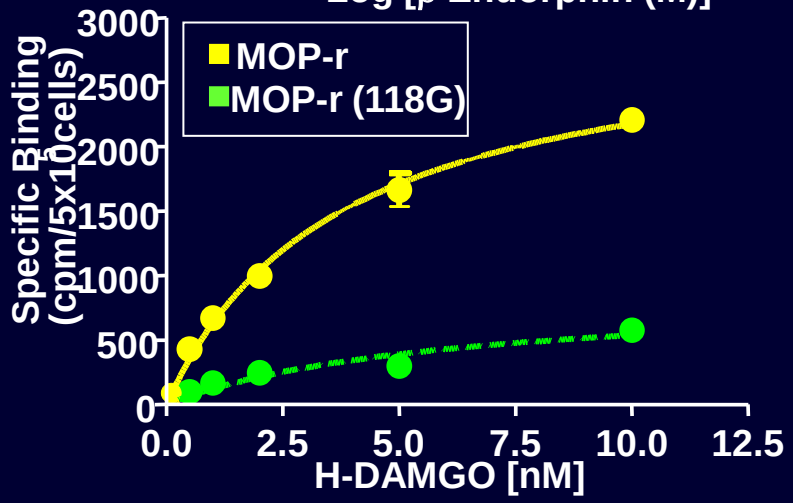


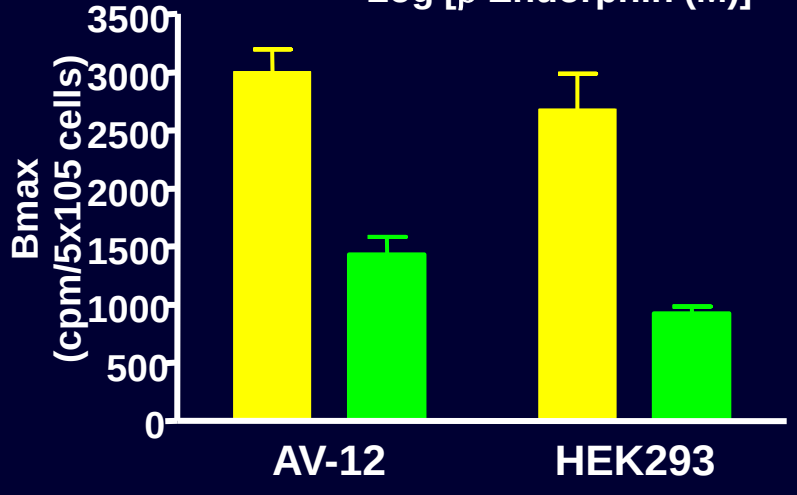
Bond, LaForge… Kreek, Yu, PNAS, 95:9608, 1998

FUNCTIONAL MOP-r (A118G) VARIANT – Increased Binding and Coupling to G Protein-Activated, Inwardly Rectifying K^+(GIRK) Channels by Beta-Endorphin at the Prototype A118A and A118G Variant of the Mu Opioid Receptor, but Lower Cell-Surface Receptor Binding and Bmax Levels and Lower Forskolin-Stimulated cAMP Accumulation than MOP-r Prototype (Stably Expressed in AV-12 or HEK293 Cells)



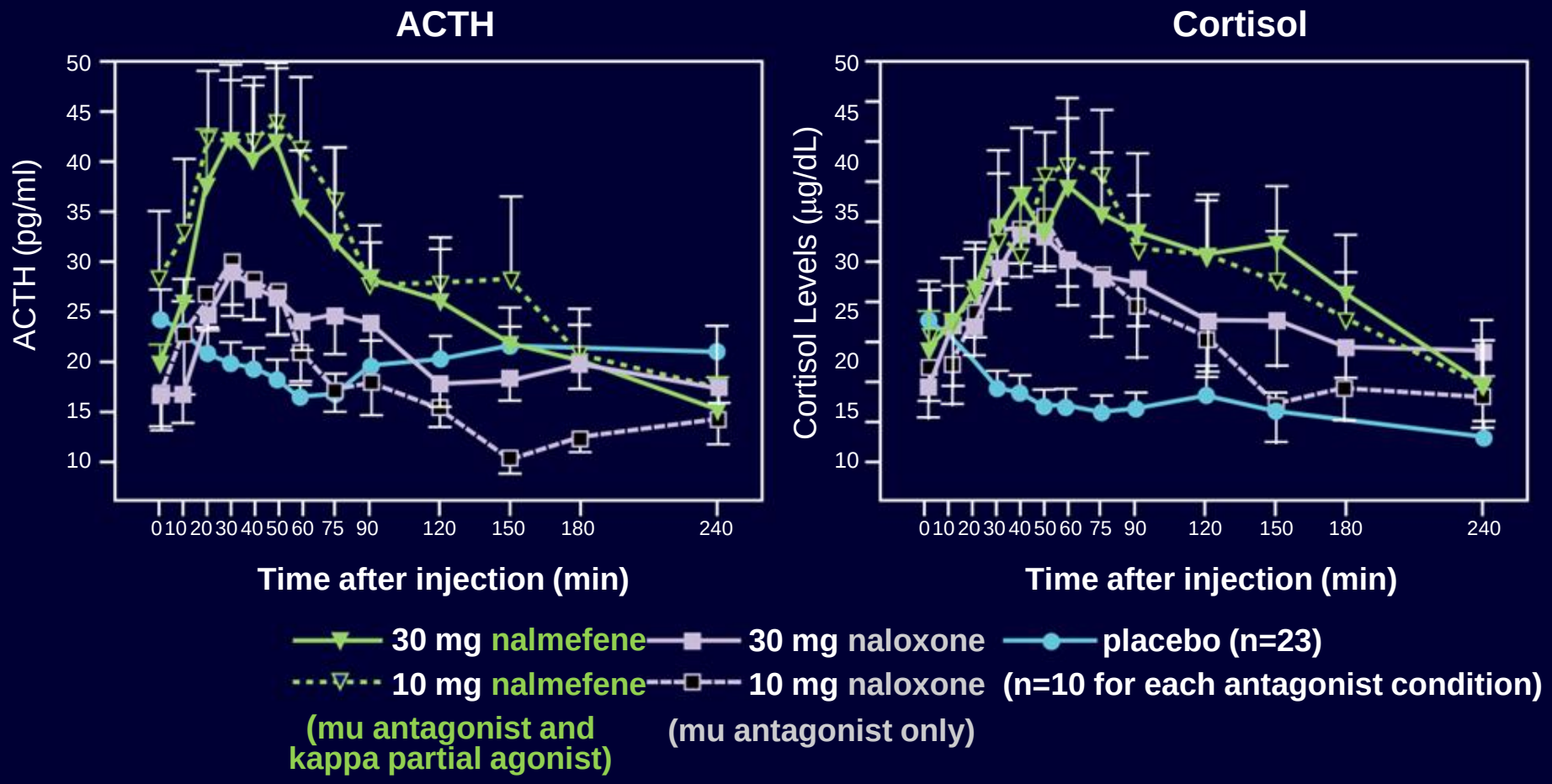






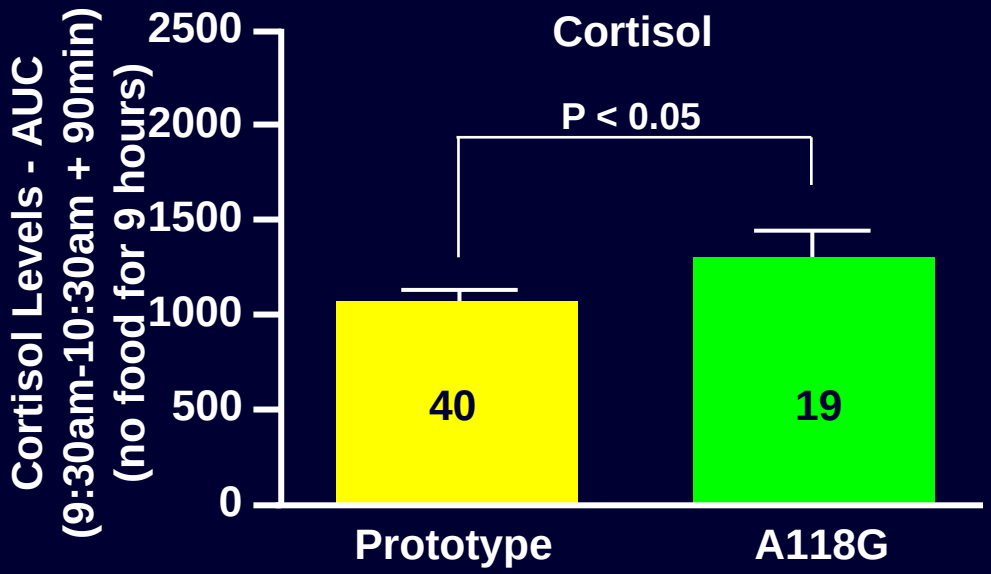
Bond, LaForge… Kreek, Yu, PNAS, 95:9608, *1998;*
Kreek, Yuferov and LaForge, Eur. J. Pharmacol. 410 *2000; Kroslak et al.,* J. Neurochem. 103:77, *2007*

