Opioid Receptors – The Basis of Pain Relief and Addiction: Bidirectional Translational Research

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Pain, Opioids, and Addiction:
An Urgent Problem for Doctors and Patients
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Pain, Addictions, and the Endogenous Opioid System

- Pain is modulated by endogenous opioid peptides
- Exogenous opioids are effective analgesic agents
- Many drugs of abuse act at or impact upon the endogenous opioid system

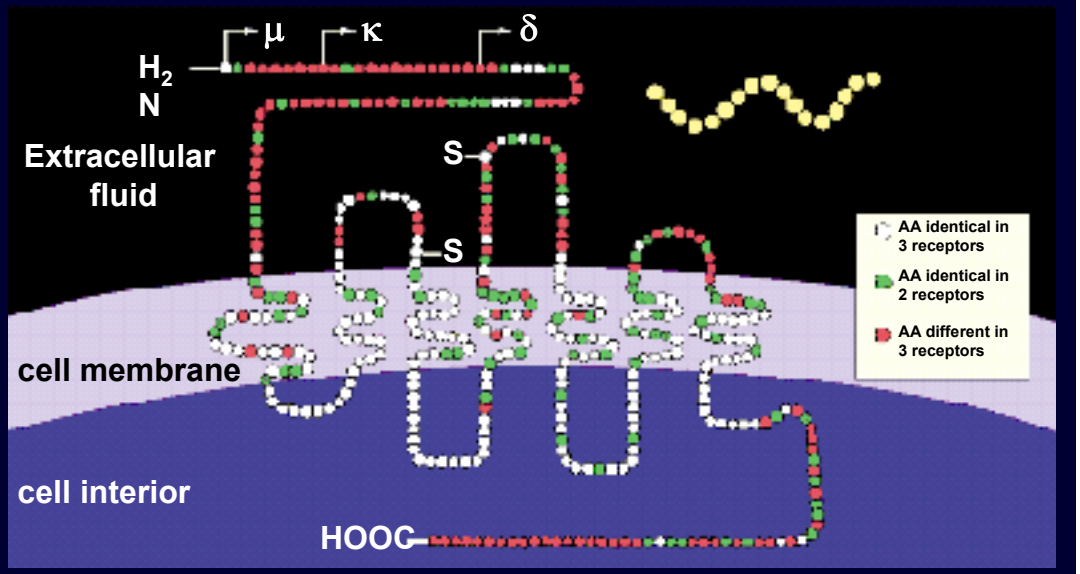
  Three major addictive diseases under study:
  - Heroin Addiction
  - Cocaine Dependency
  - Alcoholism

  Role of endogenous opioid system in each:
  - Extent of Role?
  - Precise Mechanism of Role?

Kreek, 2007

Endogenous Opioids and their Receptors

Opioid Classes	Opioid Receptor Types
Endorphins	Mu
Enkephalins	Delta
Dynorphins	Kappa
Endomorphins (?)	



LaForge, Yuferov and Kreek, 2000

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 hypothalamic-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, 2007

Prevalence of Specific Drug Abuse and Vulnerability to Develop Addictions

National Household Survey and Related Surveys – 1996 – 2002

Alcohol Use – ever	~ 177 million
Alcoholism	~ 15 million
Cocaine Use – ever	~ 26 million
Cocaine Addiction	~ 2 to 3 million
Heroin Use – ever	~ 2.5 to 3 million
Heroin Addiction	~ 0.5 to 1 million
Illicit Use of Opiate Medication – ever	~ 4.4 million
Resultant Opiate Medication Addiction	?

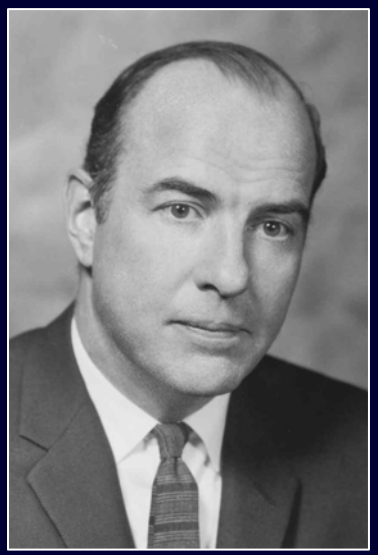
Development of Addiction After Self Exposure

Alcoholism	~ 1 in 8 to 1 in 15
Cocaine Addiction	~ 1 in 8 to 1 in 15
Heroin Addiction	~ 1 in 3 to 1 in 5

NIDA, SAMHSA Reports, 1998-2005

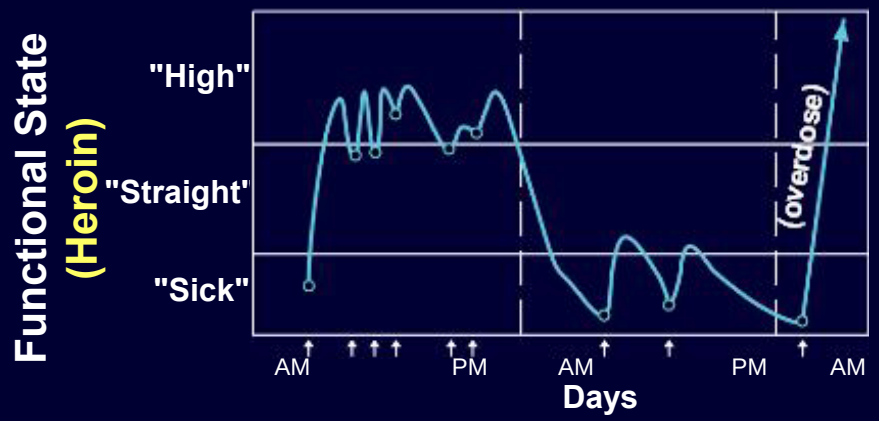
Hypothesis (1964) Leading to Development of Methadone Maintenance Treatment

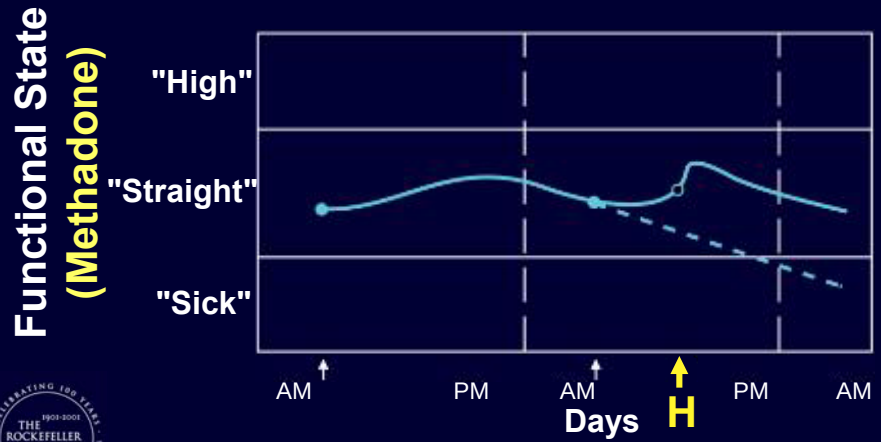
Heroin (opiate) addiction is a disease – a "metabolic disease" – of the brain with resultant behaviors of "drug hunger" and drug self-administration, despite negative consequences to self and others. Heroin addiction is not simply a criminal behavior or due alone to antisocial personality or some other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



Dole, V.P., Nyswander, M.E. and Kreek, M.J.: Narcotic blockade. Arch. Intern. Med., 118:304-309, 1966; 2006

Impact of Short-Acting Heroin versus Long-Acting Methadone Administered on a Chronic Basis in Humans - 1964 Study and Opioid Agonist Pharmacokinetics: Heroin Versus Methadone





Systemic Bioavailability After Oral Administration	Apparent Plasma Terminal Half-life ($t_{1/2}$ Beta)	Major Route of Biotrans-formation
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
Essentially Complete (>70%)	24h (48h for active [R](l)-enantiomer)	N-demethylation

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

"On-Off" *versus* "Steady-State": Relationship Between Blood (and Brain) Levels of Drugs of Abuse and Their Effects on Events Related to Addictions

Disruption *versus* Normalization

- **levels of gene expression**
- **receptor mediated events**
- **physiology**
- **behaviors**

Rates of rise of blood (and presumable brain) levels of drugs of abuse are related positively to their reinforcing effects

Rates of fall of blood (and presumably brain) levels of drugs of abuse are related positively to the onset of withdrawal symptoms and/or acute "craving"

Kreek, 1978;1987;1991;1992; 2001

Methadone Maintenance Treatment for Opiate (Heroin) Addiction

Number of patients currently in treatment: 212,000 (USA)
>500,000 (worldwide)

Efficacy in "good" 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 mu receptor sites.

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

Kreek, 1972; 1973; 2001; 2005; Inturrisi et al, in progress; Evans et al, 2003

Mu Opioid Agonist and Antagonist Pharmacotherapies: Opiate Treatment Outcome* and Numbers Seeking Treatment***

OPIATE ADDICTION

Long-Acting Mu Opioid Receptor Agonist or Partial Agonists	
Methadone Maintenance	50 – 80%
Buprenorphine-Naloxone Maintenance	40 – 50%**
Mu Opioid Receptor Antagonists	
Naltrexone Maintenance	10 – 20%
Other	
"Drug Free" (non-pharmacotherapeutic)	5 – 30%
Short-term Detoxification (any mode)	5 – 20%

Illicit Opiate Users Seeking Treatment

Numbers	1995	2005
Heroin (%OTR***)	227,989	254,345 (30.1)
Prescription Opiates (%OTR***)	16,121	67,887 (19.9)

