Opioids and the future of pain therapy: Hope is on the horizon

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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

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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 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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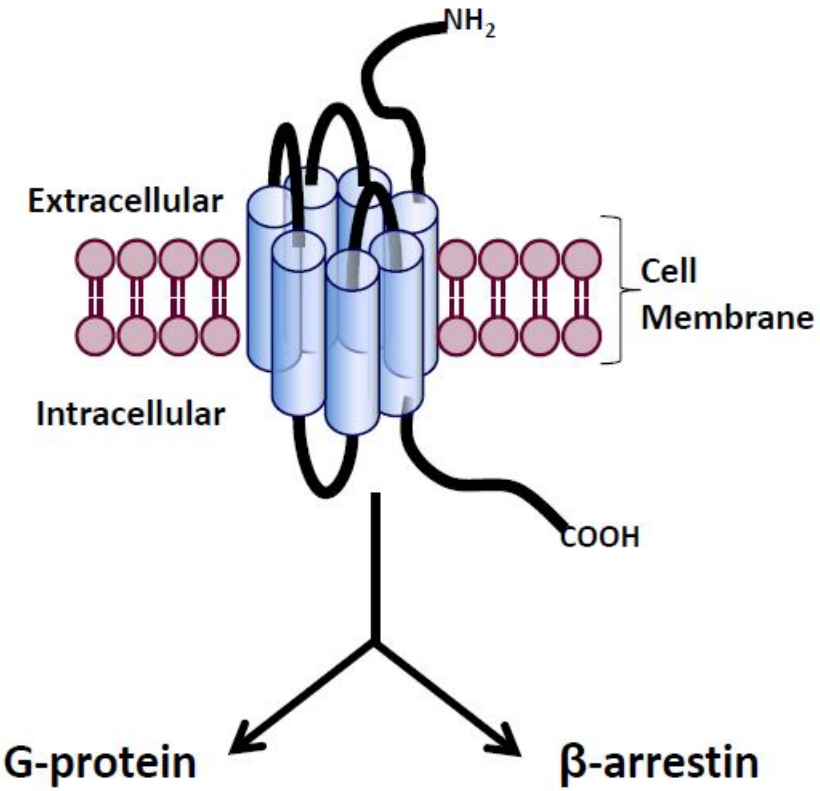
Biased Signaling

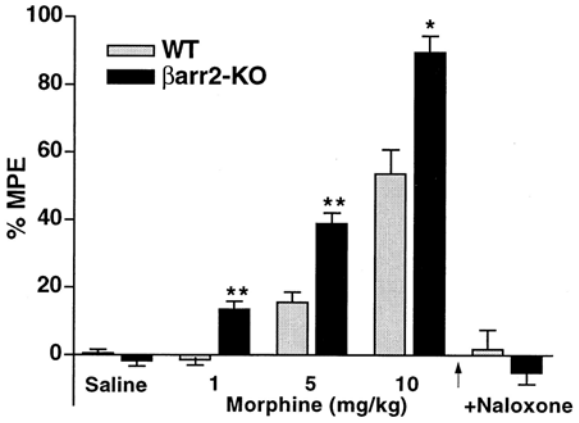
- Opioid receptors are members of the GPCR family
- Humans have approximately 1000 different GPCR genes (~50% taste/smell)
- 40% of all drugs target GPCR's
- Full length variants have the traditional 7 transmembrane (TM) domains
- Interact with G-proteins and with β–arrestin