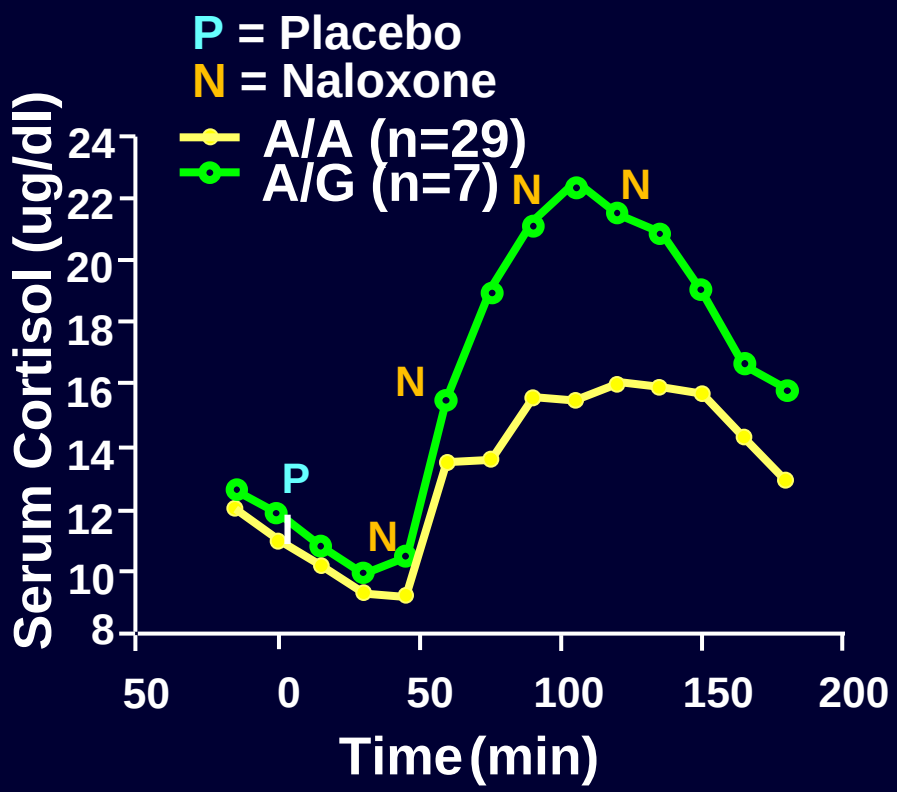
STRESS RESPONSIVITY – High Dose Opiate Antagonist Studies: Nalmefene (mu/kappa Directed) Causes Greater HPA Axis Activation Than Naloxone (mu Directed) in Normal Human Volunteers



Schluger, Ho, Borg, Porter, Maniar, Gunduz, Perret, King, and Kreek, Alcohol. Clin. Exp. Res., 22,1430, 1998

FUNCTIONAL MOP-r (A118G) VARIANT – "Physiogenetics" Related to A118G Variant of Human Mu Opioid Receptor Gene – Altered Stress Responsivity in Healthy Control Volunteers

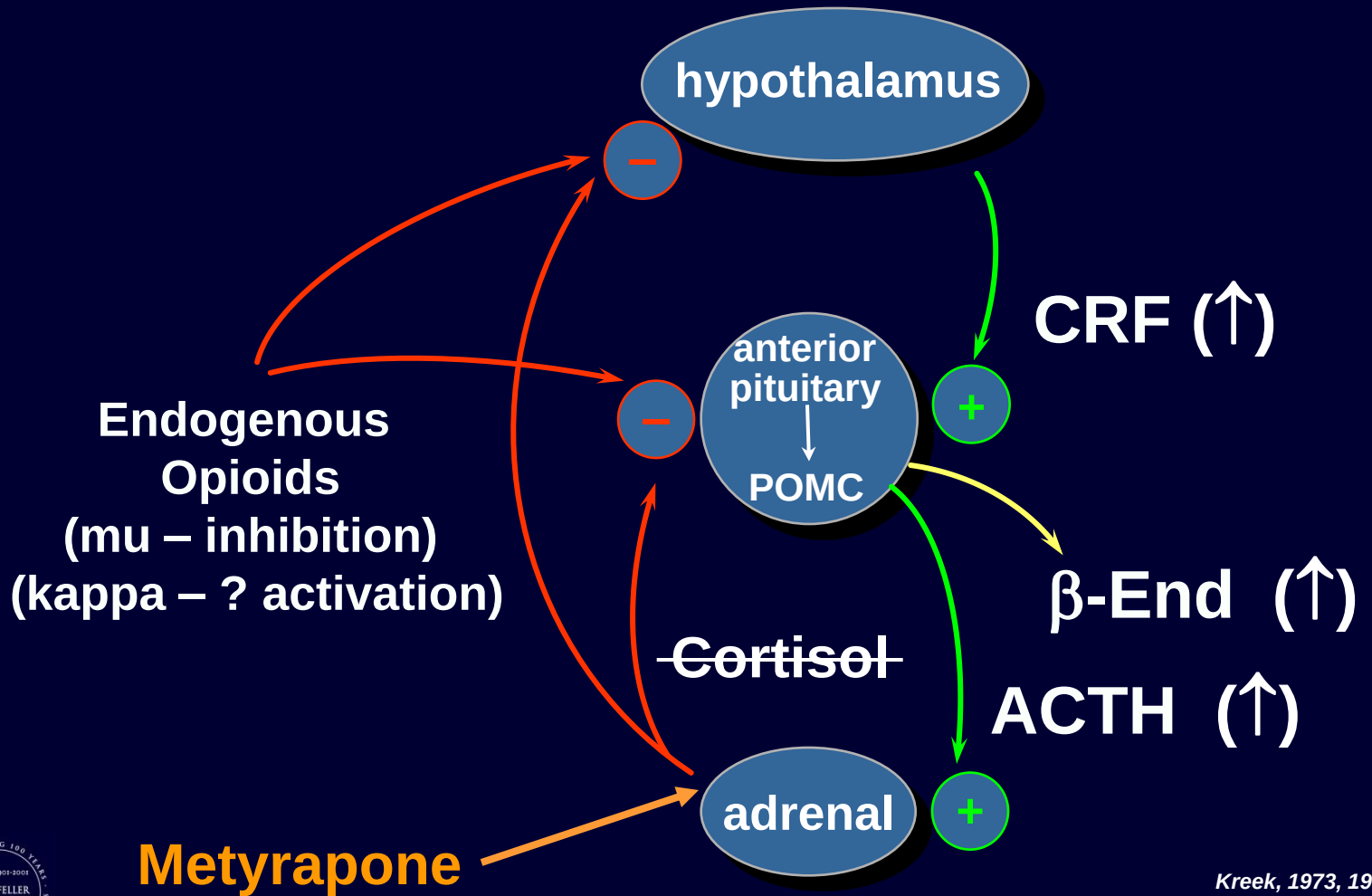




Bart et al. Neuropsychopharmacology, 31:2313-2317, 2006

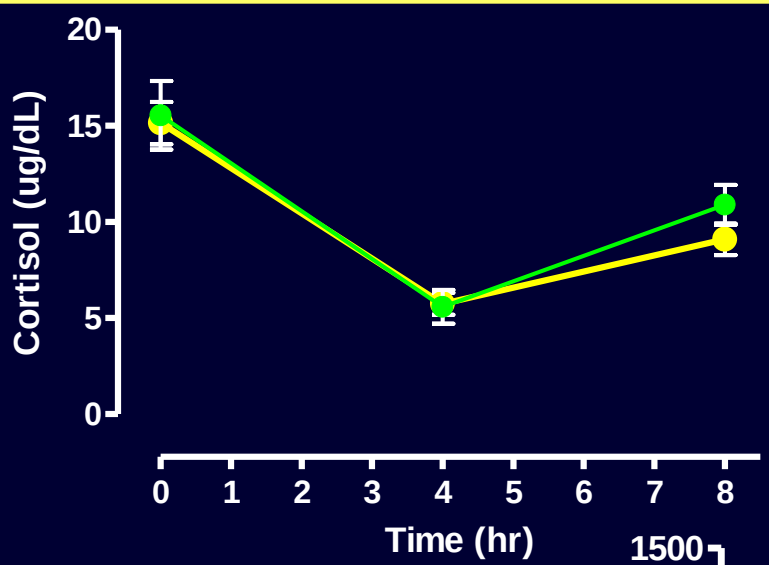
Wand et al., Neuropsychopharmacol, 26:106, 2002
Chong…Wand, Neuropsychopharmacology, 31:204, 2006