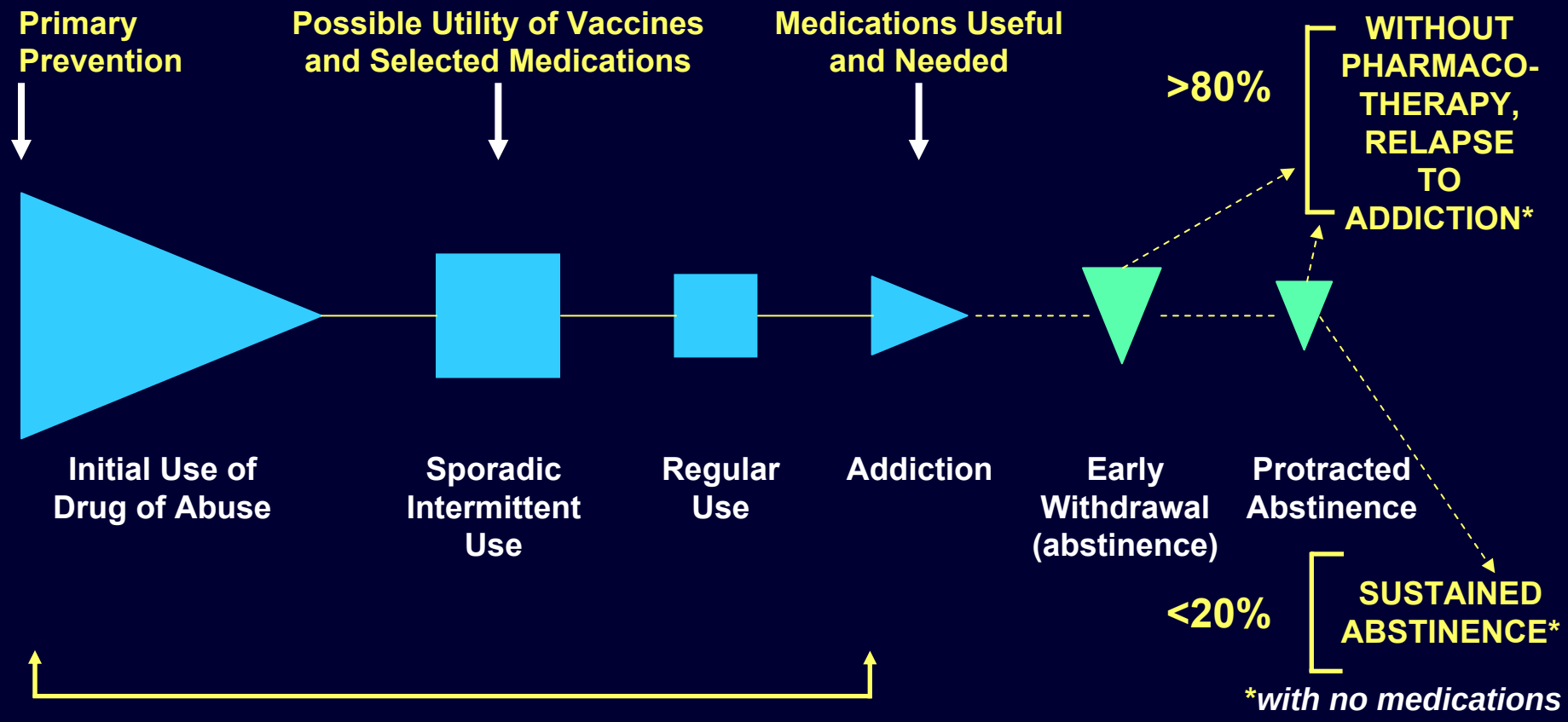
** One year retention in treatment and/or follow-up with significant reduction or elimination of illicit use of opiates*

*** Maximum effective dose (24 to 32 mg sl) equal to 60 to 70 mg/d methadone. Data based on 6 month follow-up only.*

**** OTR – Pharmacotherapy with methadone or buprenorphine maintenance*

Kreek, 1996; 2001; 2004; 2006; Treatment Episode Data Set (TEDS), SAMHSA, 2005

Natural History of Drug Abuse and Addictions

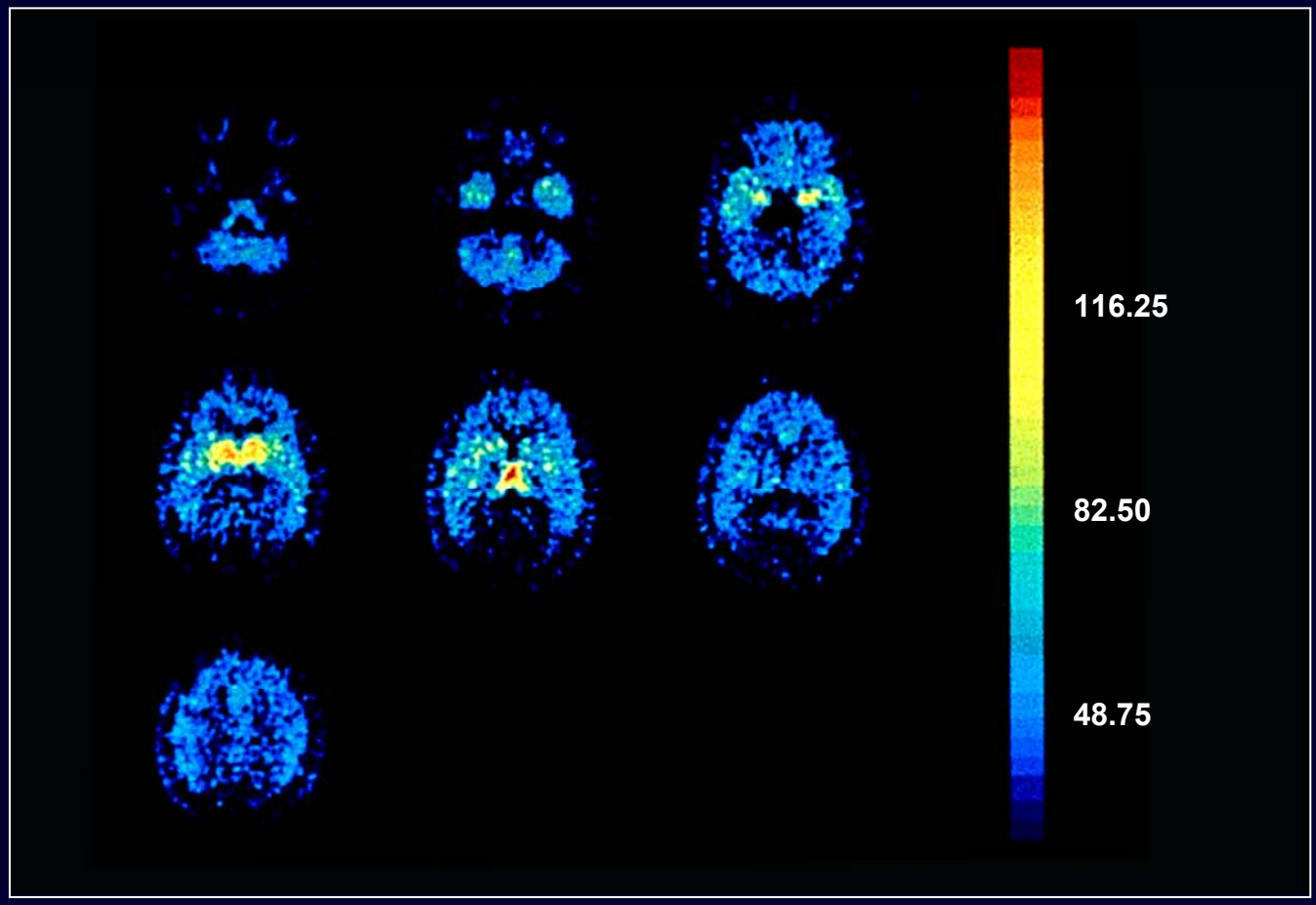


ADDICTION: Compulsive drug seeking behavior and drug self-administration, without regard to negative consequences to self or others (adapted from WHO).

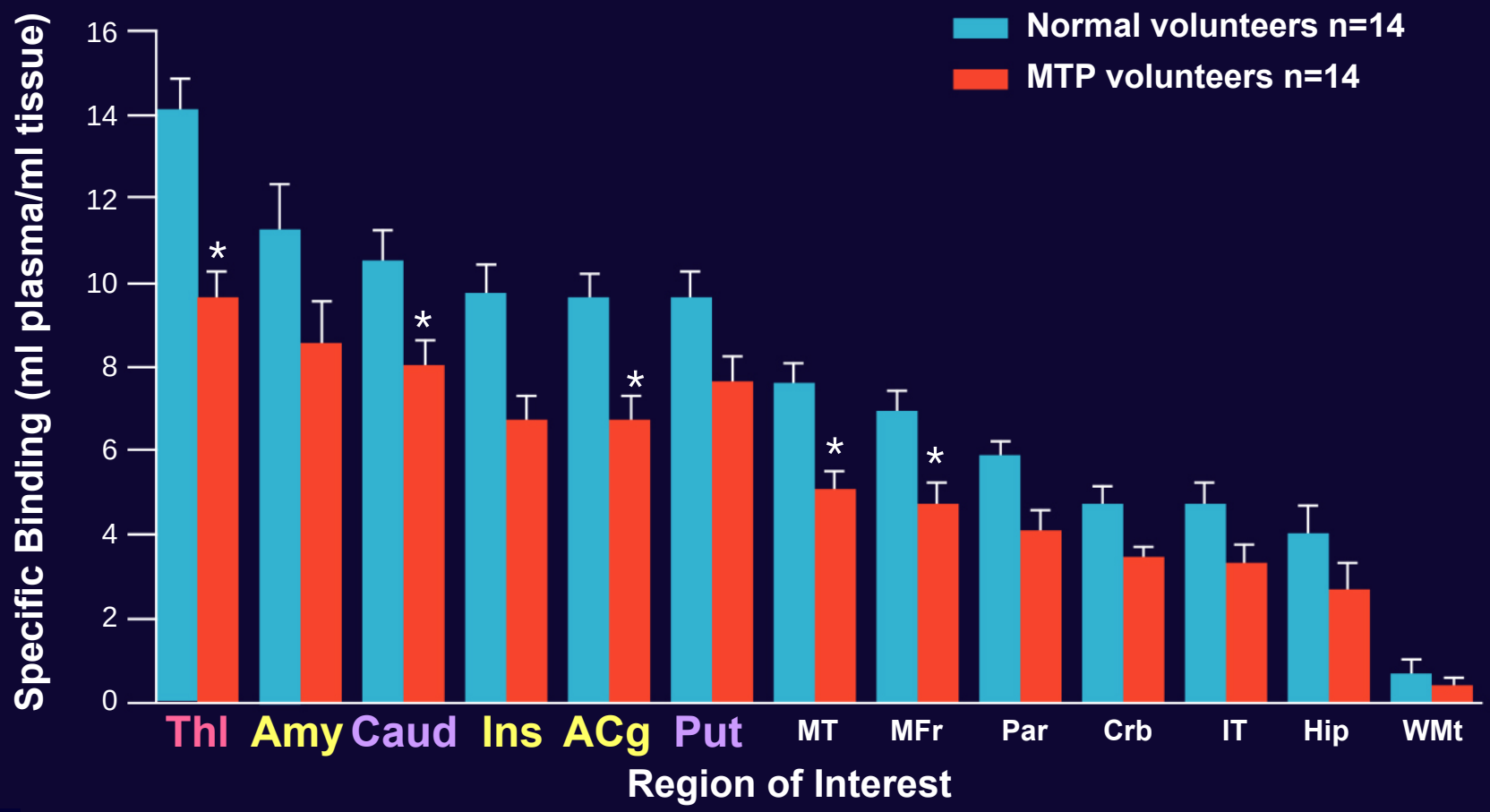
For entry into opioid agonist pharmacotherapy (methadone or LAAM maintenance) (U.S. Federal Regulations), above criteria, plus multiple daily self-administration of opiates for one year or more. For entry into opioid partial agonist pharmacotherapy (buprenorphine), meeting DSM-IV criteria for dependence.

