The promises of opioids: Future and present

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Dedication

E. Leong Way

(1915-2017)

This talk is dedicated to Eddie Way

Eddie provided a foundation for opioid pharmacology

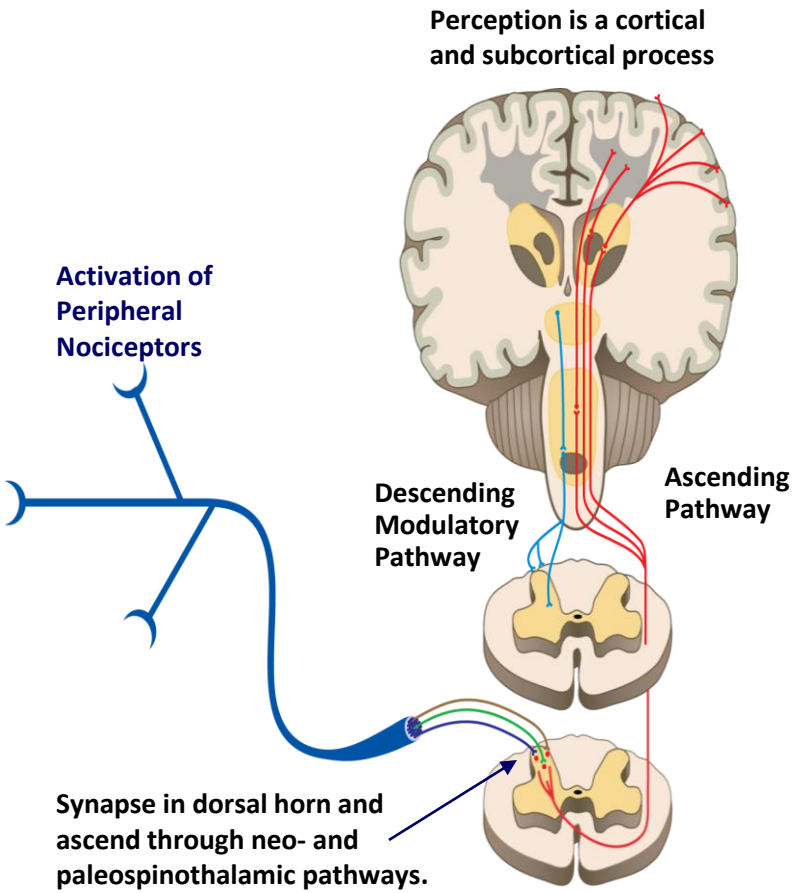
His work and insights have brought us to where we are today

Pain

"And only YOU can hear this whistle?"

The study of pain is difficult due to its subjective nature and the unpredictable contributions of genetics

Opioid analgesia



Opioid are active:

- Spinally
- Supraspinally
- Peripherally

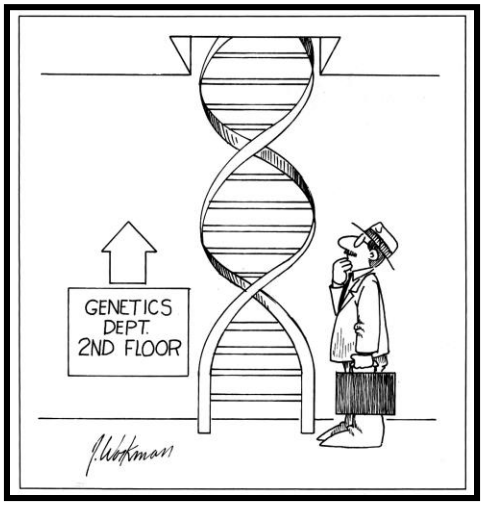
There is synergy among sites:

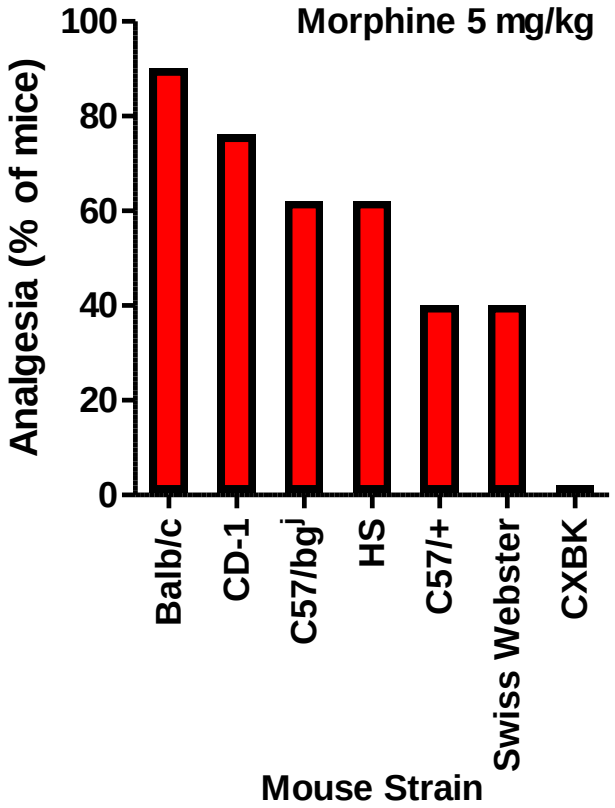
- Spinal/supraspinal
- Systemic/spinal

Complexity of opioid analgesia

Genetic backgrounds impact potency

Genetic backgrounds impact selectivity





Complexity of opioid analgesia

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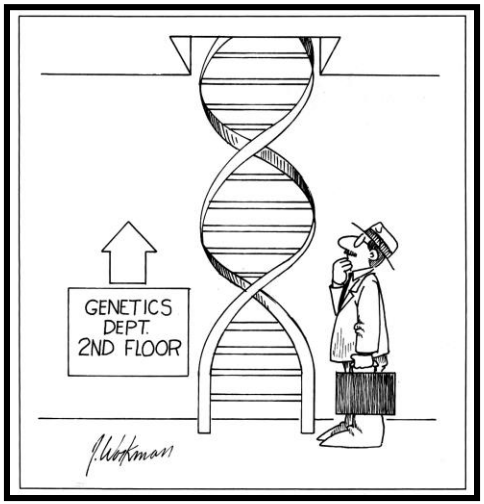
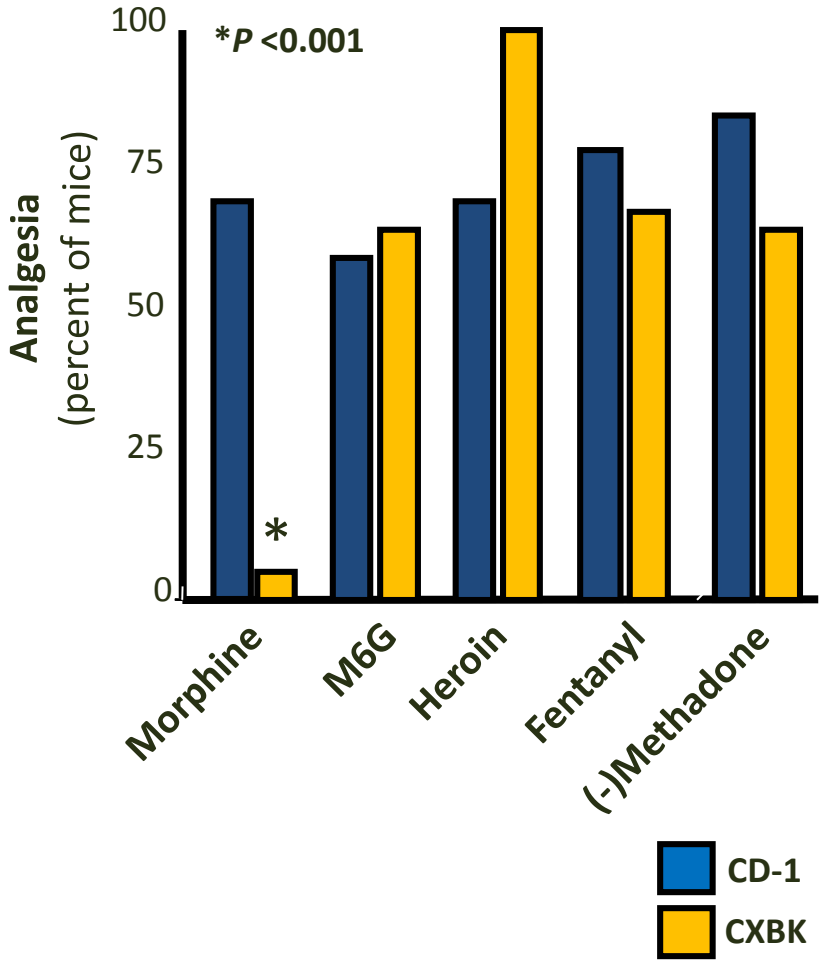




Why Mu Opioids Differ?