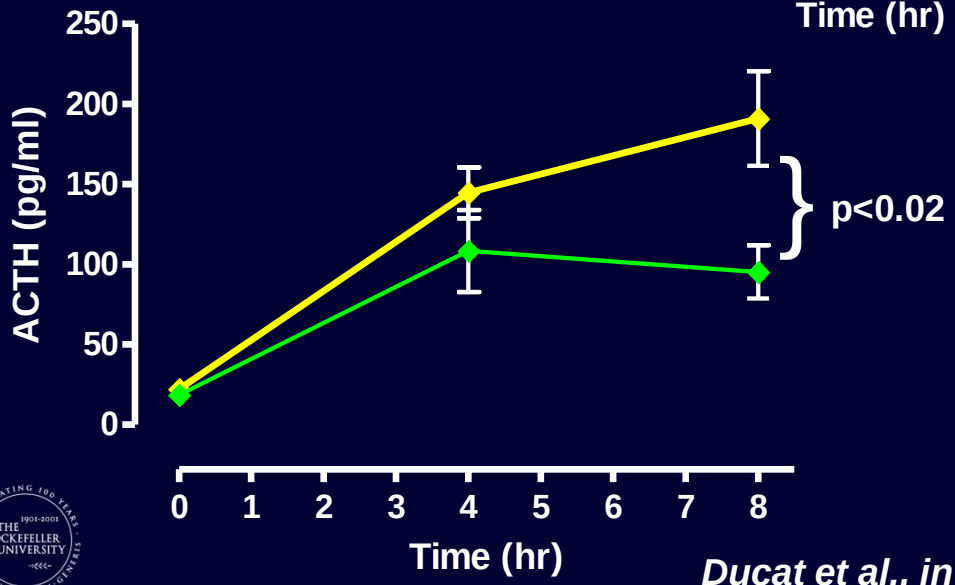
Dissecting the Hypothalamic-Pituitary-Adrenal Axis in Humans: Single-Dose (2.25g) Metyrapone Effects

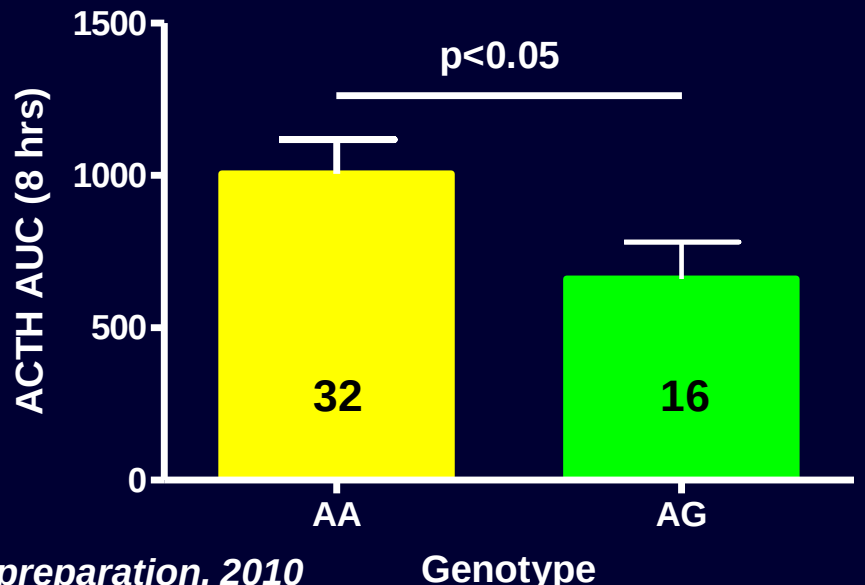


Kreek, 1973, 1978, 2006; Kreek et al.1984; Schluger et al, Neuropsychopharmacology, 24:568, 2001; 2006

FUNCTIONAL MOP-r (A118G) VARIANT– Metyrapone Testing in Normal Volunteers: Plasma cortisol levels and resultant plasma ACTH levels and AUC at 9 a.m. (prior to metyrapone) and after 4 and 8 hours







Ducat et al., in preparation, 2010

Association Between a Functional (A118G) Polymorphism in the mu Opioid Receptor Gene and Opiate Addiction in Central Sweden

Genotype	All Subjects		Swedish with Both Parents Swedish	
	Controls (n=170)	Opiate Dependent (n=139)	Controls (n=120)	Opiate Dependent (n=67)
A/A	147	98	104	46
A/G	21	39	15	19
G/G	2	2	1	2

RR = 2.86 $\chi^2_{(1)}$= 13.403 P = 0.00025*

RR = 2.97 $\chi^2_{(1)}$= 8.740 P = 0.0031*

	Opiate Dependent (n=139)	Control (n=170)
G/G; A/G	41	23
A/A	98	147
118G Allele Frequency	0.155	0.074

Thus, in the entire study group in this central Swedish population,

Attributable Risk due to genotypes with a G allele in this population: 18%

Attributable Risk due to genotypes with a G allele in Swedes w/ Swedish parents: 21%

(with confidence interval ranges from 8.0 to 28.0%)

Bart G , Heilig M, LaForge KS… Ott J, Kreek MJ, et al., Molecular Psychiatry, 9:547, 2004

Association Between a Functional (A118G) Polymorphism in the mu Opioid Receptor Gene and Alcoholism in Central Sweden

	Swedish with two Swedish parents		Non-Swedish without Swedish Parents	
	Alcohol Dependent (n=193)	Control (n=120)	Alcohol Dependent (n=196)	Control (n=50)
A118	158	104	141	43
A118G, G118G	35	16	55	7

OR=1.92 $\chi^2_{(1)} = 7.18$, p = 0.0074

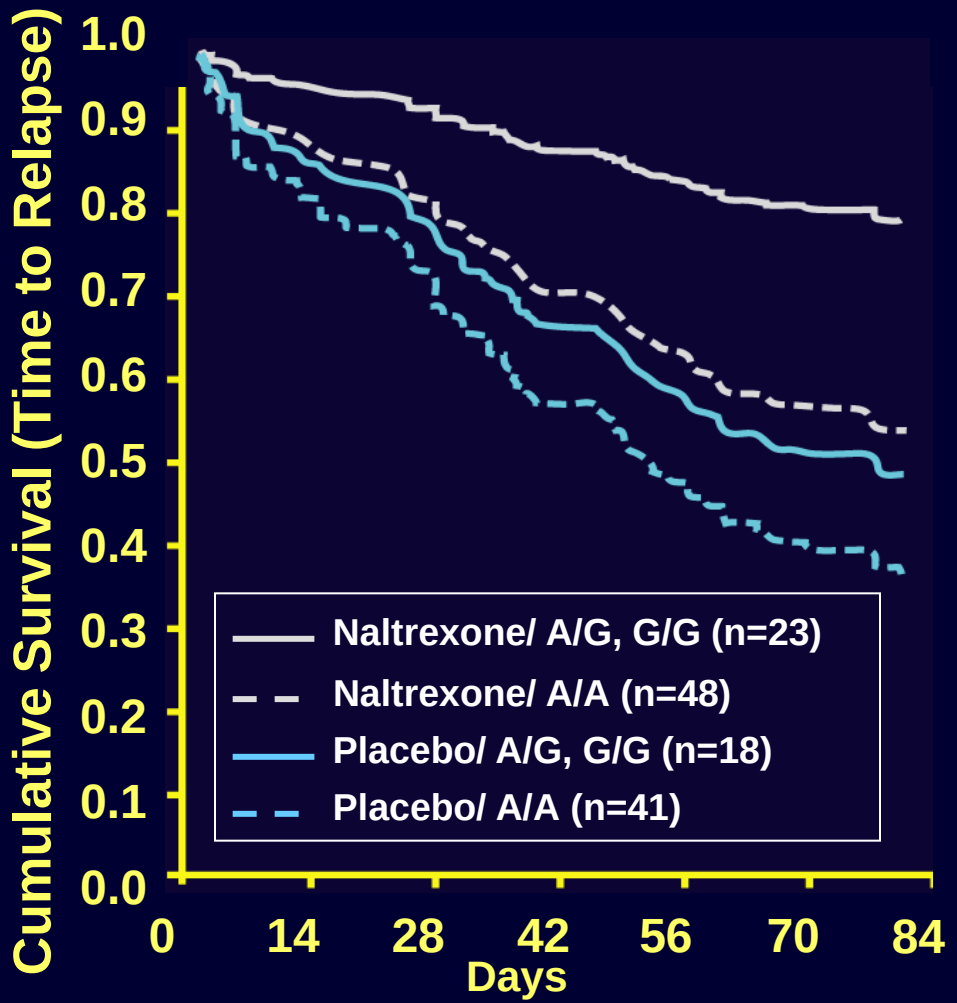
	Alcohol Dependent (n=389)	Control (n=170)
G/G; A/G	90	23
A/A	299	147
118G Allele Frequency *	0.125	0.074