Kreek et al., Nature Reviews Drug Discovery, 1:710, 2002; 2007

[^{18}F] Cyclofoxy (a Selective Opioid Antagonist) Binding in Human Brain: Normal Volunteer PET Study - NIH



Mu Opioid Receptor Density in Humans: Specific Binding of [^{18}F] Cyclofoxy (mean + S.E.M.) in 13 Brain Regions of Normal Volunteers and Long-Term, Methadone Treated Former Heroin Addicts - PET Study



Area related to pain response

Dopaminergic terminals of VTA neurons (mesolimbic-mesocortical dopaminergic system regions)

Dopamine terminals of substantia nigra neurons

Kling et al., 2000

Methadone Maintenance Treatment Allows Normalization of Endogenous Opioid-Related Physiological Functions Disrupted During Chronic Heroin Use

Neuroendocrine Function

- **Hypothalamic-Pituitary-Adrenal Axis – Stress Responsivity** levels and circadian rhythm of release of POMC peptides (β Endorphin; ACTH and cortisol)
- **Hypothalamic-Pituitary-Gonadal Axis – Reproductive Biology** levels and pulsatile release of LH and testosterone levels

Immune Function

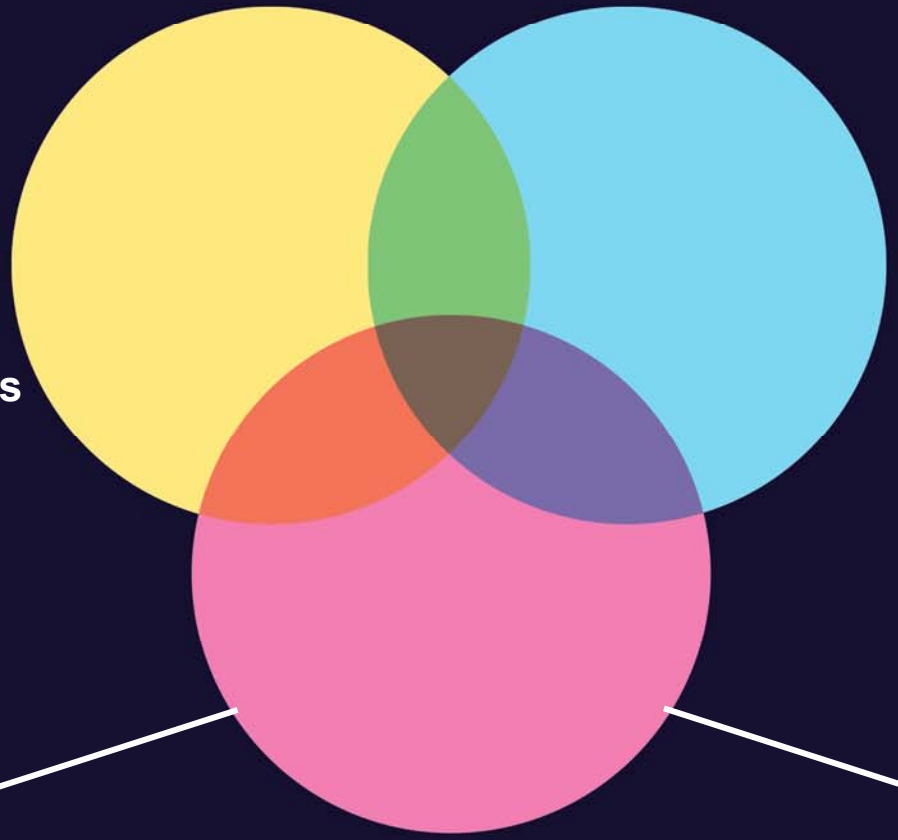
- **Natural Killer Cell Activity**
- **Absolute Numbers of Cells** — T cells; T cell subset levels; B cells; NK cells
- **Immunoglobin Levels (M and G)**

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

Kreek, 1972; 1973; 1978; 1987; 1992; 2001; Novick et al., 1989

Factors Contributing to Vulnerability to Develop a Specific Addiction

use of the drug of abuse essential (100%)



Genetic (25-60%)
- DNA
- SNPs
- other polymorphisms

Environmental (very high)
- prenatal
- postnatal
- contemporary
- cues
- comorbidity
- stress-responsivity

- mRNA levels
- peptides
- proteomics

Drug-Induced Effects (very high)

- neurochemistry
- synaptogenesis
- behaviors

Kreek et al., 2000; 2005

Primary Site(s) of Major Drugs of Abuse

Heroin	***Depressant***	• Acts primarily on endogenous opioid system (mu opioid receptor) • Also affects dopaminergic system • Enhances dopamine by inhibition of inhibitory GABAergic neurons
Cocaine	***Stimulant***	• Acts primarily on dopaminergic, as well as on serotonergic and noradrenergic presynaptic reuptake transporters • Also affects mu and kappa opioid systems
Alcohol	***Stimulant & Depressant***	• Undefined primary site of action • Affects dopaminergic, serotonergic and opioid systems

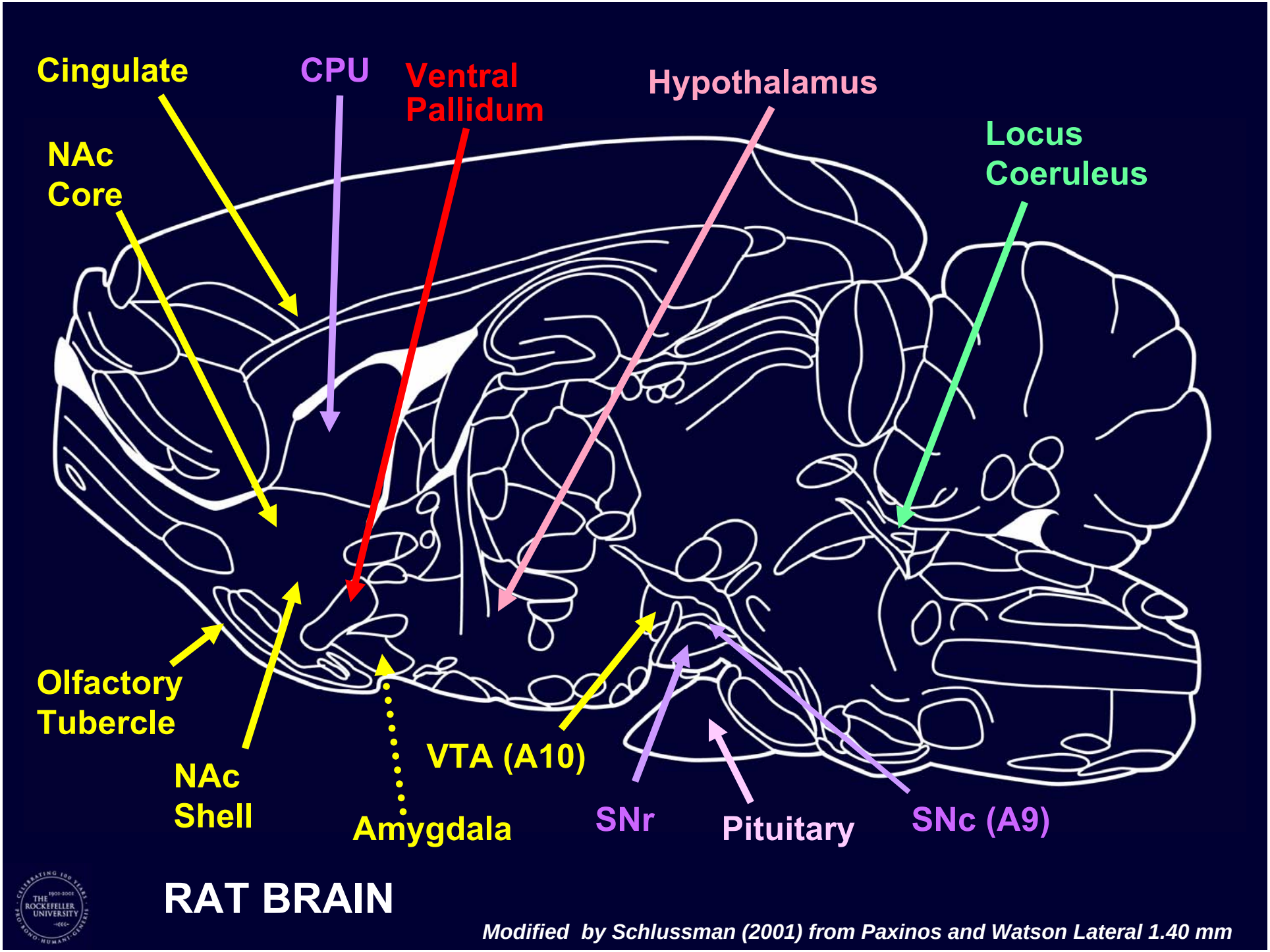
Kreek, 1978, 1987, 2005

Reinforcing or "Reward" Effects of Drugs of Abuse

Initial exposure to a drug of abuse may produce effects which are interpreted by the individual as "desirable" or "pleasurable", i.e., "rewarding". These effects may lead to "craving" or "hunger" for the drug, with resultant spontaneous activity or work for drug acquisition and self-administration.

Primary sites of actions of drugs of abuse with respect to their reward or reinforcing effects have been identified as specific brain regions, rich in dopamine nerve terminals or cell bodies, the mesolimbic and mesocortical dopamine systems especially the nucleus accumbens, as well as the amygdala, the anterior cingulate and the insula, with related actions in the nigrostriatal dopaminergic regions. Each of these areas also has abundant receptors and peptides of the endogenous opioid system.