- Different pharmacokinetics
- Different metabolic profile
- Differential function activation of the receptor (**Biased Signaling**)
- Differential activation of subtypes of receptors

Defining Opioid Receptors

Opioid receptors were originally defined by ligands.

- 1965: 'Opioid' defined by sensitivity to naloxone

Subtypes of opioid receptors were defined by agonists:

- 1967: Receptor Dualism: M & N: morphine & nalorphine
- 1976: Mu and kappa: morphine and ketocyclazocine
- 1977: Delta: Enkephalins
- 1983: Kappa: U50,488H

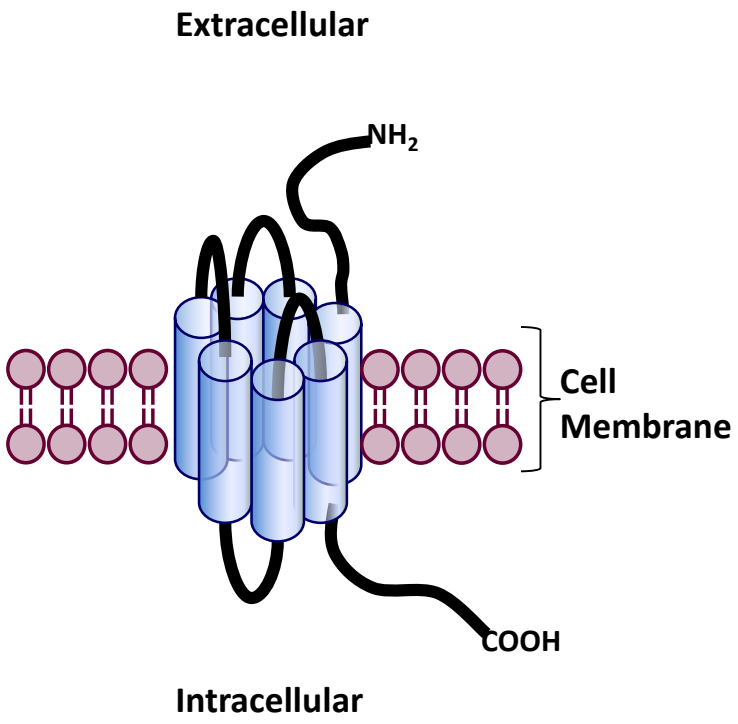
Subsequent studies utilized antagonists:

- 1980: Mu: β-Funaltrexamine (β-FNA)
- 1980: Mu subtypes: naloxazone/naloxonazine
- 1987: Kappa: norBinaltorphimine (norBNI)
- 1988: Delta: naltrindole

After the cloning of the mu, delta and kappa receptor genes, subtypes have been defined molecularly at the genetic level

G-Protein Coupled Receptors

- Traditional GPCR have 7 transmembrane (TM) domains
- Splicing, both 5’ and 3’, yields both 7TM and truncated forms
- Evidence now shows that these truncated forms may be important physicologically and pharmacologically
- GPCRs interact with G-proteins
  - Both α and βγ subunits are released by agonist and each has its own transduction pathways
- Agonists induce phosphorylation of the receptor by receptor kinases, leading to β–arrestin binding, which in turn induces a separate transduction cascade involving MAPK, Src and Akt
- Biased signaling results from the differences in the ability of an individual ligand to activate G-protein vs β–arrestin pathways



The future of opioids: Time to abandon pessimism

British Journal of Pharmacology (2006) 147, S153–S162

www.nature.com/bjp

75 years of opioid research: the exciting but vain quest for the Holy Grail

[1]Alistair D. Corbett, [2]Graeme Henderson, *Alexander T. McKnight & [3]Stewart J. Paterson

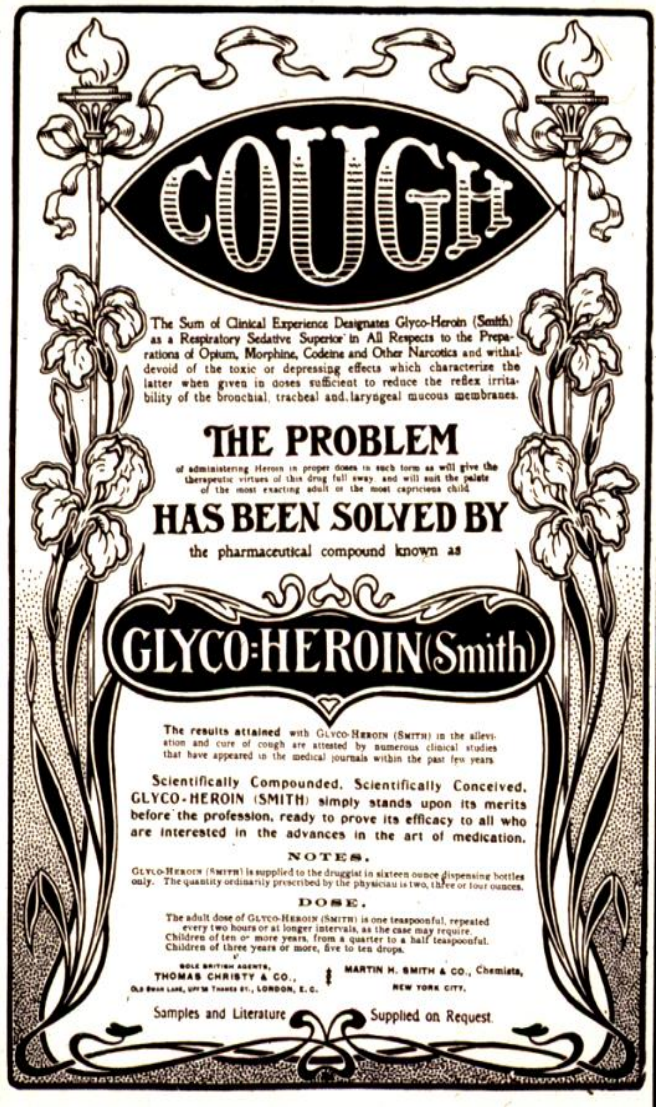
[1]Department of Biological & Biomedical Sciences, Glasgow Caledonian University, Glasgow G4 0BA; [2]Department of Pharmacology, University of Bristol, University Walk, Bristol BS8 1TD and [3]Kings College London, Department of Pharmacology and Therapeutics, GKT School of Biomedical & Health Sciences, Guy's Campus, London Bridge, SE1 1UL

Over the 75-year lifetime of the British Pharmacological Society there has been an enormous expansion in our understanding of how opioid drugs act on the nervous system, with much of this effort aimed at developing powerful analgesic drugs devoid of the side effects associated with morphine – the Holy Grail of opioid research. At the molecular and cellular level multiple opioid receptors have been cloned and characterised, their potential for oligomerisation determined, a large family of endogenous opioid agonists has been discovered, multiple second messengers identified and our understanding of the adaptive changes to prolonged exposure to opioid drugs (tolerance and physical dependence) enhanced. In addition, we now have greater understanding of the processes by which opioids produce the euphoria that gives rise to the intense craving for these drugs in opioid addicts. In this article, we review the historical pathway of opioid research that has led to our current state of knowledge.