** Overall 118G Allele Frequency = 0.109*

Thus, in the entire study group in this central Swedish population:
Attributable Risk due to genotypes with a G allele: 11.1%
(with confidence interval ranges from 3.6 to 18.0%)

Bart G , Kreek MJ, LaForge KS… Ott J, Heilig M, Neuropsychopharmacology, 30:417, 2005

FUNCTIONAL MOP-r (A118G) VARIANT – "Pharmacogenetics" Related to A118G Variant of Human Mu Opioid Receptor Gene – Altered Stress Responsivity: Naltrexone Treatment of Alcoholics



Oslin et al., Neuropsychopharmacology, 28: 1546, 2003; similar findings by Anton… Goldman et al., Arch Gen Pscyh, 65:135, 2008

? FUNCTIONAL MOP-r (C17T) VARIANT–

Association with Alcohol and with Cocaine Dependence in HIV+ or HIV- African American Women (Based on KMSK Cut-Off Scores)

	Unadjusted Odds Ratio for TT Genotype	Adjusted for HIV-serostatus	Adjusted for HIV, age, income, and education
KMSK alcohol	3.7 (1.6-8.4) p = 0.003	3.6 (1.5-8.3) p = 0.003	3.0 (1.1-8.0) p = 0.03
KMSK cocaine	2.8 (1.8-6.4) p = 0.014	2.7 (1.2-6.2) p = 0.02	2.0 (0.8-5.2) p = 0.14
KMSK opiates	1.5 (0.4-5.1) p = 0.53	1.6 (0.5-5.6) p = 0.46	1.4 (0.3-6.1) p = 0.65

THE ROCKEFELLER UNIVERSITY 1901-2001 CELEBRATING 100 YEARS PRO BONO HUMANI GENERIS

Crystal et al., Addiction Biology, in press, 2011

GWAS (10K) ARRAY – Genes with Possible Association with Opiate Addiction in Caucasian Subjects: Top Hypothesis-Generated "Hits"

101 Controls: No drug addiction *(ASI criteria)*
104 Former Severe Heroin Addicts

Gene	Product	Description	P-Value
CRY1	Cryptochrome 1 (photolyase-like)	*Transports PER proteins to nucleus*	0.0040 (1)
GRM8	Metabotropic glutamate receptor subunit 8	*Presynaptic cleft in multiple brain regions*	0.0052 (2)
OPRM1	**Mu opioid receptor**	*Site of action of opiates/ opioids, enkephalin, β-endorphin, morphine, etc.*	0.0055 (3)
GRM6	Metabotropic glutamate receptor subunit 6	*Post-synaptic cleft of ON-bipolar cells*	0.0071 (4)
NR4A2 (NURR1)	Nuclear receptor subfamily 4, group A, member 2	*Coexpressed with TH Activates DAT*	0.0312 (11)

Nielsen et al, Molecular Psychiatry 13: 417, 2008

HYPOTHESIS-DRIVEN SNP ARRAY (Using Illumina® GoldenGate Custom Array – 130 Genes, 1350 SNPs) – Study of Heroin Dependence in Caucasians

SNP	Gene	nominal P value*
rs510769	mu-opioid receptor	0.0003
rs3778151		0.0007
rs6473797	kappa-opioid receptor	0.0009
rs2236861		0.0029
rs2236857	delta-opioid receptor	0.0125
rs3766951		0.0165
rs1534891	casein kinase 1, epsilon	0.0016
rs694066	galanin	0.0019
rs3758987	serotonin receptor 3, subunit B	0.0170

* Allele test

Levran…Rotrosen, Casadonte…Adelson and Kreek *Genes Brain Behav.*, *7*:720, 2008

HYPOTHESIS-DRIVEN SNP ARRAY (Using Illumina® GoldenGate Custom Array – 130 Genes, 1350 SNPs) – Study of Heroin Dependence in African Americans

SNP	Gene	nominal P value*
rs731780	Solute carrier family 29 member 1	0.0006
rs1650420	Glutamate receptor 2A	0.0006
rs6497730		0.0015
rs1070487		0.0022
rs4587976		0.0039
rs5326	Dopamine D(1) receptor	0.0029
rs971074	Alcohol dehydrogenase 7	0.0035
rs1176724	Serotonin receptor 3, subunit A	0.0048
rs2289948	Diazepam binding inhibitor	0.0170

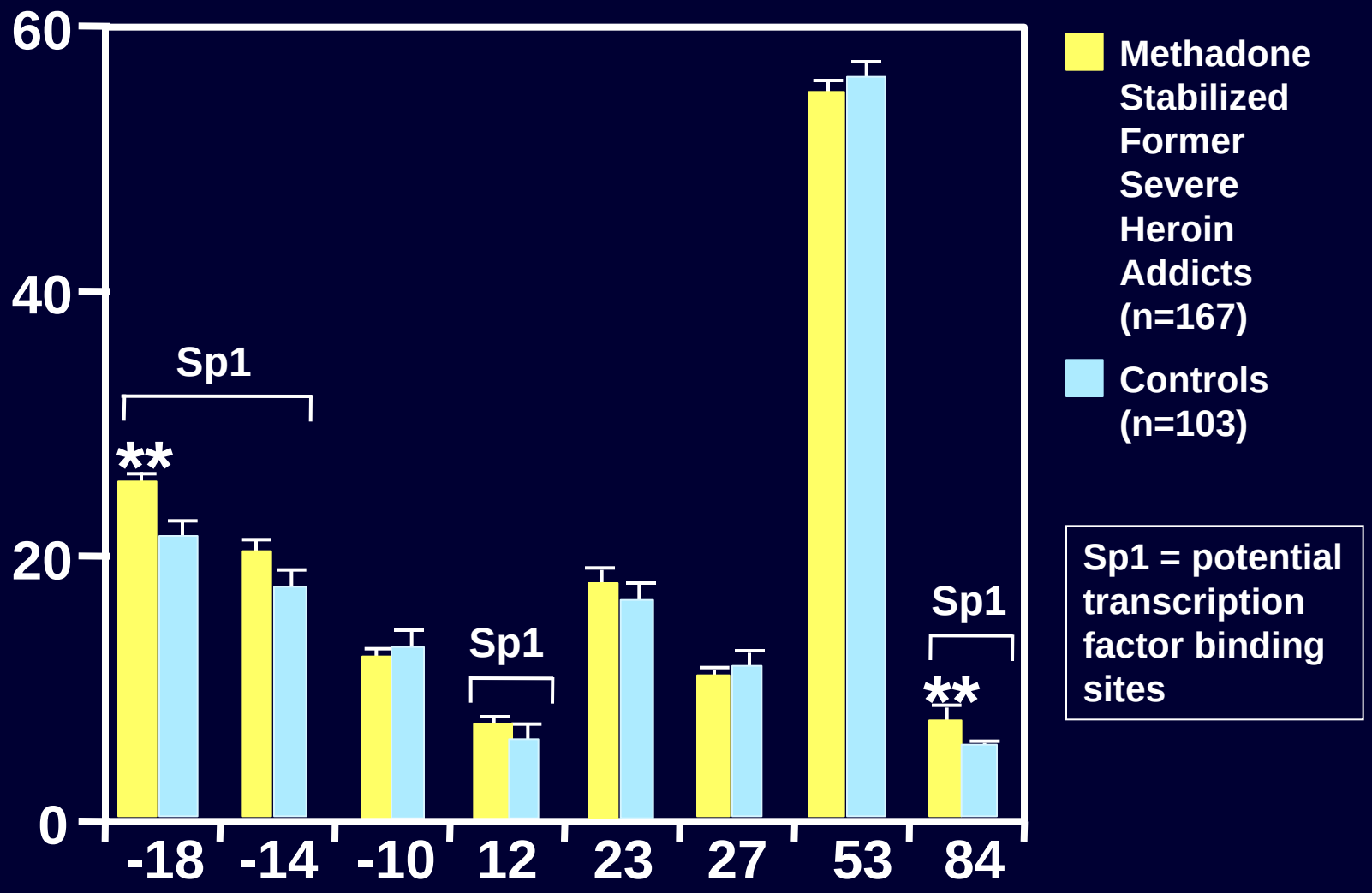
Levran et al, Genes Brain Behav. 8:531, 2009