Kreek, 1987; 2005


Cingulate
CPU
Ventral Pallidum
Hypothalamus
Locus Coeruleus
NAc Core
Olfactory Tubercle
NAc Shell
Amygdala
VTA (A10)
SNr
Pituitary
SNc (A9)
RAT BRAIN
Modified by Schlussman (2001) from Paxinos and Watson Lateral 1.40 mm

Bidirectional-Translational Research: Novel and Conventional Animal Models

- **Intermittent Morphine (Heroin) Administration Model:**
  Constant or Ascending Dose
  (mimics most common pattern of human use in addiction)
- **Pump Methadone Administration Model:**
  (converts short-acting pharmacokinetic properties of opioid agonist in rodent to long-acting human pharmacokinetic profile)
- **Extended Access Self-Administration Without or With High-Dose Drug** (Cocaine or Opiate)
- **"Binge" Pattern Cocaine Administration Model:**
  Constant or Ascending Dose
  (mimics most common pattern of human use in addiction)
- **"Binge" Pattern Oral Ethanol Administration Model:**
  (mimics common pattern of human excessive use)

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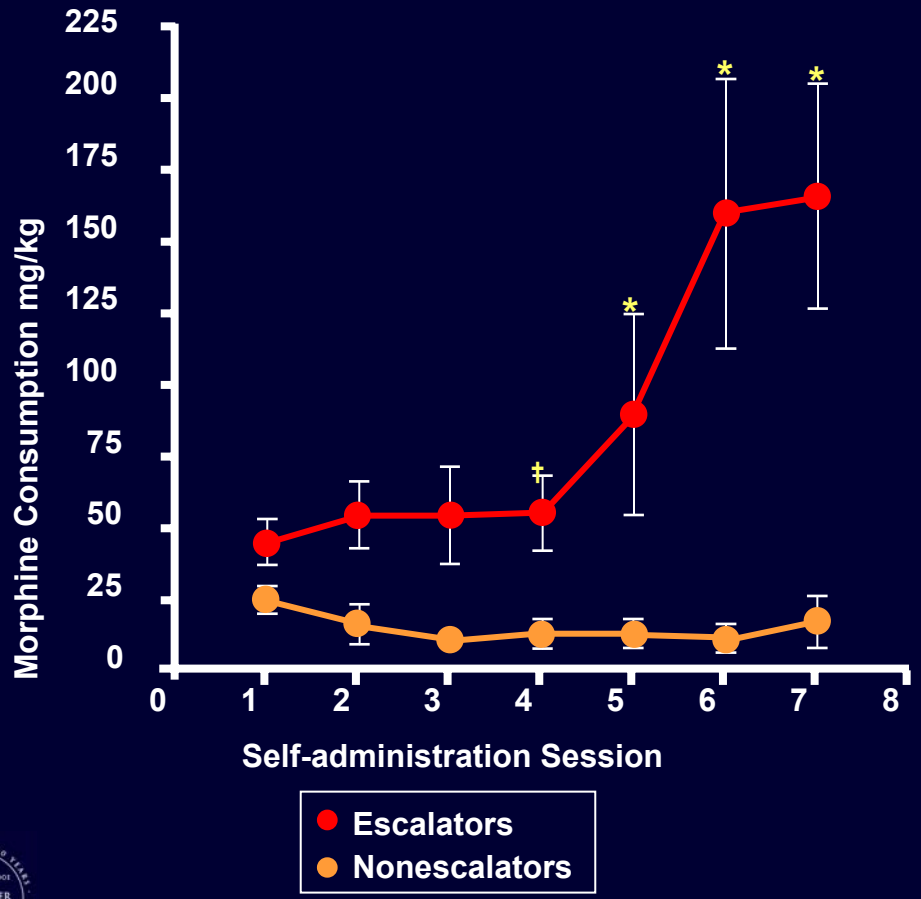
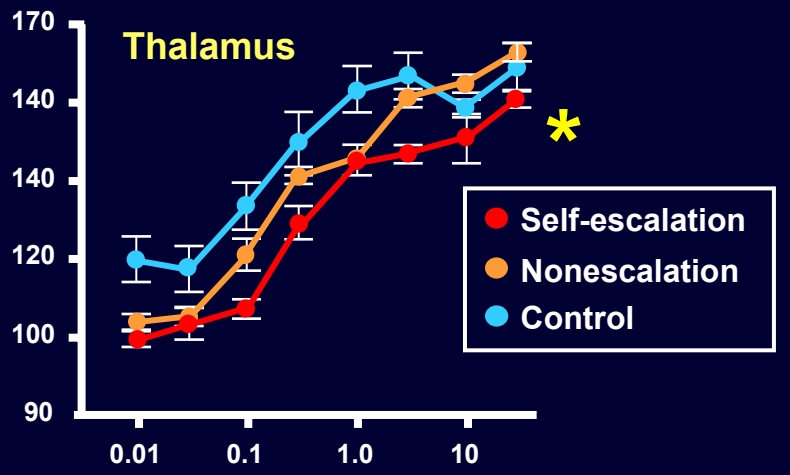
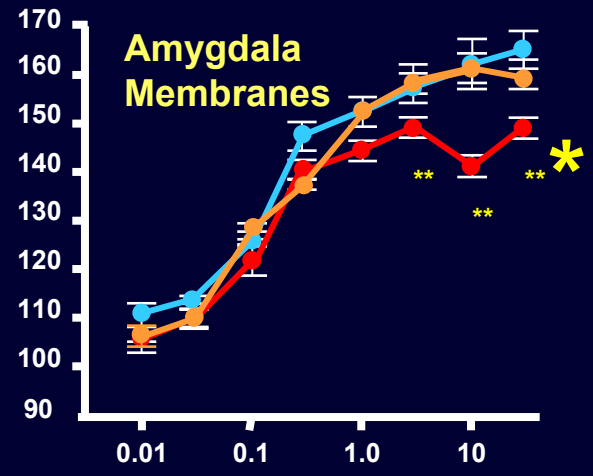
Kreek et al., 1987; 1992; 2001; 2005

Extended Session (18h) Morphine Self-administration in Rats: Dose Escalation by Choice or No Possible Escalation: Effects on [^{35}S]GTPγS binding in the thalamus and amygdala membranes

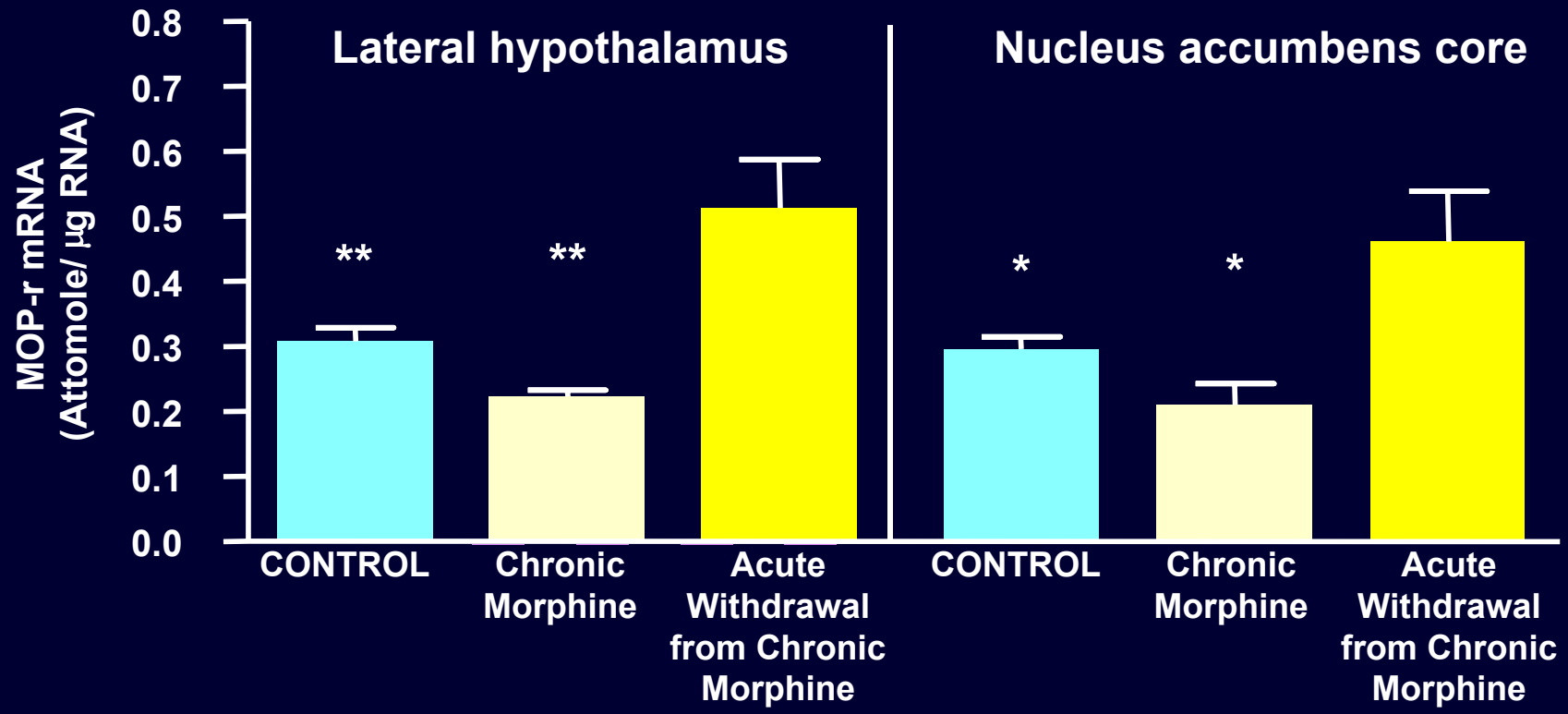






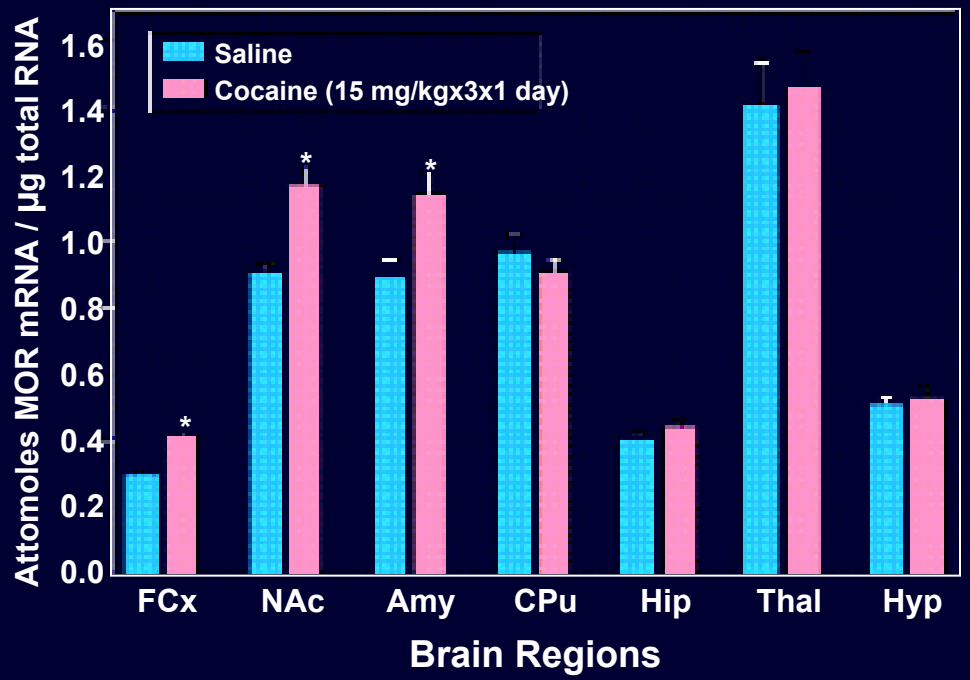
Kruzich, Chen, Unterwald & Kreek, 2001; Kruzich et al, Synapse, *2003*