British Journal of Pharmacology (2006) **147**, S153–S162. doi:10.1038/sj.bjp.0706435

Once dismissed as an impossibility, approaches are arising to develop mu opioids lacking many of the adverse effects of current agents, yielding safer and more efficacious compounds.

The Holy Grail of Opioid research



Heroin: "The non-addictive cure for the Soldiers Disease"
(morphine addiction)

The essence of opioid therapy is selectivity

Selectivity can be achieved by:

Route/site of administration

Topical, epidural/intrathecal

Peripherally restricted (agonist or antagonists)

Functional bias at the receptor

(Biased Signaling)

Allosteric modulation of transduction

(PAM)

Alternative receptor targets

(Receptor subtypes)

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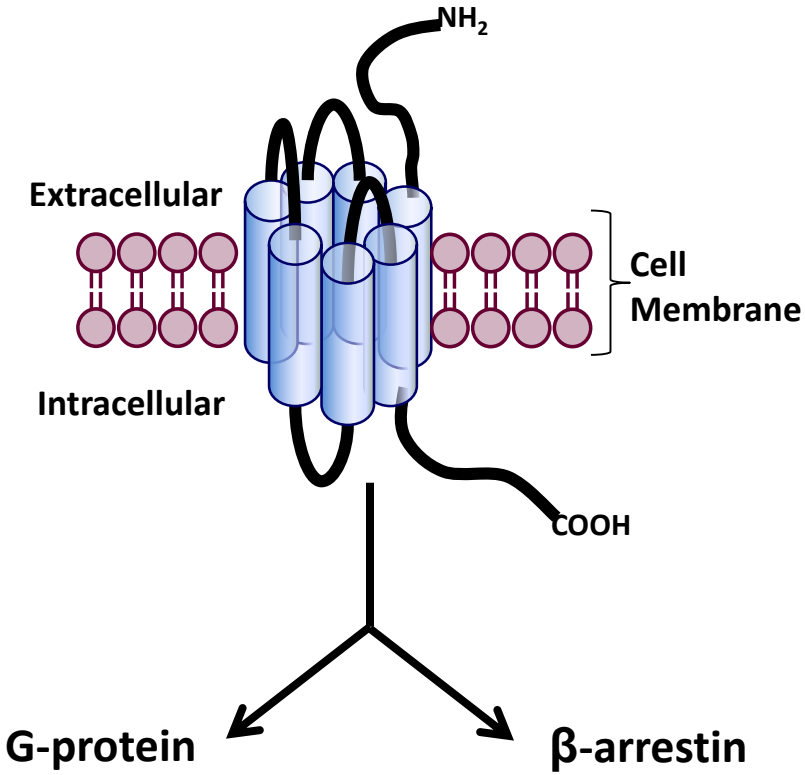
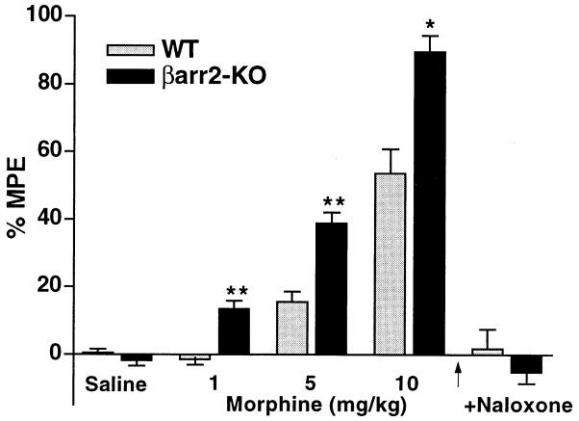
Biased Signaling



The ratio of G-protein/β-arrestin provides a measure of 'bias'