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

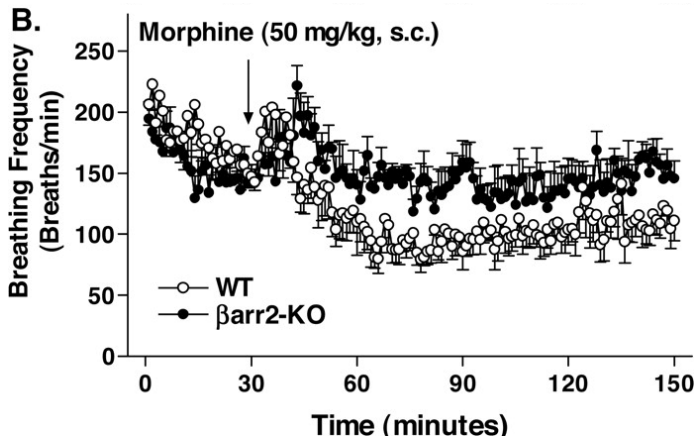
β-Arrestin2 knockout mice

Enhance morphine analgesia



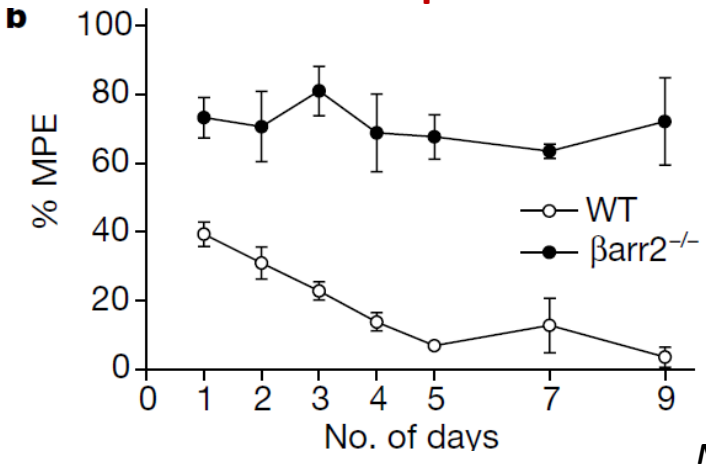
Science 286:2495, 1999

Diminish morphine respiratory depression



JPET 314:1195, 2005

Diminish morphine tolerance



Nature 408:720, 2000

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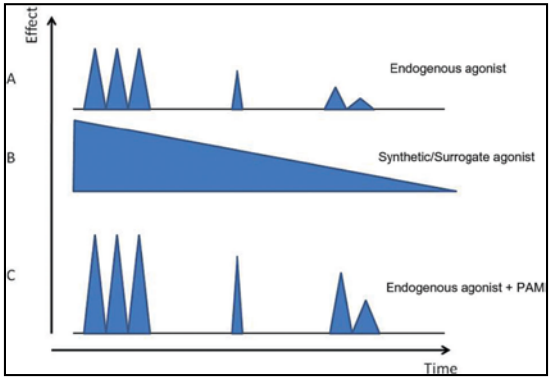
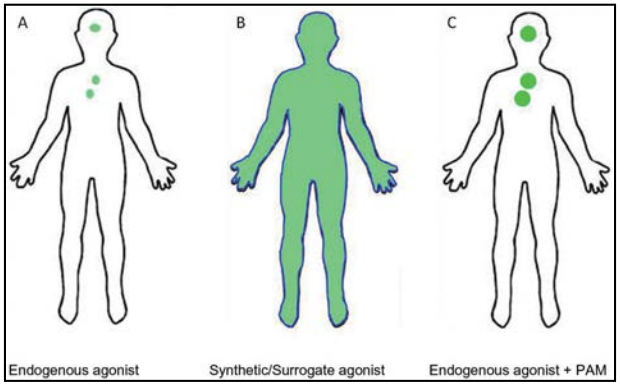
Positive Allosteric Modulators