Epigenetic Inheritance

- The transmission of information to a daughter cell or from generation to generation that is not encoded in the DNA sequence
- DNA methylation and covalent histone modifications are the primary sources of epigenetic inheritance

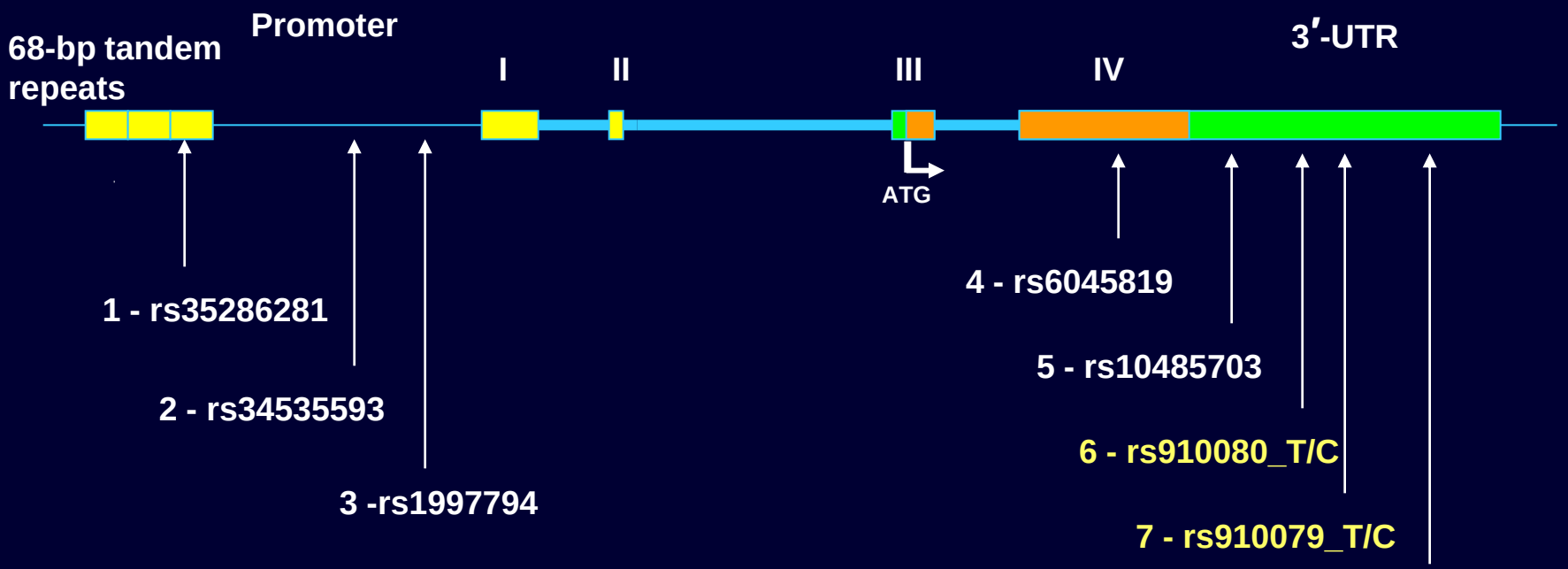
Nielsen, Neuropsychopharmacology 34:867-873, 2009

Increased Methylation at Two of Eight CpG Dinucleotides in the OPRM1 Promoter Region in Caucasian Former Severe Heroin Addicts versus Controls



Nielsen, Neuropsychopharmacology 34:867-873, 2009

Human prodynorphin gene: Chr 20pter-p12 Exon / intron organization and single nucleotide polymorphisms



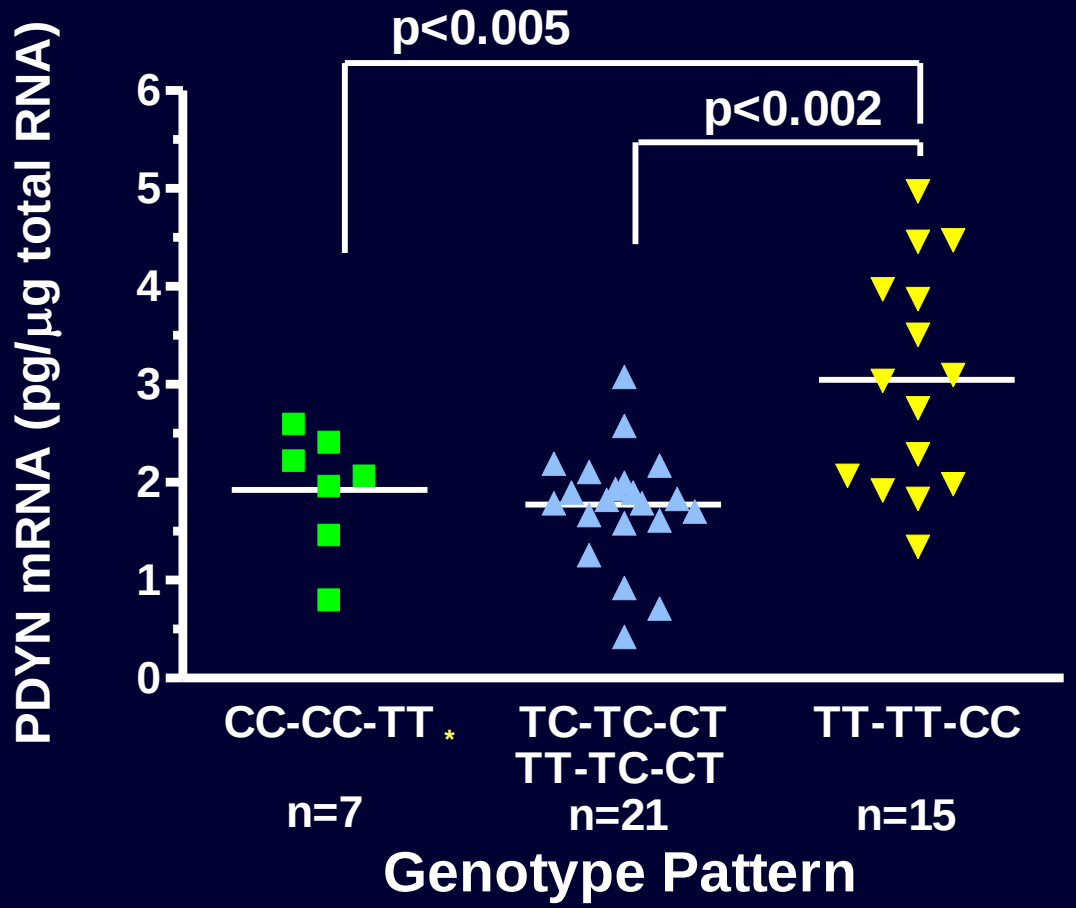
Human prodynorphin gene: Chr 20pter-p12
Exon / intron organization and single nucleotide polymorphisms

- Three 3'UTR SNPs (rs910080, rs910079, and rs2235749) are in complete linkage disequilibrium (LD), and comprise two haplotype blocks: T-T-C or C-C-T;

- The haplotype C-C-T was significantly associated with cocaine dependence and cocaine/alcohol codependence (OR=2.32, experiment-wise p=0.015) in Caucasians.

Yuferov et al, Neuropsychopharmacology, 34:1185, 2009

Preprodynorphin mRNA levels in the caudate from human post-mortem brains stratified by genotypes of PDYN gene



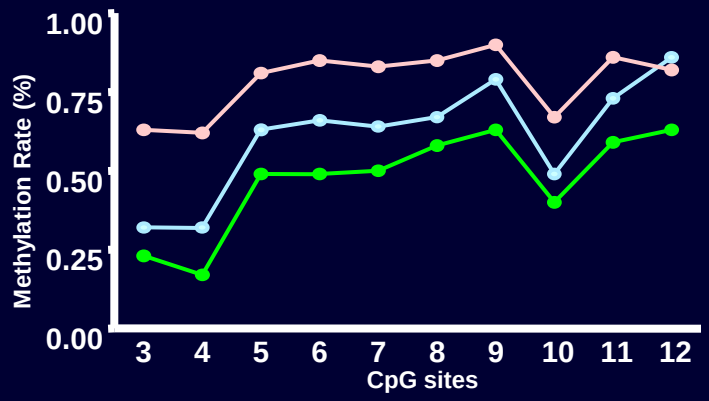
* Haplotype C-C-T significantly associated with cocaine dependence and cocaine/ alcohol codependence (OR=2.32, experiment-wise p=0.015) in Caucasians.