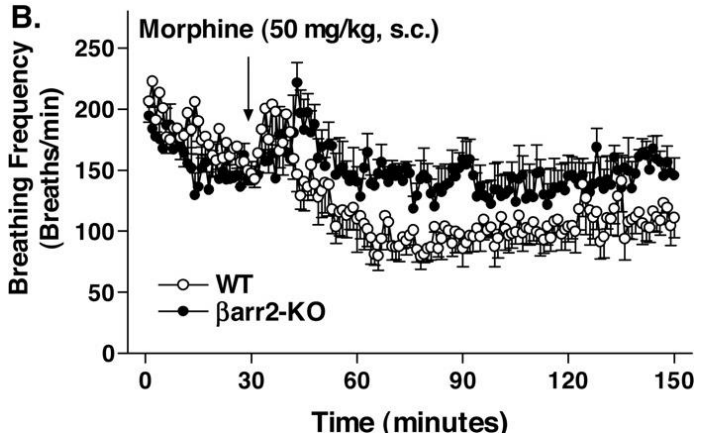
β-Arrestin2 knockout mice

Enhance morphine analgesia



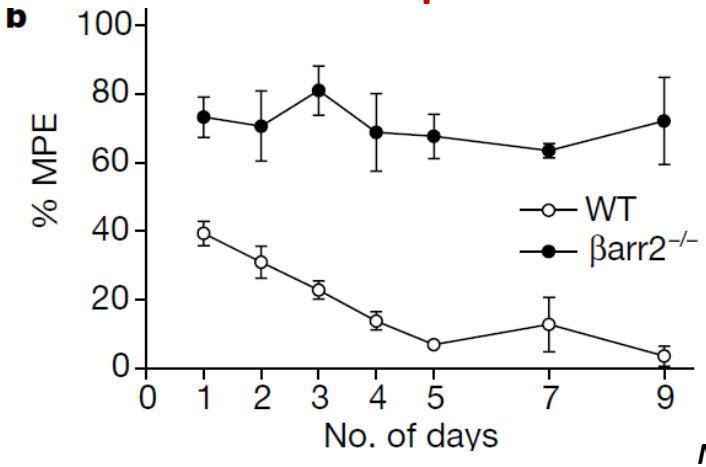
Science 286:2495, 1999

Diminish morphine respiratory depression



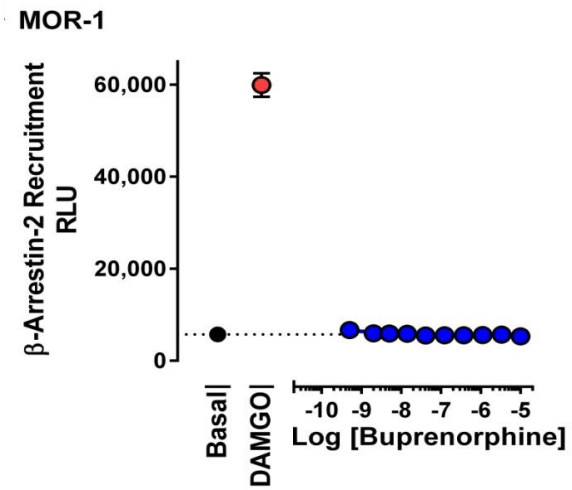
JPET 314:1195, 2005

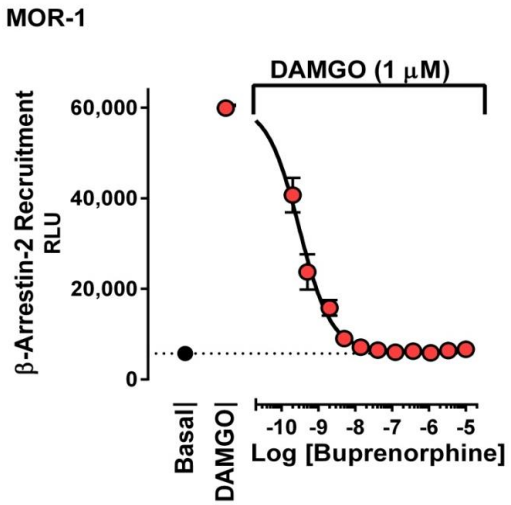
Diminish morphine tolerance

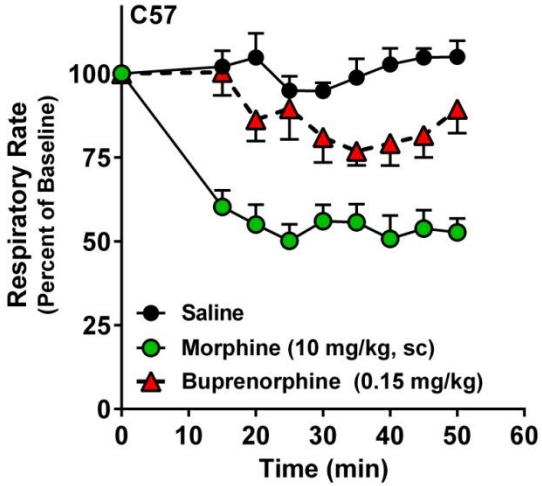


Nature 408:720, 2000

Buprenorphine and β–arrestin-2 recruitment

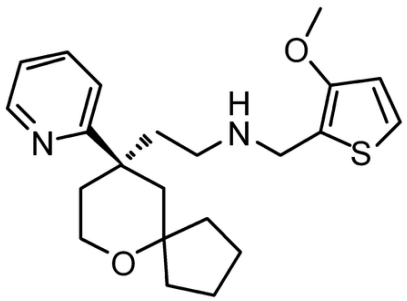


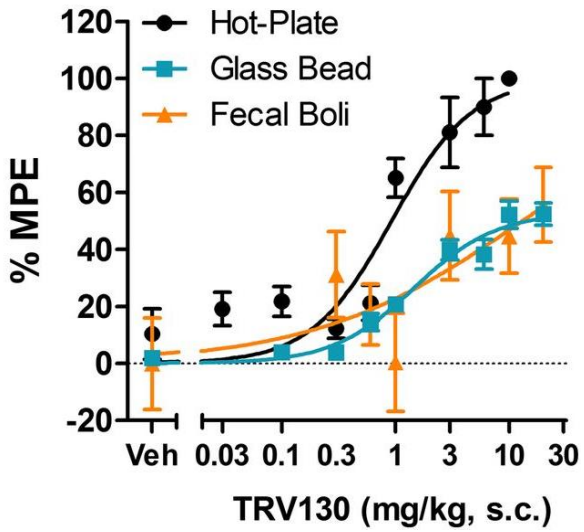


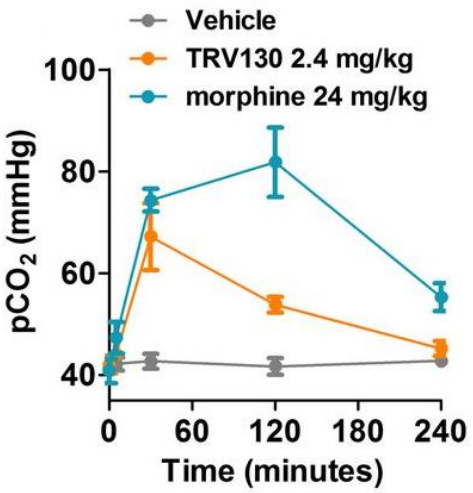


- Buprenorphine does not recruit β–arrestin2 and antagonizes the DAMGO recruitment
- At equianalgesic doses in mice, buprenorphine has less respiratory depression than morphine

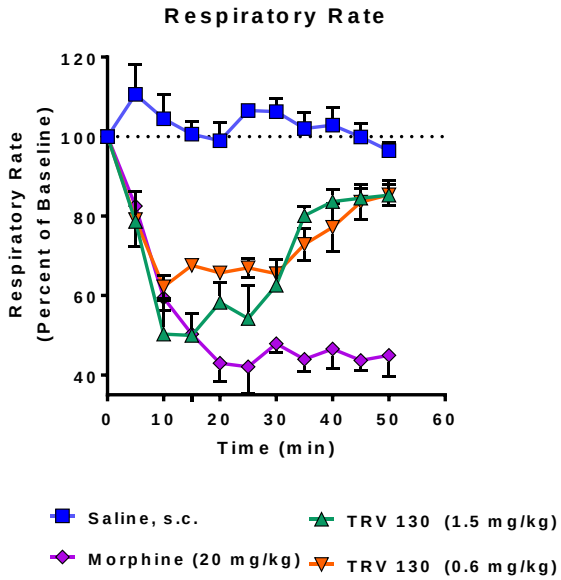
TRV130







JPET 344:708, 2013



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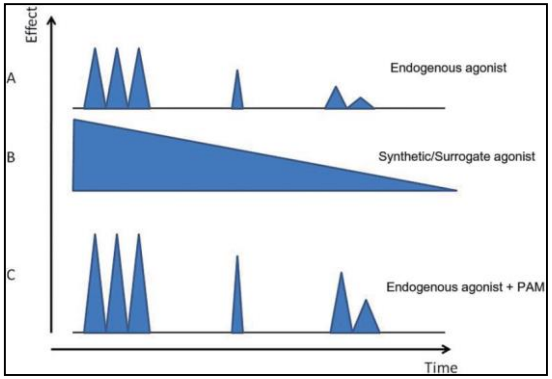
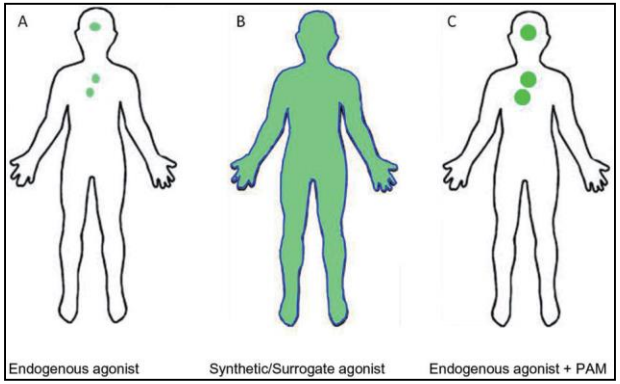
Alternative receptor targets
(Receptor subtypes)

Positive Allosteric Modulators

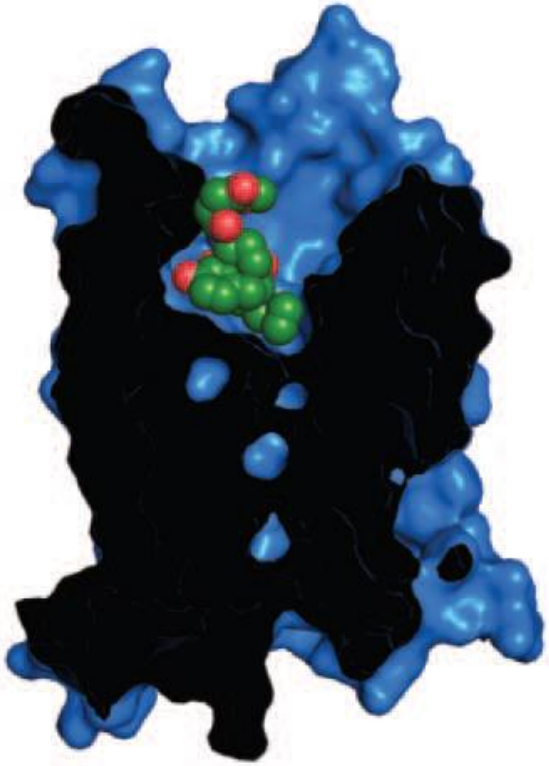
- No activity alone
- Potentiate the activity of orthosteric agonists
  - Enhance the actions of physiologically released endogenous ligand
    - Requires appropriate release of endogenous ligand
  - Advantage of use with exogenous agonists not clear