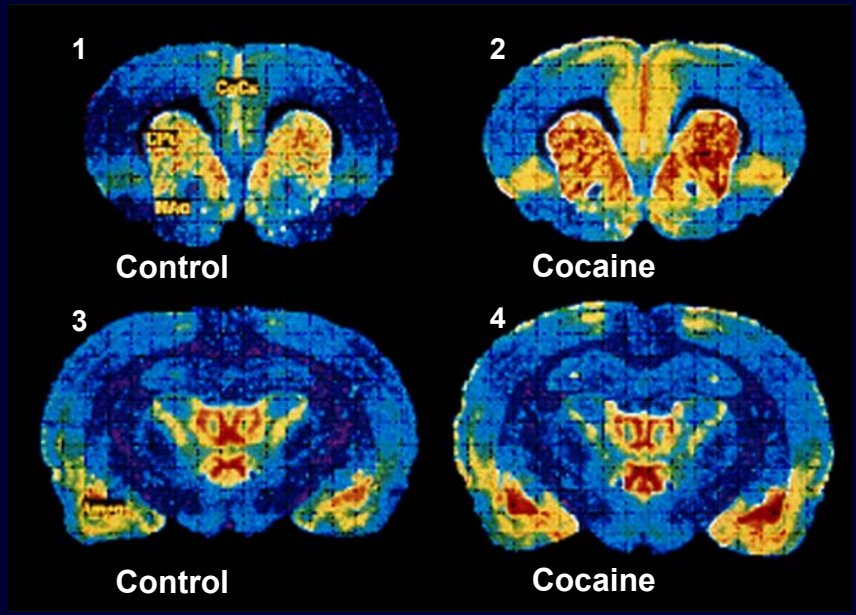
Chronic Intermittent Escalating Dose Morphine and Acute Withdrawal from Morphine: Effects on Mu Opioid Receptor mRNA Levels



Zhou et al., J. Endocrinology, 2006

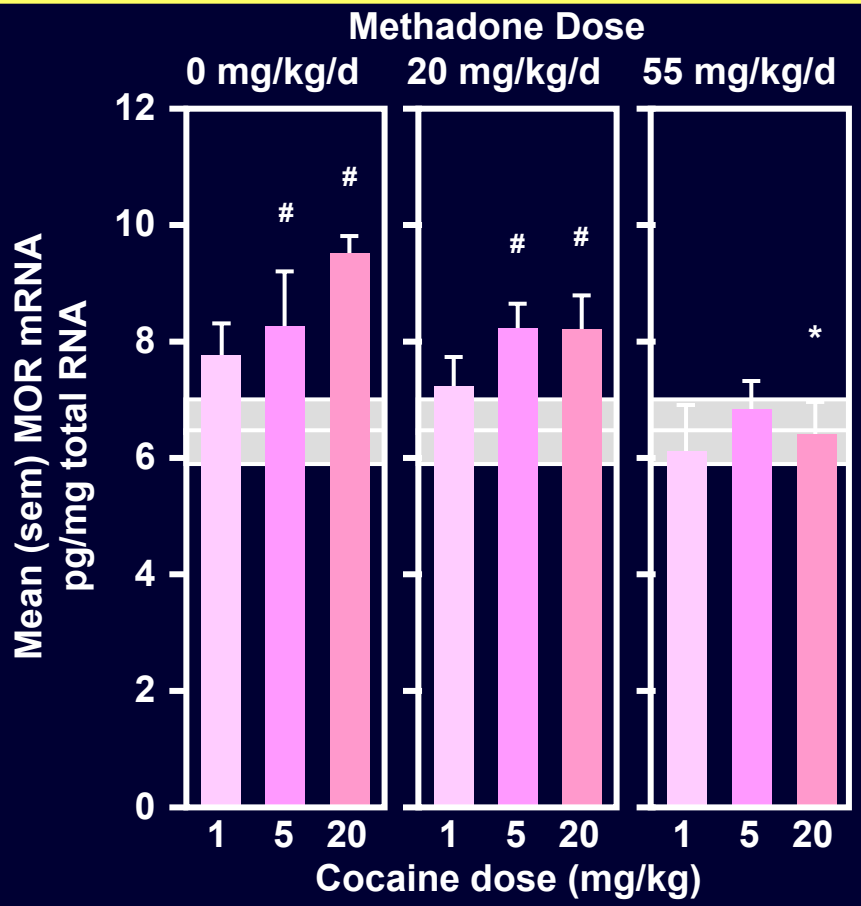
Mu Opioid Receptor-Endorphin System: REWARD — Acute "Binge" Pattern Cocaine Increases Mu Opioid Receptor mRNA Levels and Chronic Cocaine Increases Mu Opioid Receptor Density in Rat





Yuferov et al., Brain Res. Bull., 48:109 1999; Unterwald et al., Brain Res., 584:314 1992

Increased Mu Opioid Receptor mRNA Levels Induced by Subacute Cocaine Administration (3 Days) are Attenuated or Prevented by Low to Moderate Dose Methadone Infused by Pump in the Rat



Rats sacrificed 10 days following methadone-filled osmotic pumps; shaded area represents data from control group (n=8) that received no methadone and no cocaine.

* Significant difference from same cocaine-dose group in 0-dose methadone maintained group.

Leri et al., Neuropsychopharmacology, 31:1462, 2006

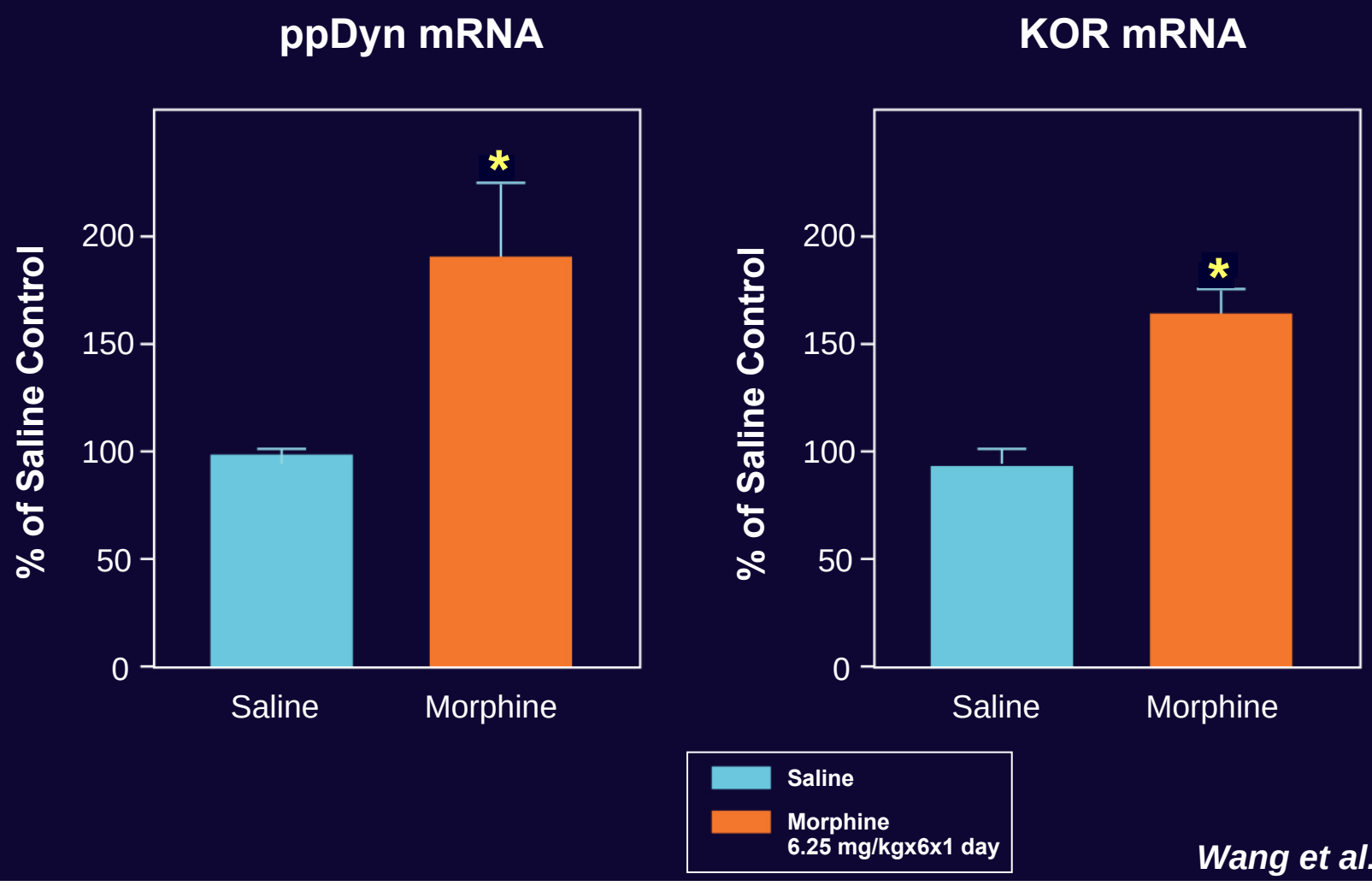
Mu Opioid Receptor Knock-Out Mice

- No morphine or other mu agonist analgesia
- No heroin or morphine self-administration
- No heroin or morphine induced conditioned place preference
- Attenuated self-administration of cocaine
- Attenuated self-administration of alcohol

[Different knock-out constructs and multiple research groups, including Kieffer, Uhl, Yu. Pintar, Loh, with, e.g., Maldonado, Pasternak, Hoellt, Roberts]

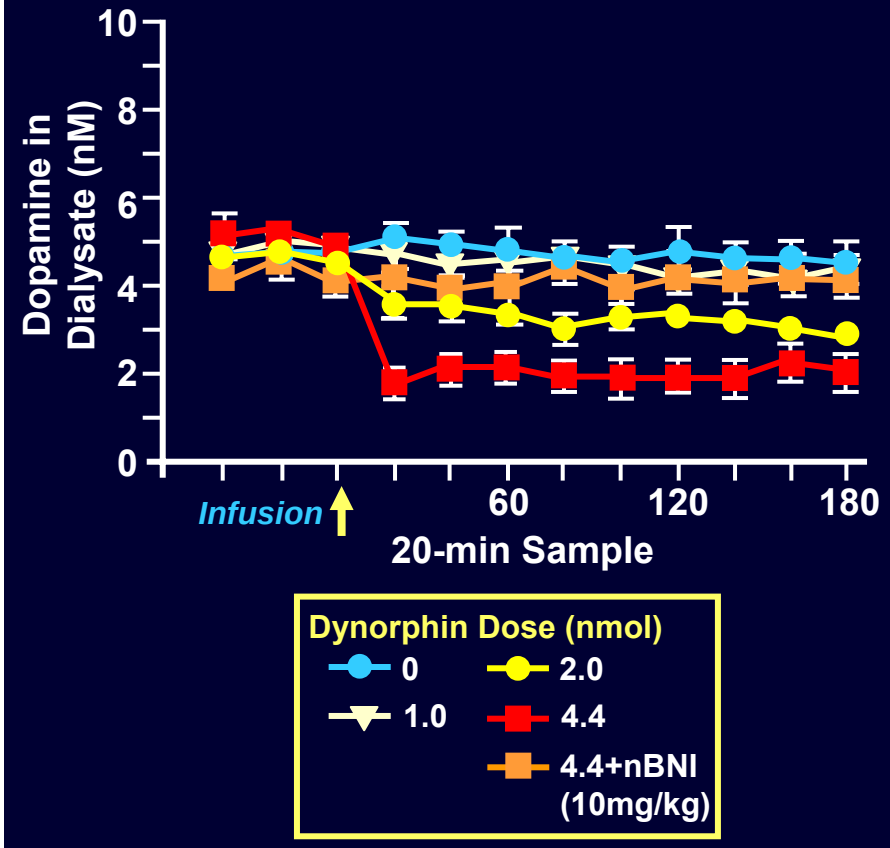
Reviewed in *Kreek et al.*, *Nature Reviews Drug Discovery*, *1*:710-726, 2002