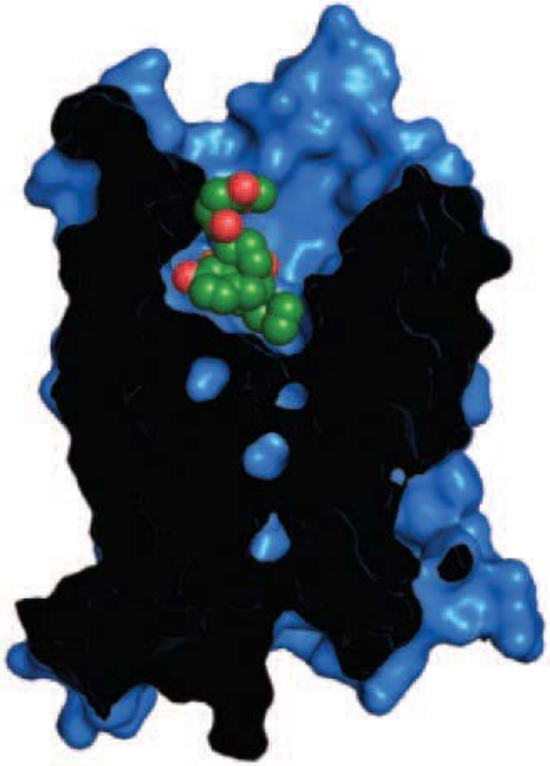
- No activity alone
- Potentiate the activity of orthosteric agonists
  - Enhance the actions of physiologically released endogenous ligand
    - Requires appropriate release of endogenous ligand
  - Advantage of use with exogenous agonists not clear



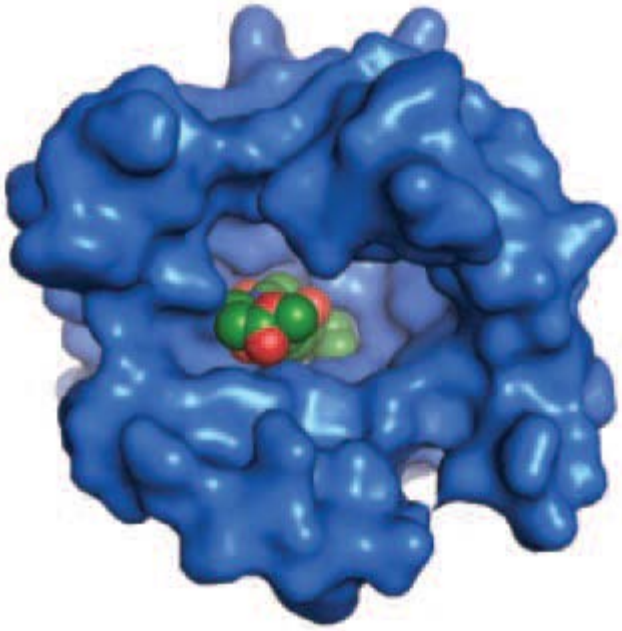


Burford et al. Br J Pharmacol 172:277, 2015

Crystal Structure of the mouse mu opioid receptor



Cross section



Top

Manglik et al, Nature, 2012
PMID: 22437502

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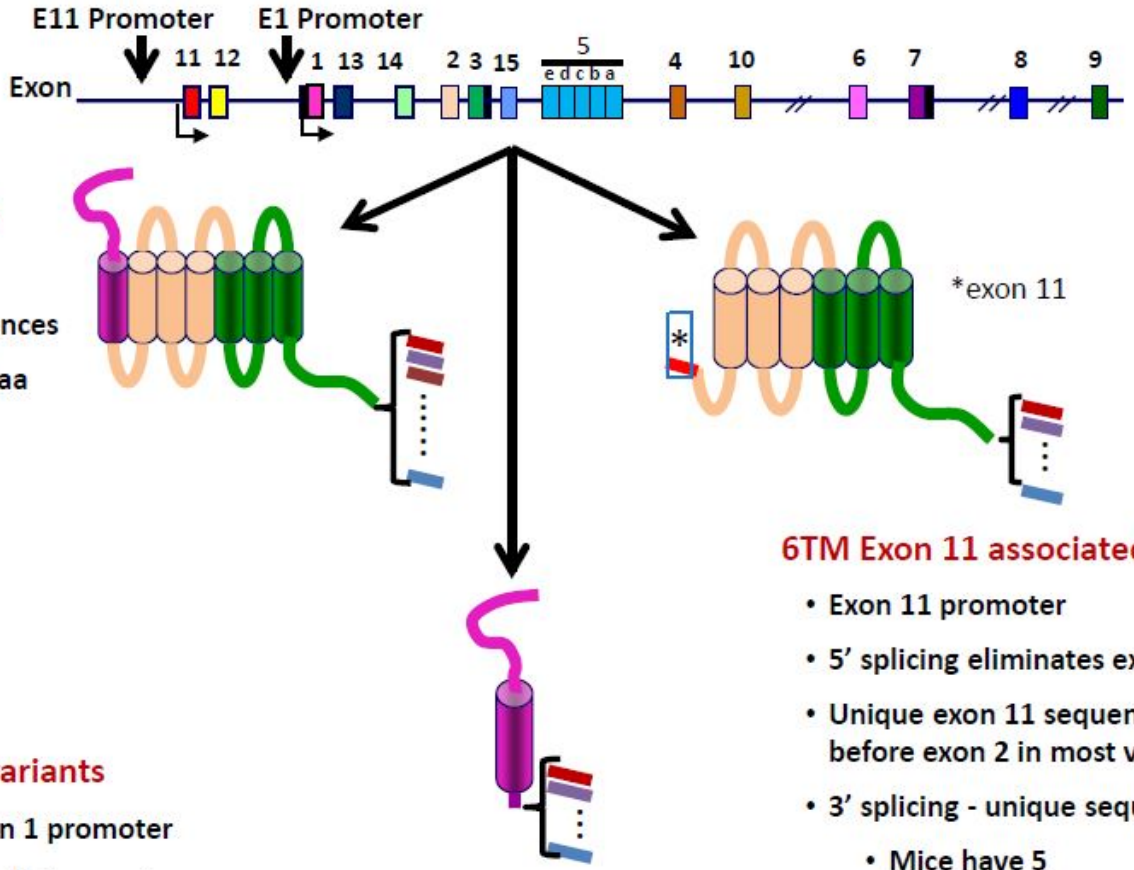
Splicing of the *Oprm1* gene