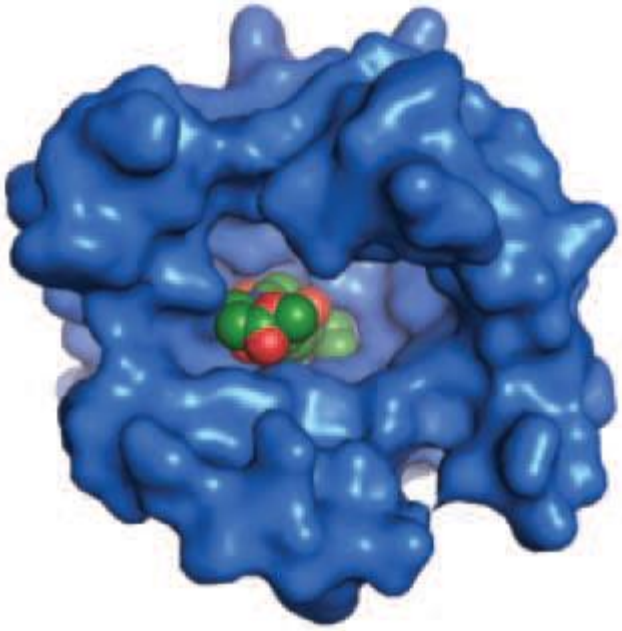


Burford et al. Br J Pharmacol 172:277, 2015

Crystal Structure of the mouse mu opioid receptor



Cross section



Top

Manglik et al, Nature, 2012
PMID: 22437502

20

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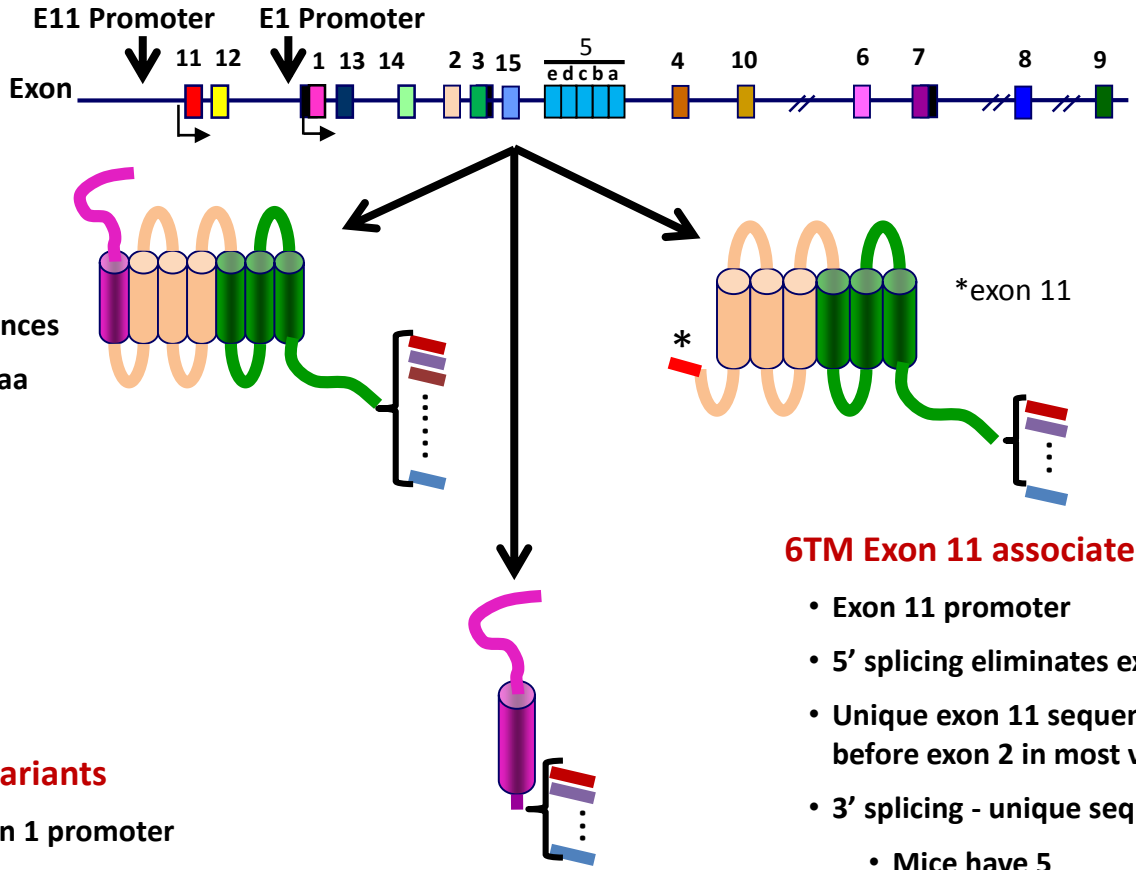
The scientific method

Splicing of the *Oprm1* gene



Full Length 7TM variants

- Exon 1 promoter
- 3’ splicing - unique sequences
- Lengths vary from 1 – 88 aa
  - Mice have 24
  - Humans have 12
  - Rats have 13

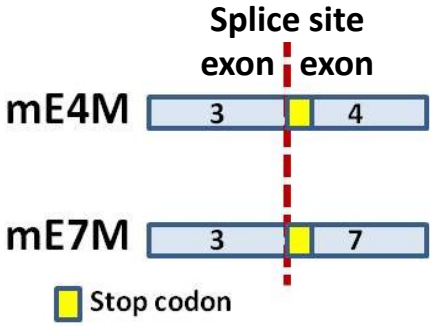
1TM variants

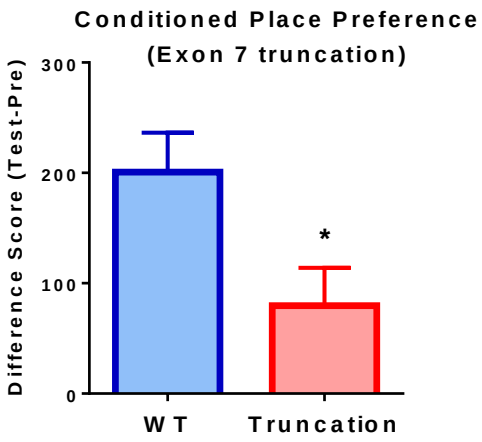
- Exon 1 promoter
- 3’ splicing - unique sequences
  - Mice have 5 (1-127 aa after exon 1)
  - Humans have 4 (1-90 aa after exon 1)
  - Rat have 2 (1 - 127 aa after exon 1)

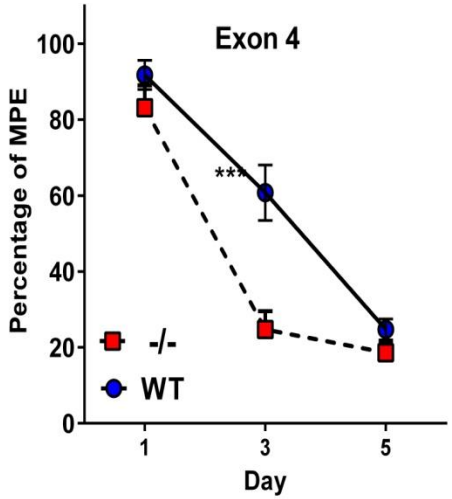
6TM Exon 11 associated variants

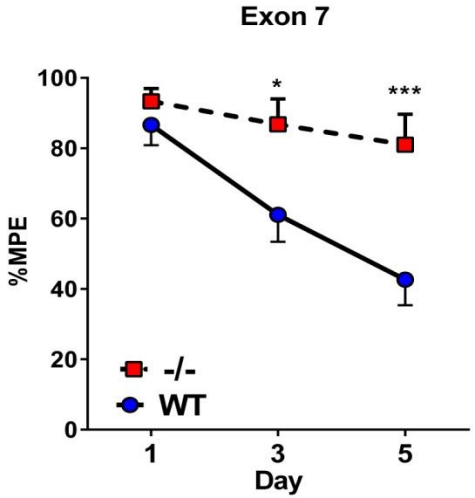
- Exon 11 promoter
- 5’ splicing eliminates exon 1
- Unique exon 11 sequence (~25 aa) before exon 2 in most variants
- 3’ splicing - unique sequences
  - Mice have 5
  - Humans have 3
  - Rats have 2