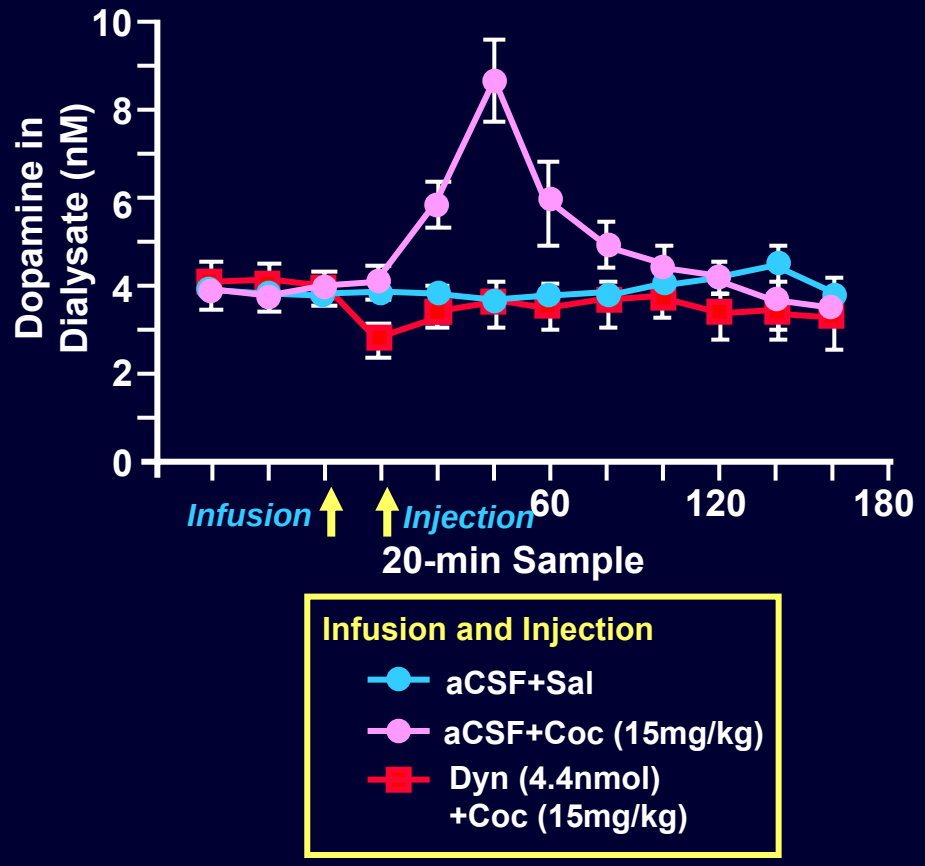
Acute Intermittent Morphine Increases Preprodynorphin and Kappa Opioid Receptor mRNA Levels in Rat Whole Brain (Minus Cerebellum)



Wang et al., 1999

Natural Dynorphin A_{1-17} Lowers Basal and Cocaine Induced Dopamine Levels in Mouse Striatum





Zhang, Butelman, Schlussman, Ho, and Kreek, Psychopharmacology, 172:422 2004

"Craving" or "Drug Hunger": Hypothesis
(with or without drug seeking and drug self-administration)

Neurochemical mediators of "rewarding" or "reinforcing" effects of drugs of abuse

- Dopamine acting at dopamine DA_1-like and DA_2-like receptors
- Mu opioid receptor agonists acting at mu opioid receptors (e.g., beta-endorphin and enkephlins)
- CRF and ACTH in stimulant and stimulant-depressant addicts only (e.g., cocaine and alcoholism)
- +/- serotonin, +/- norepinephrine

Neurochemical counter-modulators of "rewarding" or "reinforcing" effects

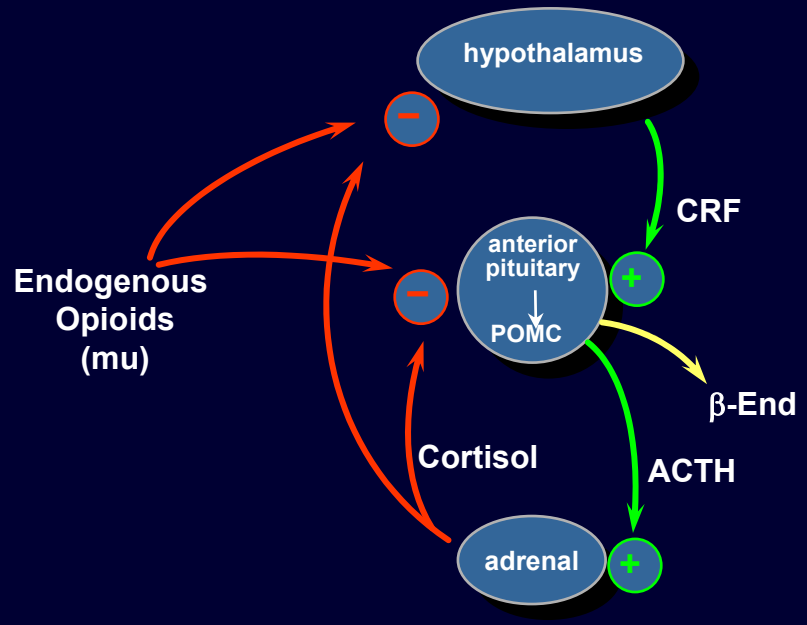
- Kappa opioid receptor agonists acting at kappa opioid receptors (e.g., dynorphins)
- Orphanin/nociception acting at orphan opioid-like receptors
- CRF and ACTH in opiate addicts (e.g., heroin)
- +/- GABA, +/- glutamate

Chronic drug use leads to persistent neurochemical and neurobiological changes, with blunting of the "rewarding" components and persistence of the counter-modulatory components (lowered dopaminergic tone and relative "endorphin deficiency"), which, when coupled with learning and memory, contribute to the resultant "drug craving" and "drug hunger."

Kreek, 2003; 2007

Hypothesis — Atypical Responsivity to Stressors: A Possible Etiology of Addictions

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; 1987; 1992; 2001; 2005

Neuroendocrine Effects of Opiates, Cocaine, and Alcohol in Humans: Hormones Involved in HPA Axis Stress Response

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

} Suppression of HPA Axis

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

} Activation of HPA Axis

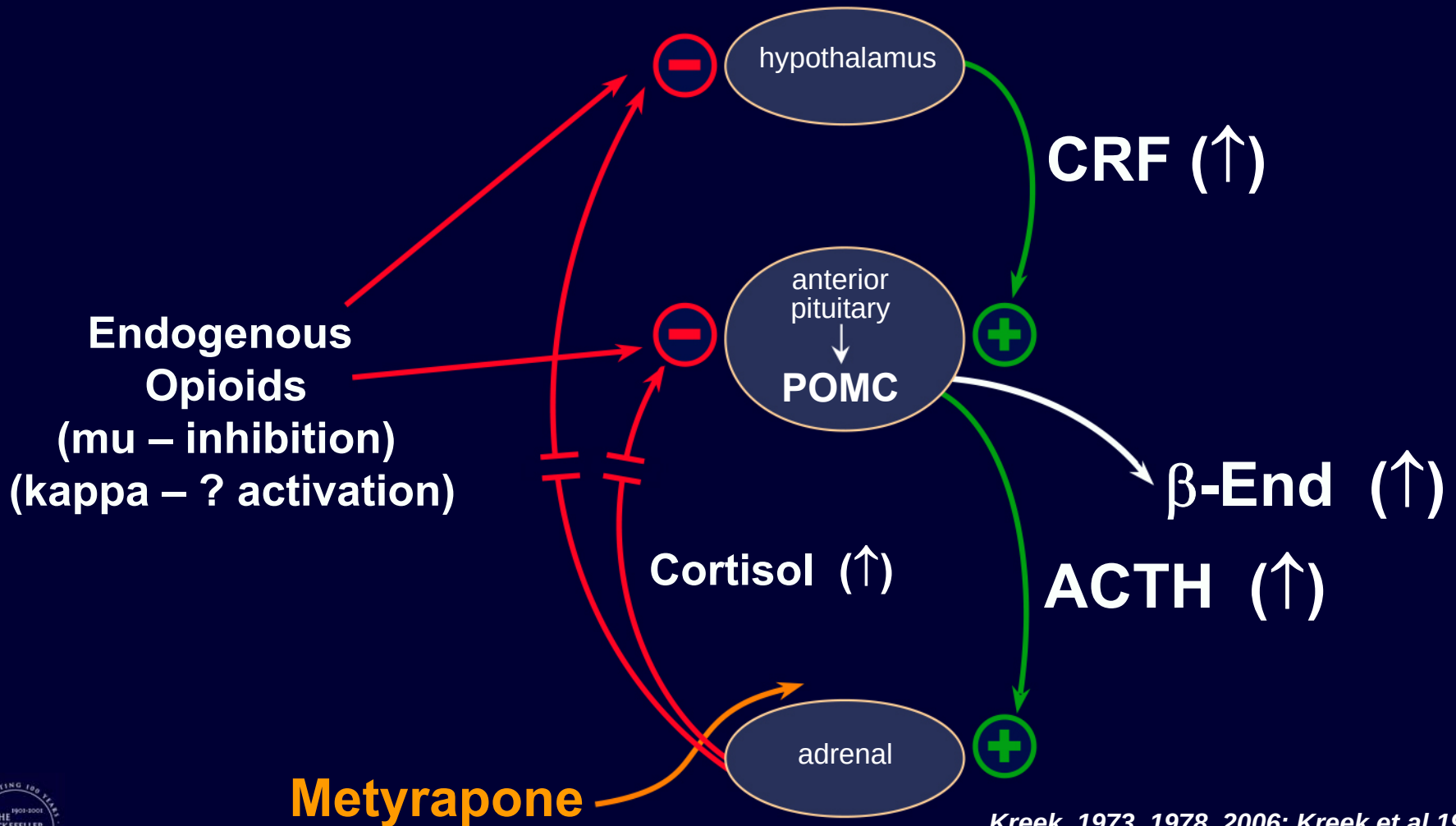
- Chronic effects of long-acting opiate (e.g. methadone maintenance treatment)

Normalization of HPA Axis

HPA – Hypothalamic-pituitary-adrenal axis (involved in stress response)