Yuferov et al, Neuropsychopharmacology, 34:1185-1197, 2009

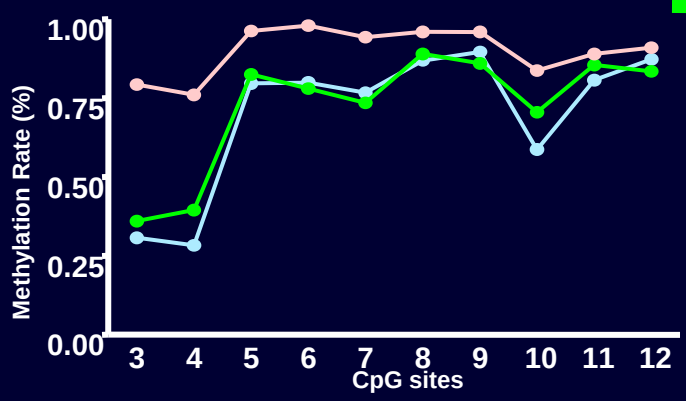
Methylation rate at specific CpG sites of the human *PDYN* gene promoter in PBMCs and two human post-mortem brain regions

ESME (sequencing) data, n=21

Cloning Data

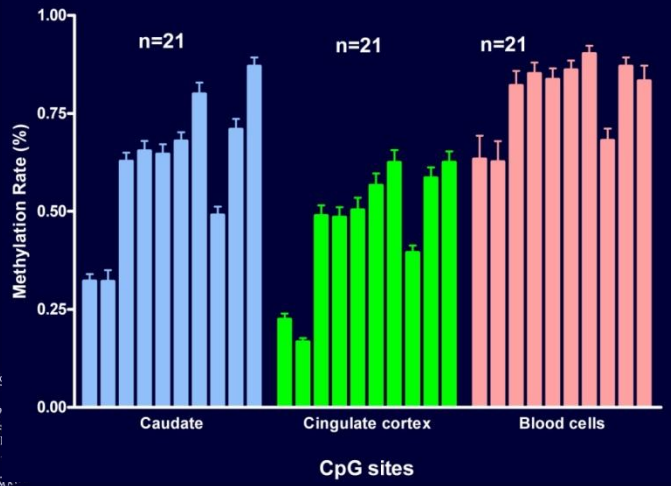


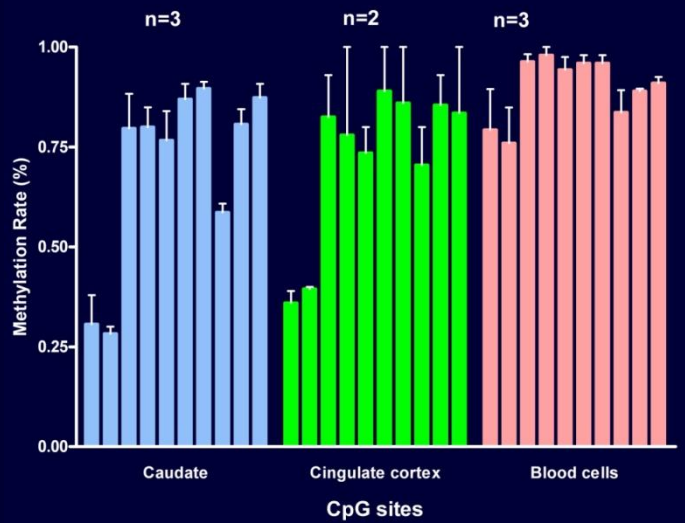
ESME (sequencing) data



Cloning data

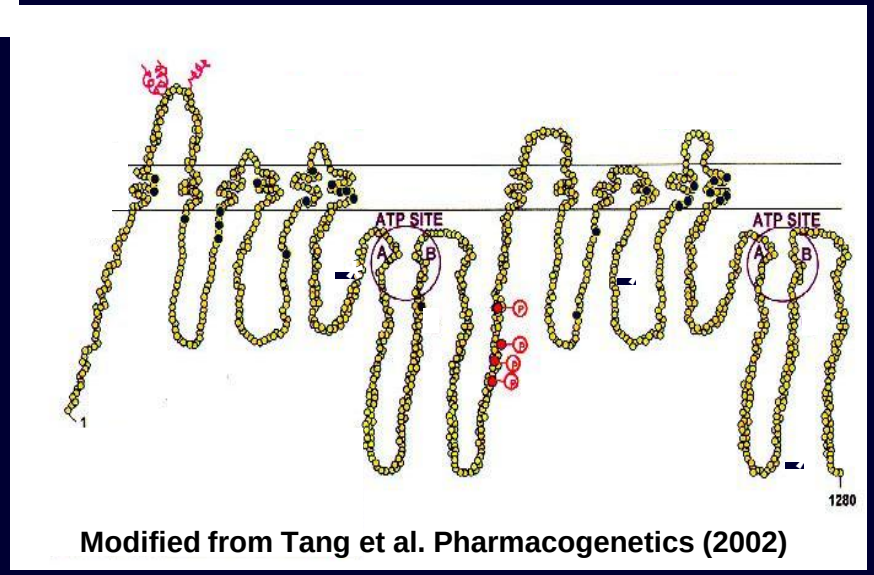
PBMC
Caudate
Cingulate cortex





Yuferov et al, ***Pharmacogenet. Genom.,*** ***21:185-196, 2011***

P-glycoprotein (MDR1, ABCB1)

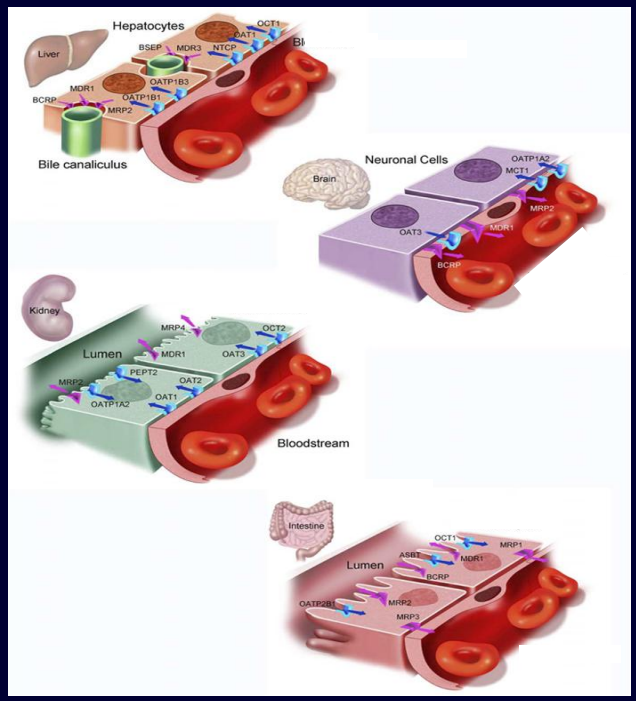


Modified from Tang et al. Pharmacogenetics (2002)