Functional Consequences of 3’ Splicing



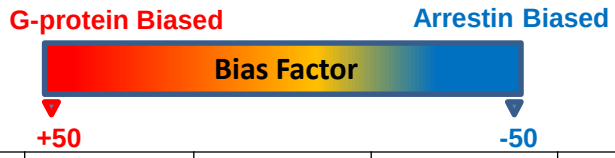






Exon 7 variants facilitate reward and tolerance
Exon 4 variants diminish tolerance

Influence of 7TM variant 3' splicing on biased signaling



Compares multiple drugs against a specific variant

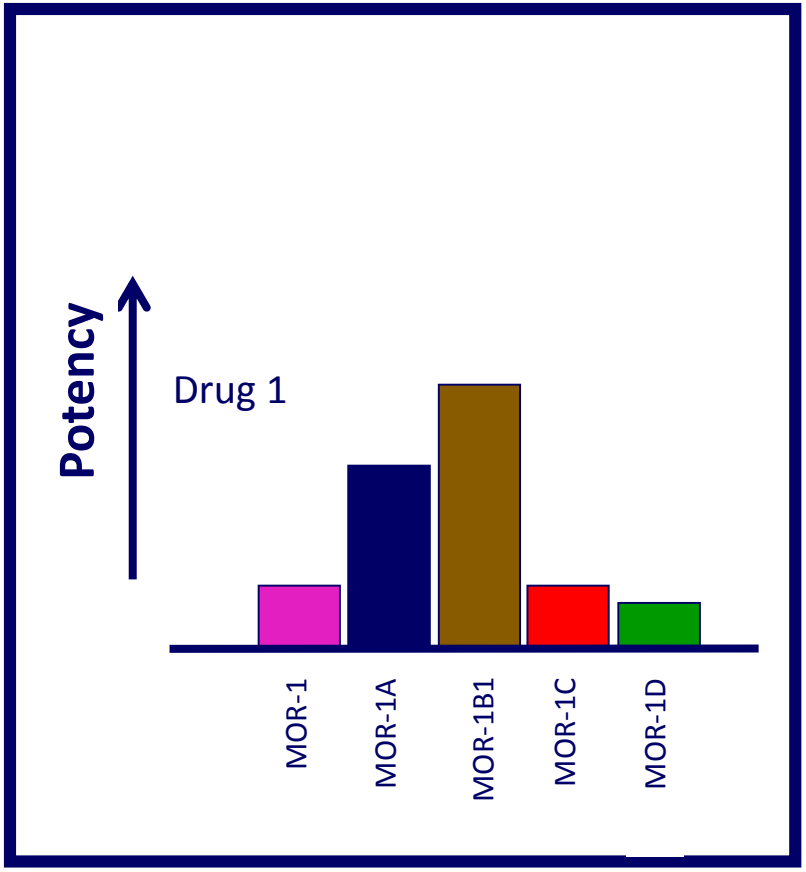
	MOR-1	MOR-1A	MOR-1B1	MOR-1E	MOR-1O
DAMGO	1.0	1.0	1.0	1.0	1.0
Morphine	-1.5	1.2	-1.2	-11.9	2.1
β-Endorphin	-2.1	1.0	1.4	1.1	1.1
Methadone	2.1	2.5	-1.9	1.8	1.7
Fentanyl	-4.4	-2.5	-6.0	-3.0	-4.3
Levorphanol	-2.6	1.6	-1.3	-1.3	95.9

Compares a specific drug against multiple variant

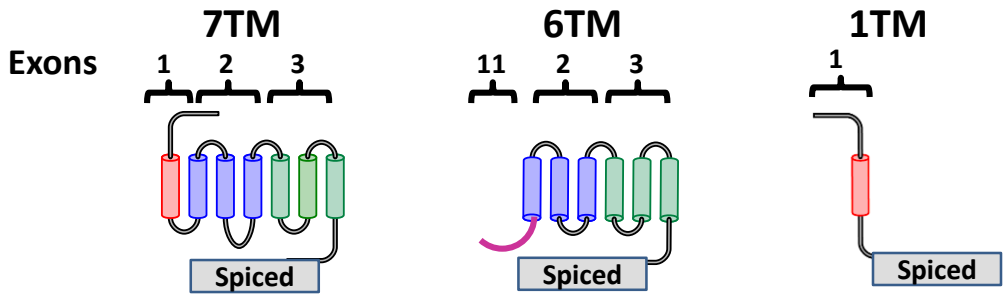
	MOR-1	MOR-1A	MOR-1B1	MOR-1E	MOR-1O
DAMGO	1.0	-1.9	-3.5	1.6	-10.2
Morphine	1.0	-1.1	-2.8	-5.0	-3.3
β-Endorphin	1.0	1.1	-1.1	3.7	-4.3
Methadone	1.0	-1.6	-13.4	1.4	-12.4
Fentanyl	1.0	-1.1	-4.7	2.3	-10.0
Levorphanol	1.0	2.1	-1.7	3.1	24.4

^{35}S-GTPγS binding and β-arrestin-2 bias was calculated for each drug and for each variant and normalized to DAMGO for each variant (top) or normalized to the specific drug and compared across the variants (bottom).

Mu opioid analgesia



Classifying mu opioid actions



Exon 1 KO (Pintar mice)	**Lost**	**Retained**	**Lost**
Exon 11	**Retained**	**Lost**	**Lost**
Exon 1/11	**Lost**	**Retained**	**Lost**

Knockout models of the mu opioid receptor can be used to genetically define the roles of different set of variants in a drugs activity

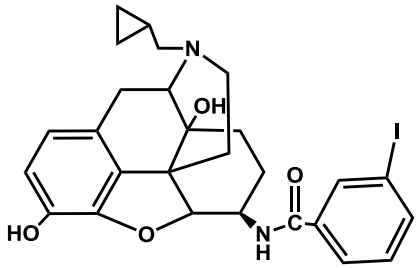
Sensitivity of mu opioids to loss of 6TM variants

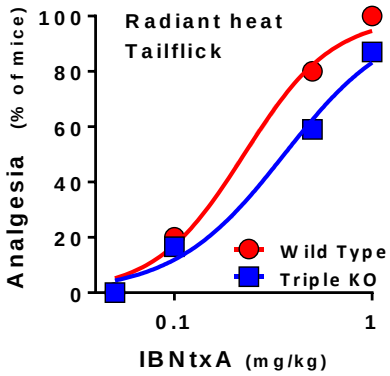
		ED_{50} (mg/kg, s.c.)		Shift
		WT	Exon 11 KO	
7TM	Morphine	1.6	2.6	1.6
7TM	Methadone	1.5	1.8	1.2
7TM + 6TM	Fentanyl	0.6	3.2	5
7TM + 6TM	Levorphanol	5	30	6
7TM + 6TM	Butorphanol	12.4	200	16
7TM + 6TM	Buprenorphine	0.2	>10	>50
6TM	IBNtxA	0.53	> 20	>35-fold

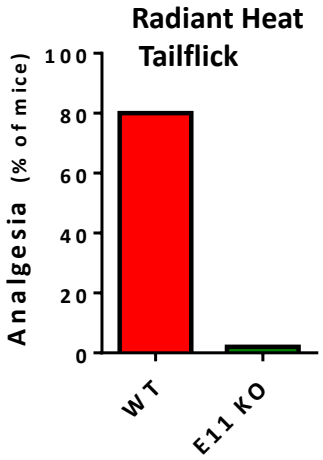
Knockout models indicate:

- Morphine and methadone analgesia are independent of 6TM
- IBNtxA analgesia is independent of 7TM
- Other drugs involve both 6TM and 7TM for analgesia

IBNtxA Analgesia







Majumdar al., PNAS 108: 19776, 2011

Assay	ED_{50} mg/kg
Tail-Withdrawal (49°C)	0.78
Hot-Plate (54°C)	0.53
Paw-Withdrawal (15%)	0.27
Formalin (late phase)	0.32
Complete Feund's Adjuvant (mechanical allodynia)	0.35
Spared Nerve Injury (mechanical allodynia)	0.35

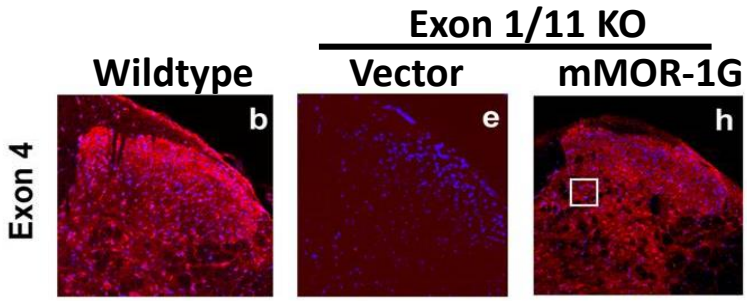
Weiskopf et al., Pain 155:2063, 2014

IBNtxA analgesia

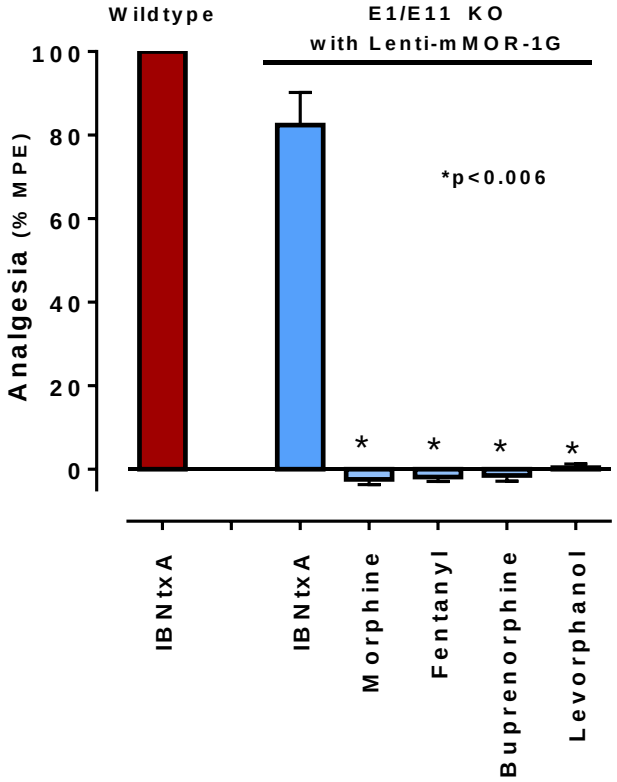
- Independent of traditional 7TM mu, delta and kappa receptors
- Totally dependent upon 6TM exon 11-associated variants
- Is more effective in neuropathic and inflammatory than thermal pain models

Rescue of IBNtxA analgesia

Dorsal Horn of Spinal Core



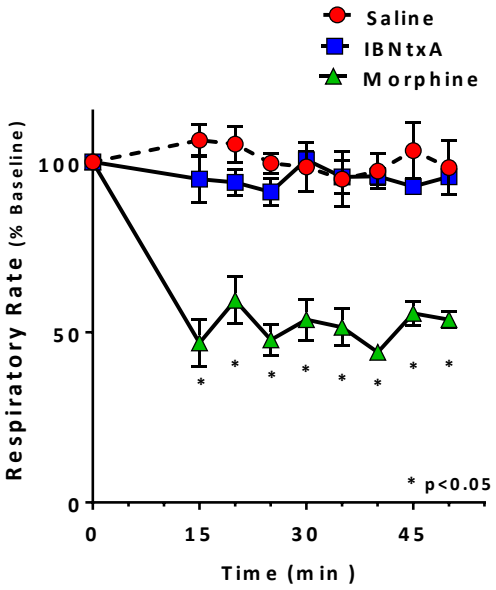
Lentivirus/mMOR-1G vector restores expression



Lentivirus/mMOR-1G vector restores only IBNtxA analgesia

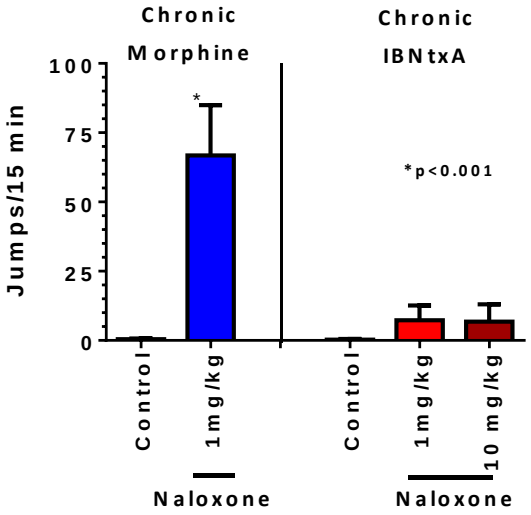
J Clin Invest 125:2626, 2015

IBNtxA (3-Iodobenzoyl-6β-naltrexamide)