Kreek, 1972; 1973; 1987; 1992; 2001; 2003

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

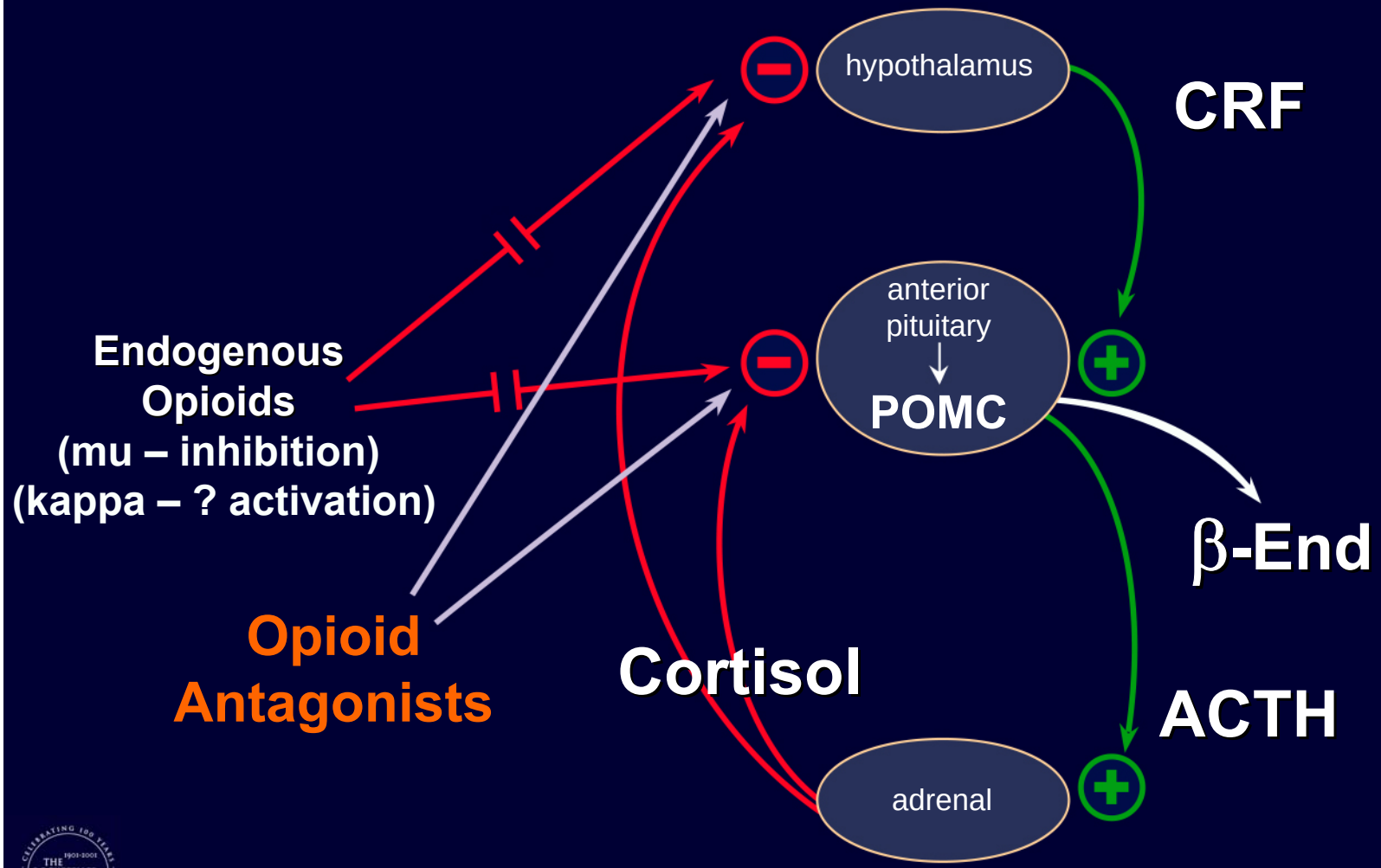
Metyrapone Testing: a Chemically-Induced "Stress"

- Heroin addicts
  - hyporesponsive
- Methadone maintained former heroin addicts
  - euresponsive
- Drug-free, opioid medication-free former heroin addicts
  - hyperresponsive
- Cocaine addicts- recently abstinent
  - hyperresponsive
- Cocaine addicted, methadone maintained former heroin addicts
  - hyperresponsive

"Hyperresponsive" indicates a relative endorphin deficiency

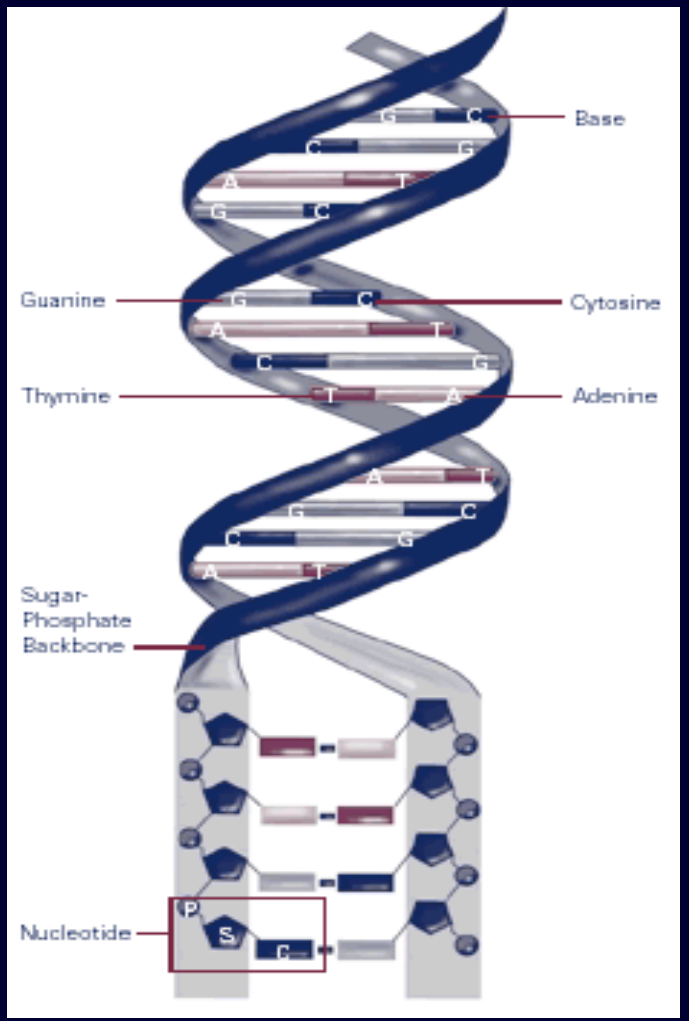
Kreek, 1972; 1973; 1984; 1987; 1992; 2005; Kreek et al., 1984; Schluger et al., 2001

Dissecting the Hypothalamic-Pituitary-Adrenal Axis in Humans: Selective Opioid Antagonist Testing



Kreek, 1984; 1998; 2006

Single Nucleotide Polymorphisms (SNPs) in Genes: Definitions



- **SNP — a single nucleotide polymorphism, that is, one nucleotide or base of any base pair**
- **Allelic Frequency:**
  - **<1% low or rare**
  - **1–5% intermediate**
  - **>5% high, frequent**

Hassin and Kreek, 2004

Hypothesis – Genetic Variability and the Mu Opioid Receptor System: Single Nucleotide Polymorphisms of Moderate Allelic Frequency in the Coding Region

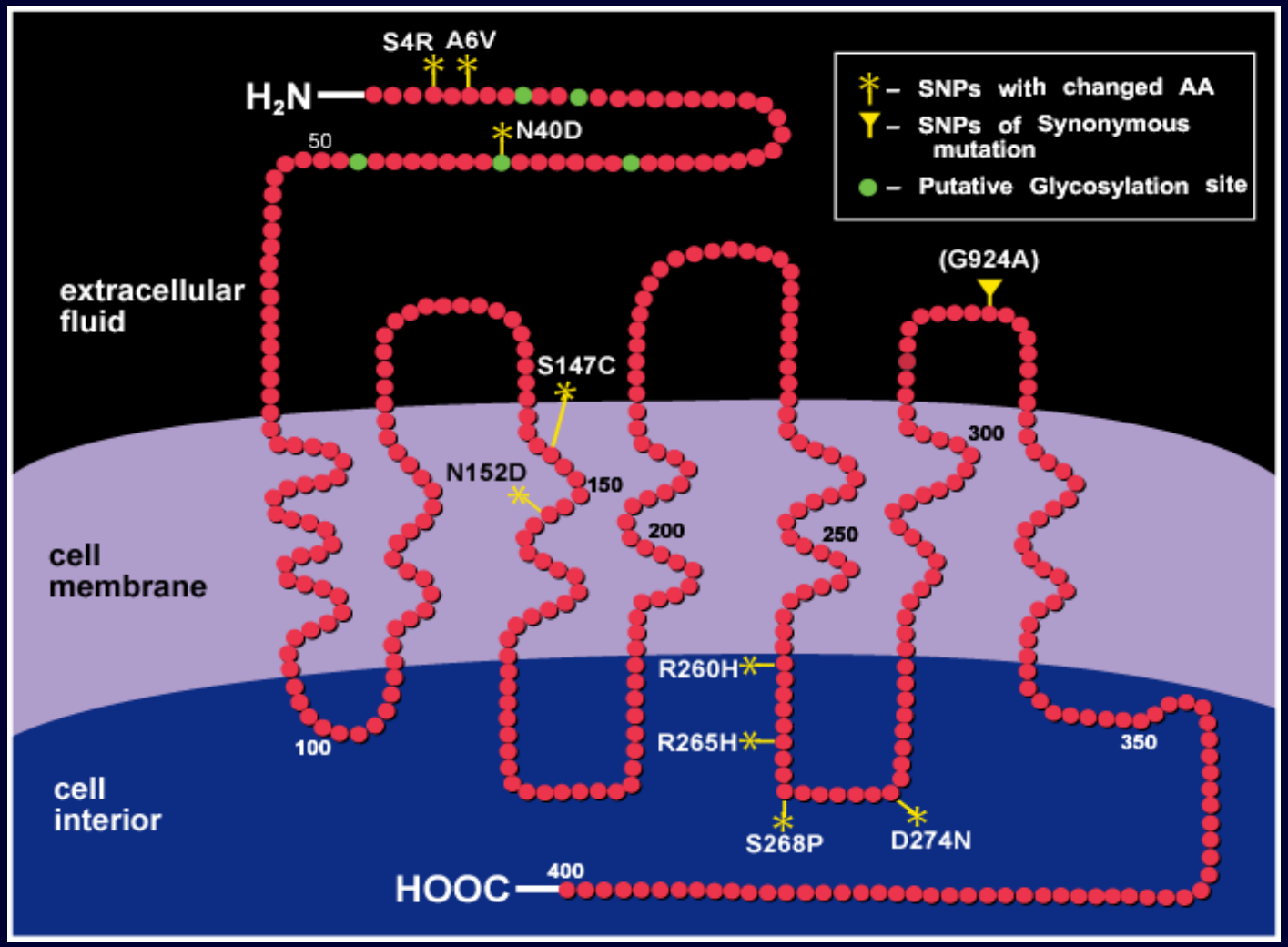
Some of the individual genetic variability in susceptibility to the development and persistence of, or relapse to, opiate addiction may be due to polymorphisms of the mu opioid receptor. Also, individual differences in responses to endogenous opioids ("physiogenetics") or pharmacotherapies ("pharmacogenetics") may be mediated by variant forms of the mu opioid receptor.