membrane

Out

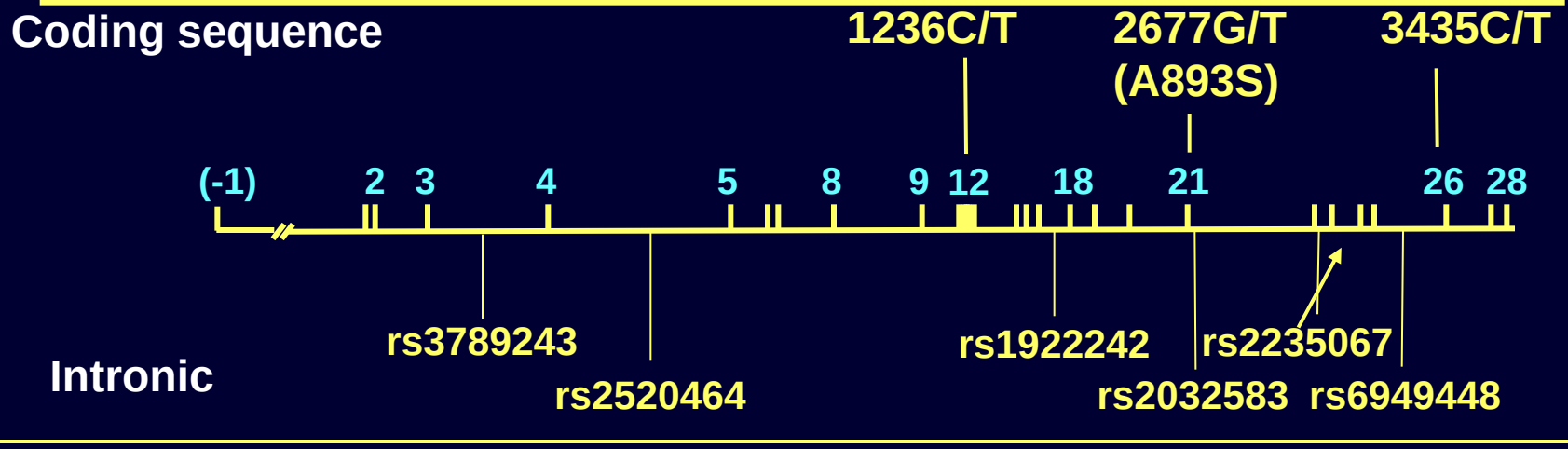
In

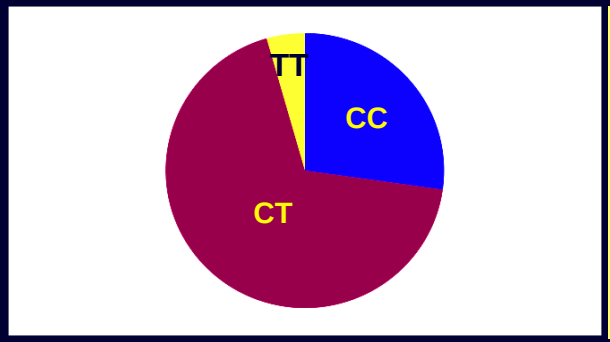


P-gp is expressed in tissues with barrier function like the endothelial cells lining of the Blood-Brain Barrier

Adapted from Ho et al., Clin. Pharm. Ther., 78: 260, 2005 and Tang et al., Pharmacogenetics, 12: 437, 2002

PHARMACOGENOMICS – P-glycoprotein (MDR1, ABCB1): SNP 1236C>T (and Related Haplotype) Associated with Higher Methadone Doses (>150 mg/day) in Maintenance Treatment Patients

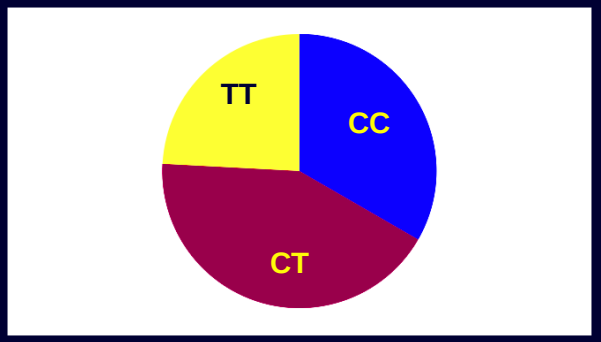




Methadone dose

← < 150 mg/day > →

"Low" | "High"

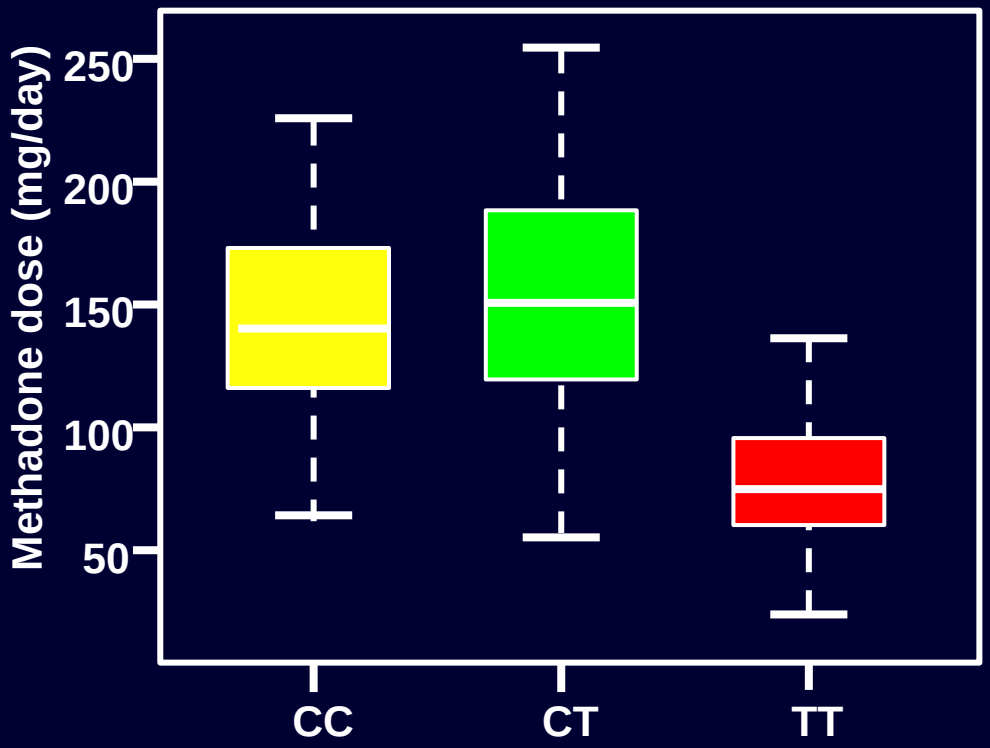


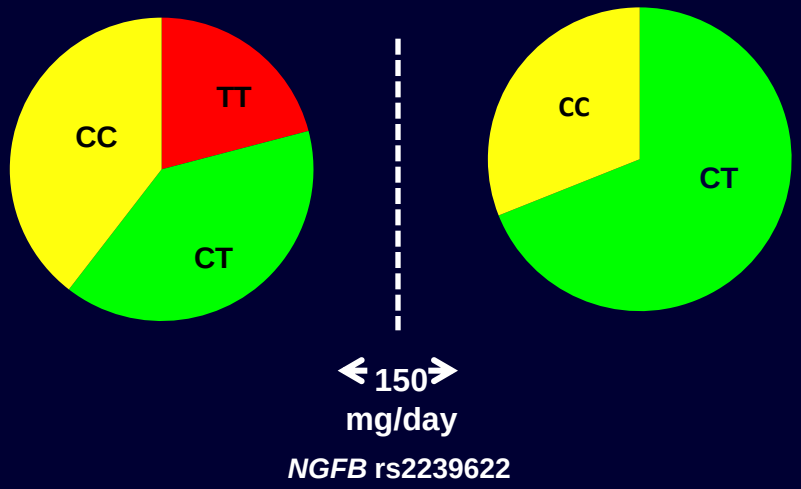
P = 0.007

P-gp is expressed in tissues with barrier function like the endothelial cells lining of the Blood-Brain Barrier

Levran… Kreek, Hum. Mol. Genet., 17:2219, 2008

PHARMACOGENETICS – Allelic Variant of *NGFB* Gene Associated with Lower Methadone Dose in Maintenance Treatment Patients (n=72)



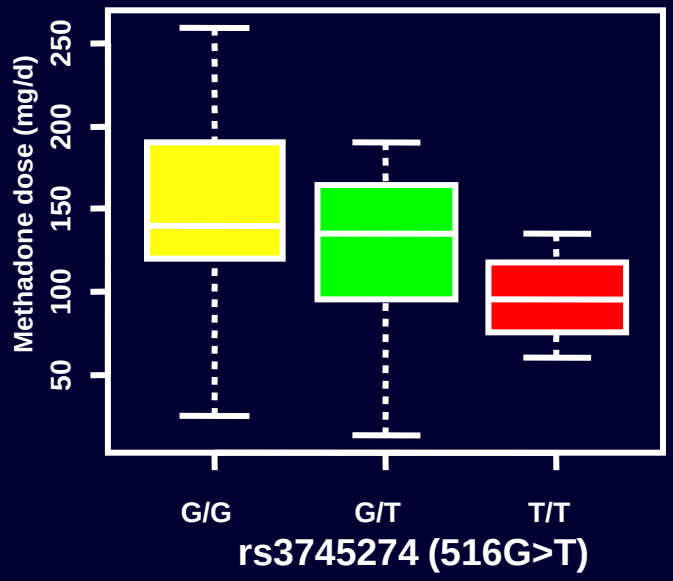


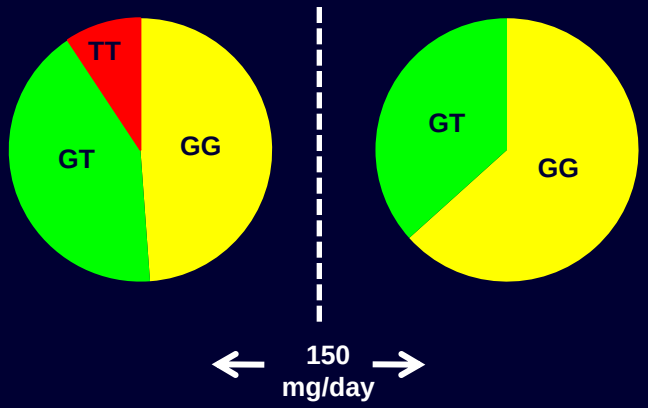
Mean Methadone Dose	
C/C	139.7 mg/d
C/T	153.1 mg/d
T/T	81.7 mg/d