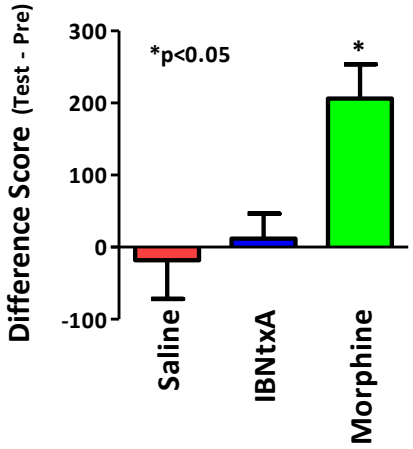
•No respiratory depression

Precipitated Withdrawal



•No physical dependence

Conditioned Place Preference

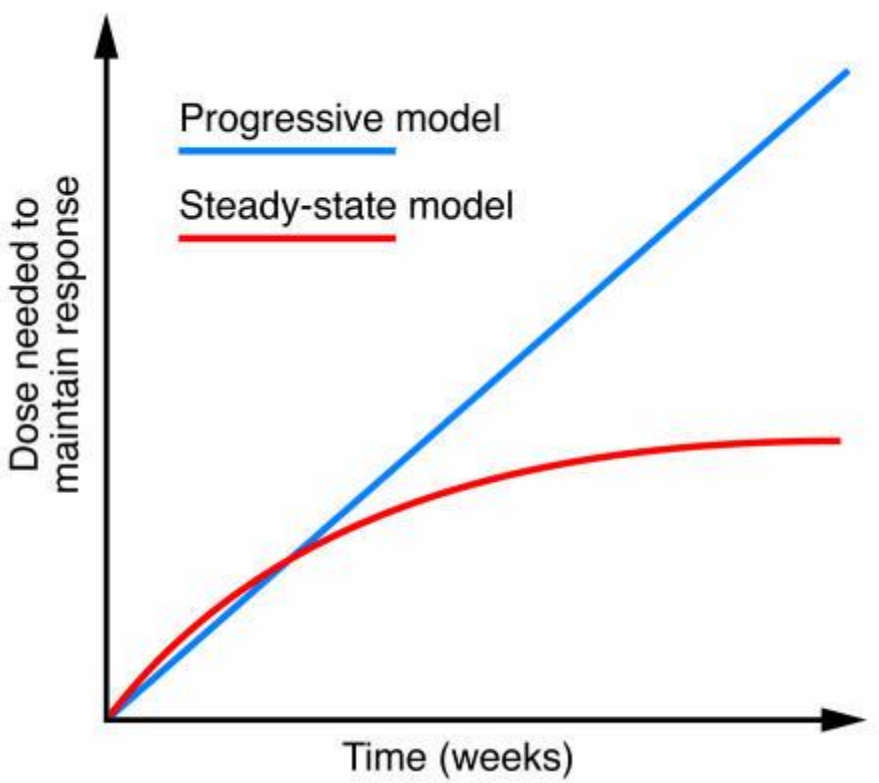


•No reward behavior

Pharmacological Profiles of 7TM and 6TM Compounds

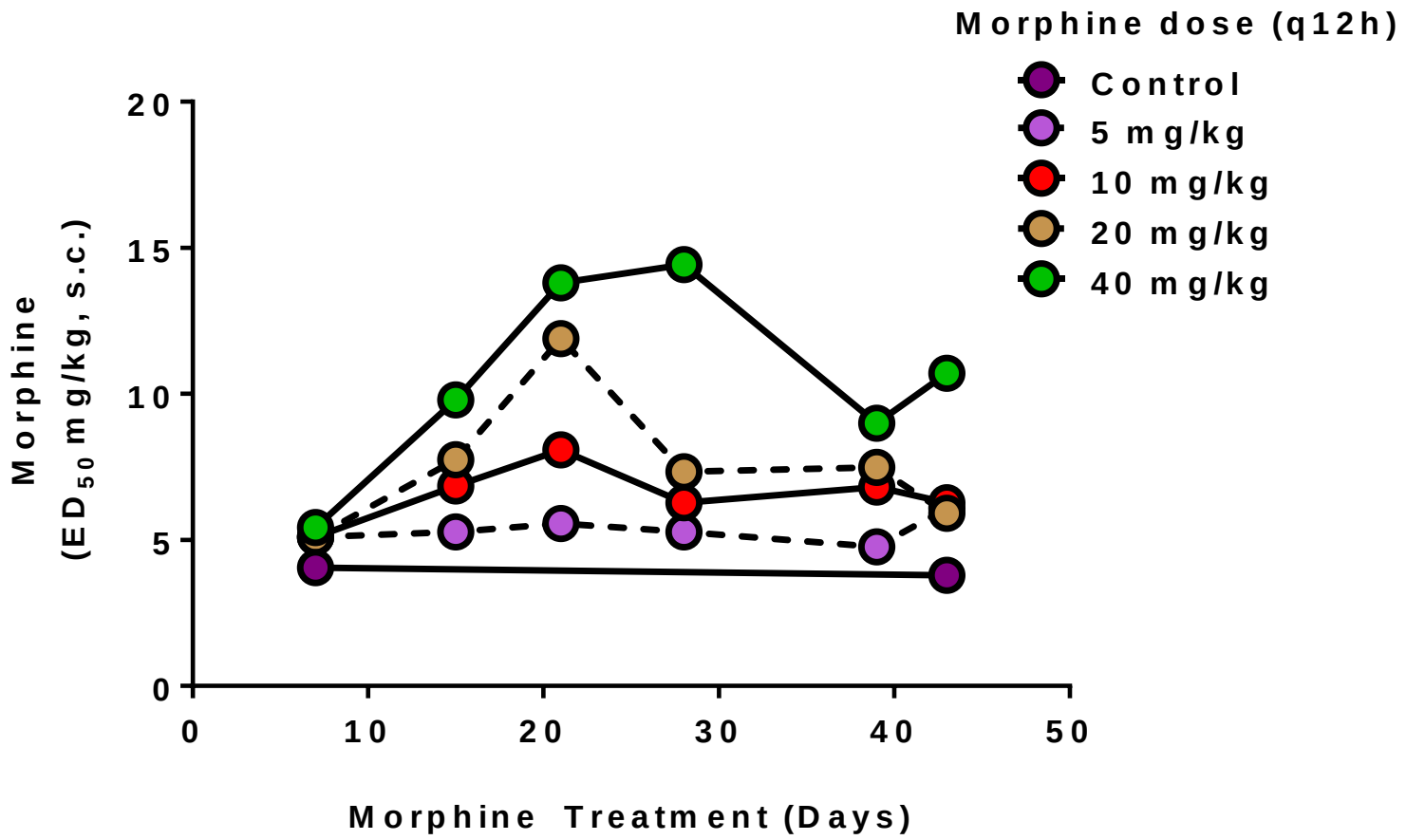
	Morphine (7TM)	IBNtxA (6TM)
Analgesia	++++	++++
Thermal	++++	++++
Inflammatory	++	++++
Neuropathic	+	++++
Respiratory depression	++++	-
Constipation	++++	+
Sedation	++++	+/-
Reward	++++	-
Physical Dependence	++++	-
Straub tail	++	-

Tolerance



A fixed dose of opioid is administered chronically

Prolonged Mophine Dosing



Opioid Tolerance

	Fold change in mRNA levels relative to saline							
Morphine (mg/kg)	Pre-Frontal cortex	Striatum	Thalamus	Hypoth	Hippo	PAG	BS	Sp Cord
	40	*5*	*40*	*40*	*40*	*40*	*40*	*40*
E1-2	7	18	23	52	13	5	5	4
MOR-1A	16	83	12	40	24	10	6	6
MOR-1B1	3	74	4	25	47	7	14	5
MOR-1B2	2	33	6	20	48	4	10	8
MOR-1B3	2	42	11	18	11	4	7	3
MOR-1B4	3	35	6	18	10	3	10	2
MOR-1C	6	45	25	58	11	8	7	3
MOR-1D	9	150	43	312	17	15	6	11
MOR-1H	1	52	12	33	22	5	31	4
MOR-1i	1	46	7	27	23	9	10	2
MOR-1J	5	56	6	34	20	10	14	5
MOR-1O	2	26	2	26	5	13	8	7
MOR-1P	4	26	7	20	49	4	11	8


THEORIES
PROOFS

Summary

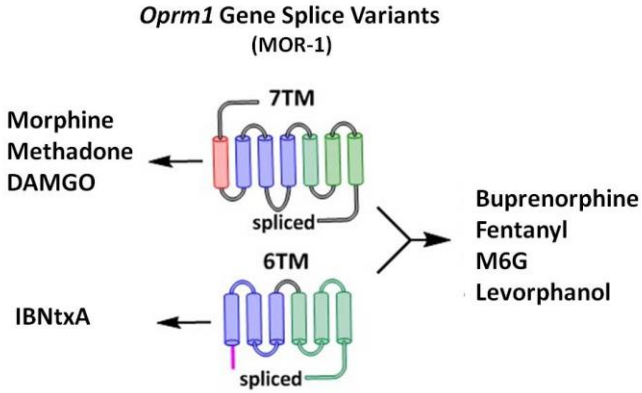
Cloning the opioid receptors has permitted the transition to a molecular classification of receptors and their subtypes

The mu opioid receptor gene *Oprm1* undergoes extensive splicing to generate three major classes of variants:

- **Full length 7TM variants**
- **Truncated 6TM variants**
- **Truncated 1TM variants**

Mu opioids can be classified into three categories:

- **Dependent upon E1, but not E11 variants**
  Morphine, Methadone
- **Dependent upon E11, but not E1 variants**
  IBNtxA
- **Dependent upon both E1 and E11 variants**
  Buprenorphine, fentanyl, M6G , levorphanol



Laboratory of Molecular Neuropharmacology

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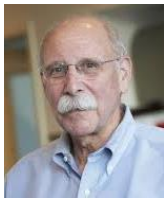

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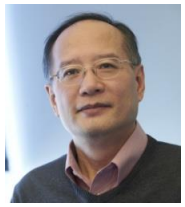

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