Variant (nucleotide position)	Exon location	Protein domain	Corresponding amino acid change	Allele frequency (overall – 3 ethnicities together)
A118G	1	N-terminus	Asn 4 Asp (N40D)	10.5% (26 heterozygous; 3 homozygous)
C17T	1	N-terminus	Ala 6 Val (A6V)	6.6% (14 heterozygous; 3 homozygous)

* *Nucleotide position 1 is first base of the start codon.*

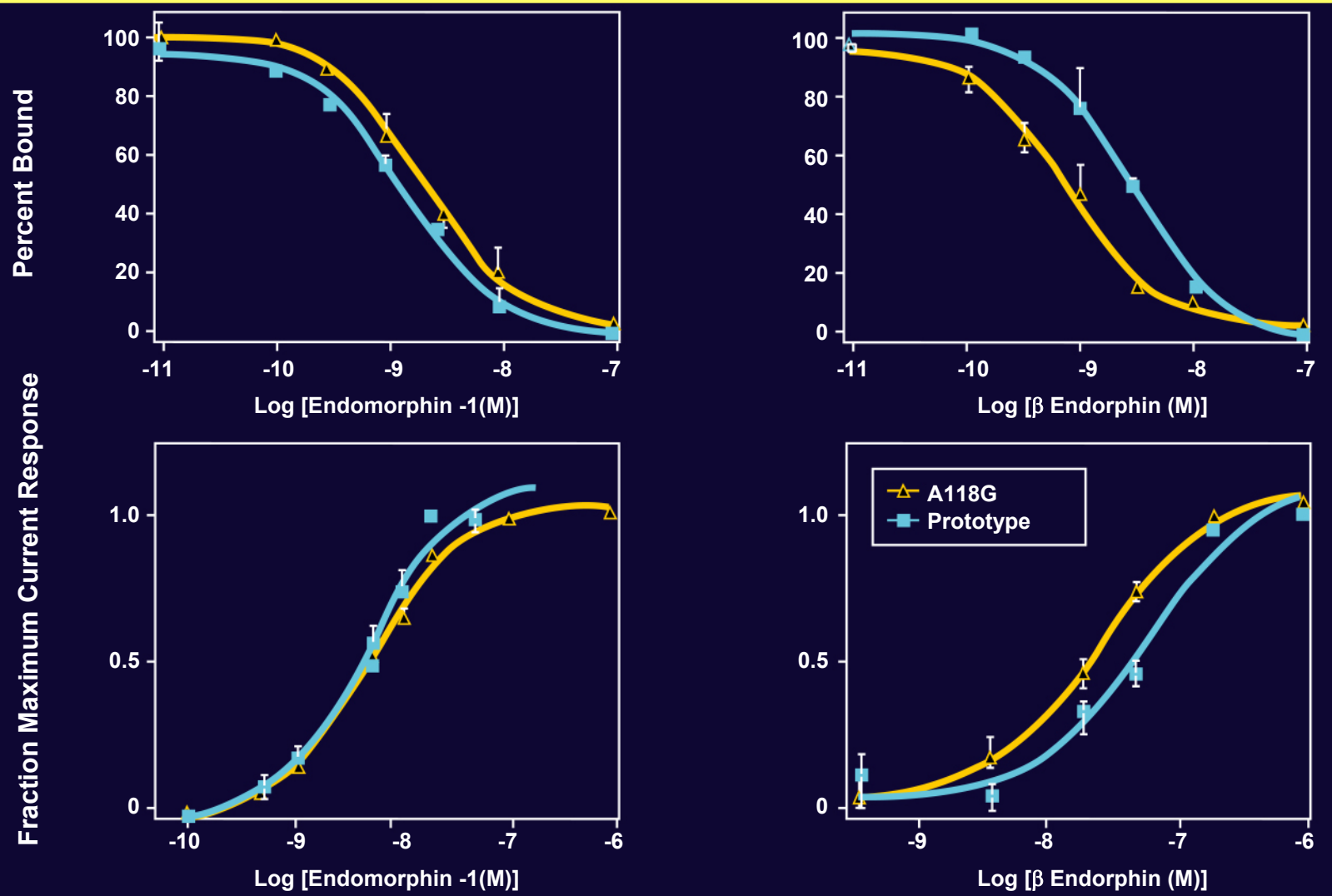
Bond, LaForge… Kreek, Yu,* PNAS, 95:9608, *1998

Human Mu Opioid Receptor: Location of Coding Region SNPs (SNPs Resulting in Amino Acids Changes or Synonymous Mutation)



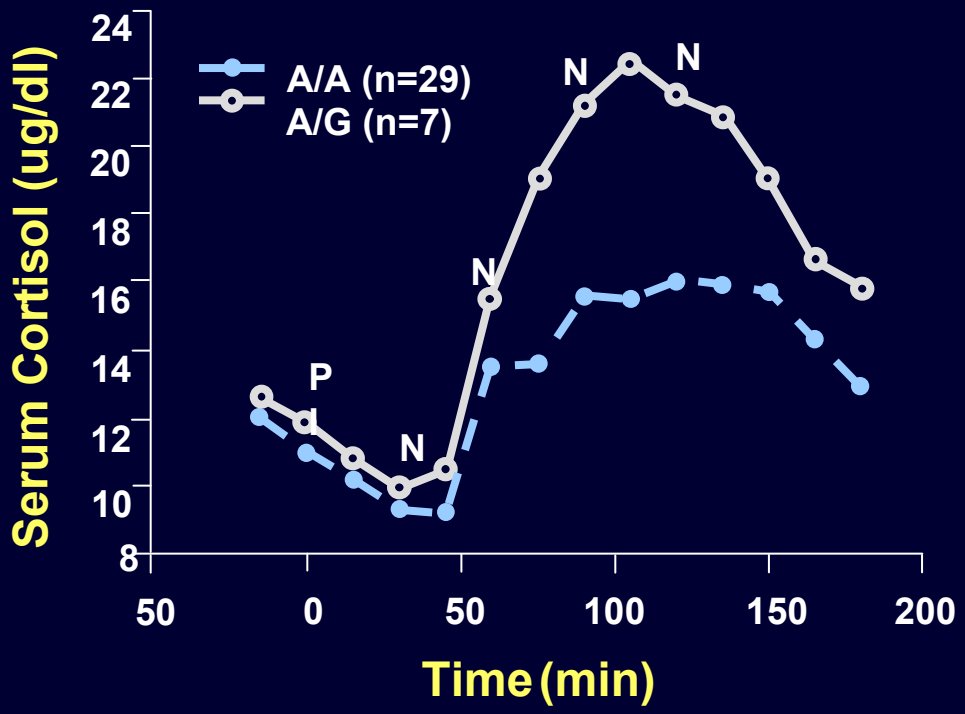
Kreek, LaForge, Yuferov, 2005

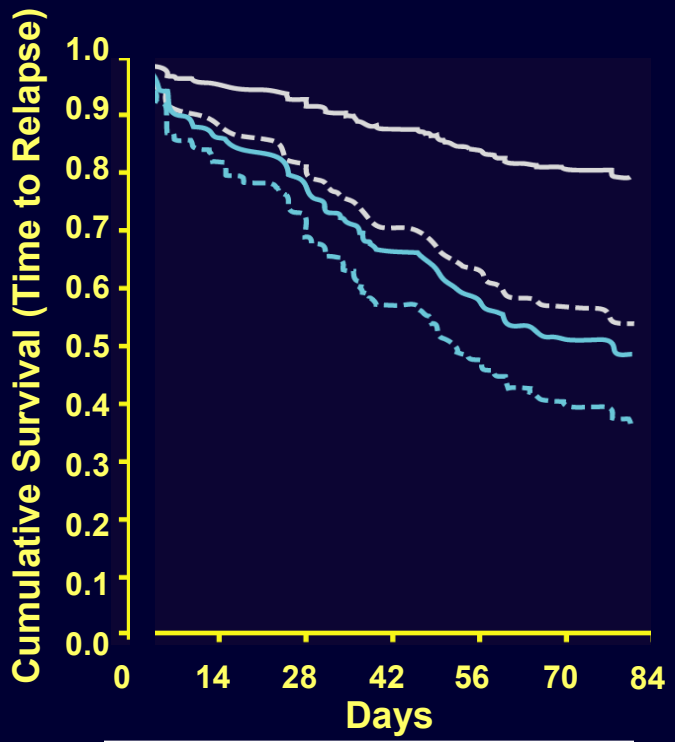
Binding and Coupling to G Protein-Activated, Inwardly Rectifying K^+(GIRK) Channels by Endogenous Opioid Peptides to the Prototype and A118G Variant Mu Opioid Receptor



Bond et al., Proc. Natl. Acad. Sci. USA, 95:9608-9613, 1998

Physiogenetics and Pharmacogenetics Related to A118G Variant of Human Mu Opioid Receptor Gene





Basal plasma levels of cortisol significantly higher in persons with the A118G variant.

Bart et al, 2006

Wand et al, 2002

Oslin et al., 2003

Association Between a Functional Polymorphism in the mu Opioid Receptor Gene and Opiate Addiction and also Alcoholism in Central Sweden

	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: 18%
(with confidence interval ranges from 8.0 to 28.0%)

Bart G , Heilig M, LaForge KS… Ott J, Kreek MJ, et al., Molecular Psychiatry, 9:547-549, 2004

	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

Hypothesis (1998-2000) and Findings (2002-2007) Concerning the Functional A118G Variant of the Mu Opioid Receptor

1) One or two copies of the functional A118G variant of the mu opioid receptor gene will result in differences in basal levels of the stress hormone, cortisol, and in stress responsivity, as objectively measured using a specific opioid antagonist (e.g., naloxone, naltrexone, nalmefene).

2) One or two copies of the functional A118G variant of the mu opioid receptor will predict a positive ("good") outcome to treatment of alcoholism with an opioid antagonist (since we hypothesized and have now shown that alcoholics seek and like modest activation of the stress-responsive hypothalamic-pituitary-adrenal axis).

3) Further, the A118G variant of the mu opioid receptor will be found to be associated with alcoholism and also opiate addiction – two addictive diseases which are characterized by disruption of HPA axis function and alter stress responsivity.

Bond et al, 1998; Kreek, 1999; LaForge, 2000; Kreek et al., Nature Neuroscience*, 2005; 2007*

LABORATORY OF THE BIOLOGY OF ADDICTIVE DISEASES, 2007
Mary Jeanne Kreek, MD – Professor and Head

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