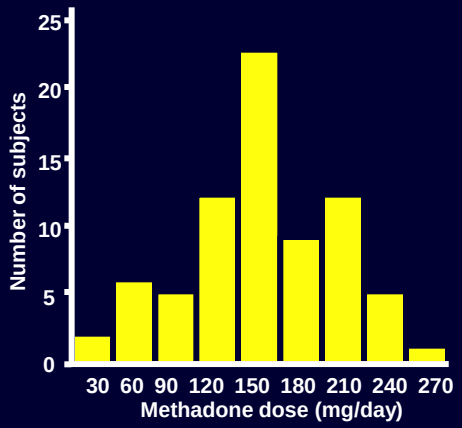
Minor Allelic Frequency (MAF) in Controls = 0.30

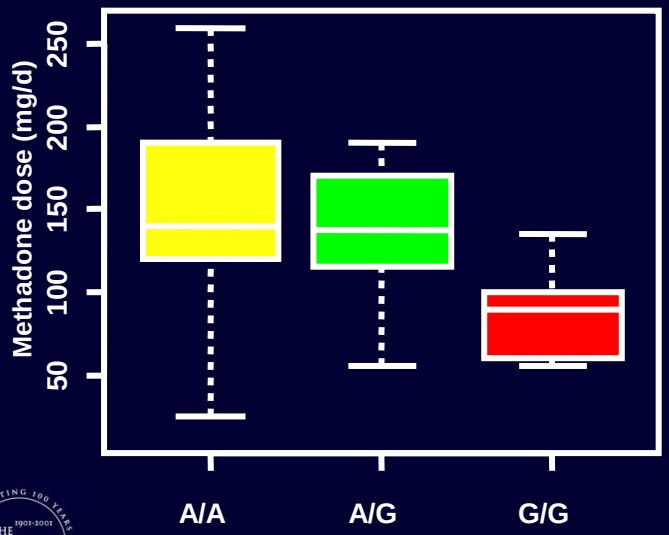
Levran… Kreek., Pharmacogenetics, in press, 2011

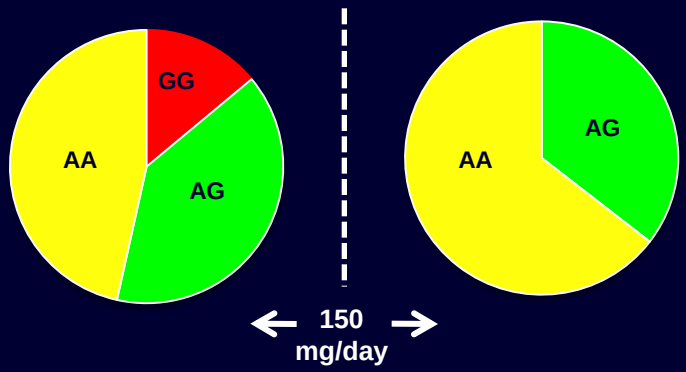
PHARMACOGENOMICS – *CYP2B6* SNPs are Associated with Effective Methadone Dose (n=74) (516G>T and 785A>G)











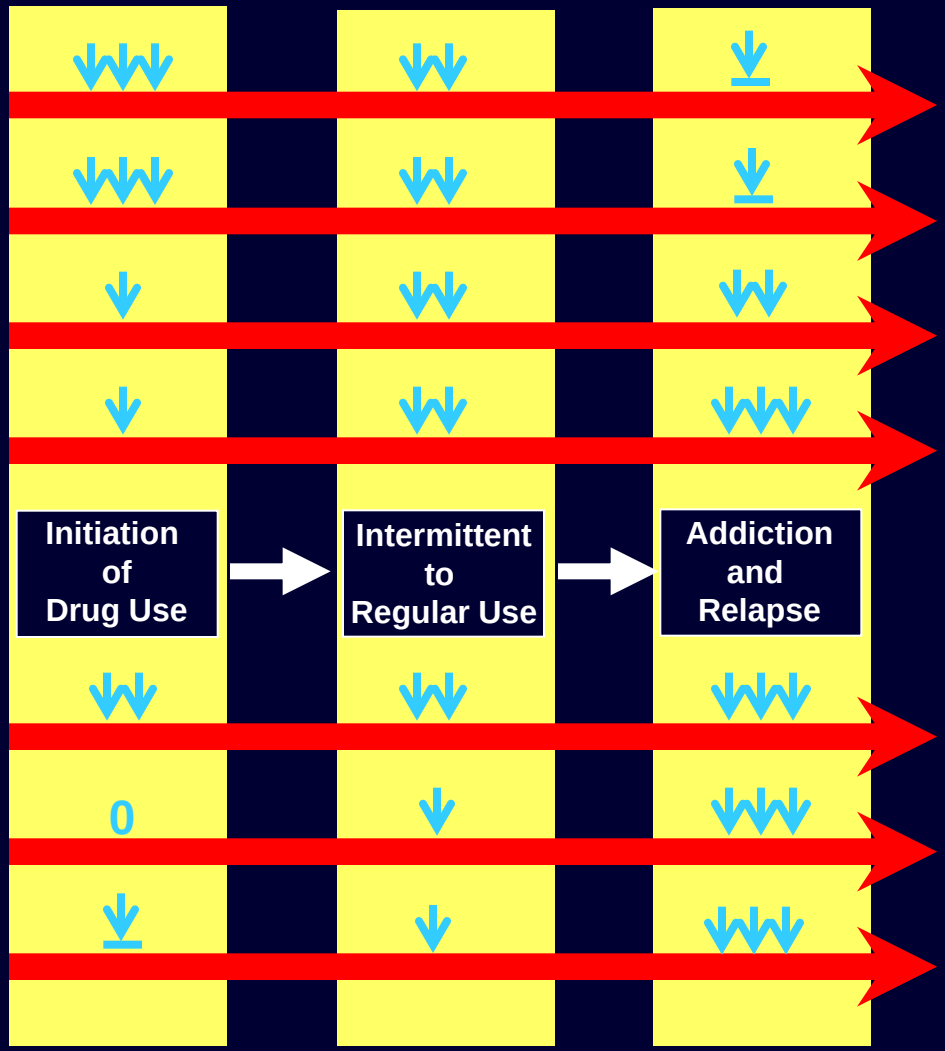
Levran … Kreek, Addiction Biology, in press, 2011

Impulsivity* (genetics?)

Risk Taking* (genetics?)

Comorbidity (genetics)

Stress Responsivity-atypical (genetics)



Environmental Factors
(~100% contribution to addiction)

Drug Induced Effects (w/ some genetic factors)
(~100% contribution to addiction)

Genetic Factors for addiction
(30-60% contribution to addiction)

** Relative scale of contributors to stage of drug use/addiction:

Kreek, Nielsen, Butelman & LaForge, Nat Neurosci., 8:1450, 2005

The Laboratory of the Biology of Addictive Diseases 2011-2012

Laboratory Scientists,
Eduardo Butelman
Yan Zhou
Orna Levran
Ann Ho
Vadim Yuferov
Dmitri Proudnikov
Yong Zhang
Brian Reed
Roberto Picetti
Stefan Schlussman

Research Physicians and Nurse Practitioners
Lisa Borg
Brenda Ray
Elizabeth Ducat

Laboratory Manager
Matthew Randesi

Postdoctoral Fellows & Graduate Students
Collene Lawhorn
Keiichi Niikura
Kate Seip
Jilda Caccavo

Assistants for Research
Adam Brownstein
Michele Buonora
Shasha Chen
Brandan Mayer-Blackwell

Administrative Staff
Kitt Lavoie
Rosanna Volchok
Susan Russo

P60-Center Collaborators/ Adjunct Faculty
Miriam Adelson*
Gavin Bart *
Paul Casadonte
Michael Glass
James Kocsis*
Diane Lane
David Nielsen*
David Novick*
Virginia Pickel*
John Rotrosen
Ellen Unterwald*

*Adjunct Faculty