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

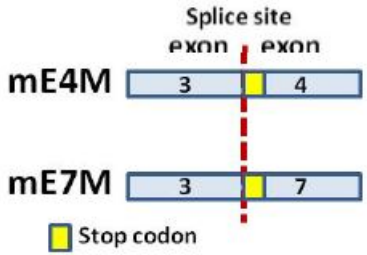
6TM Exon 11 associated variants

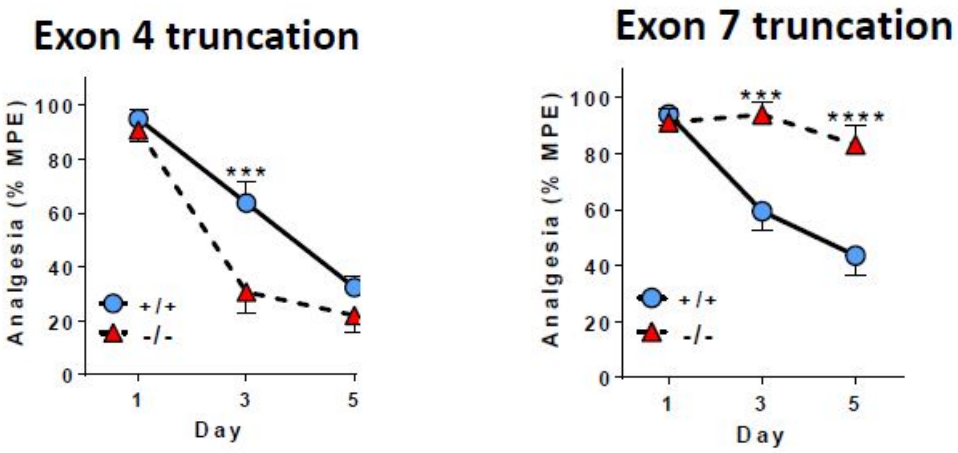
- Exon 11 promoter
- 5’ splicing eliminates exon 1
- Unique exon 11 sequence (~25 aa) before exon 2 in most variants
- 3’ splicing - unique sequences
  - Mice have 5
  - Humans have 3
  - Rats have 2

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)

Role of C-terminus on morphine tolerance in B6 mice





Exon 7 variants facilitate morphine tolerance

Exon 4 variants lower morphine tolerance

J Clin Inv 127:1561, 2017

Influence of 7TM variant 3' splicing on biased signaling

Compares multiple drugs against a specific variant

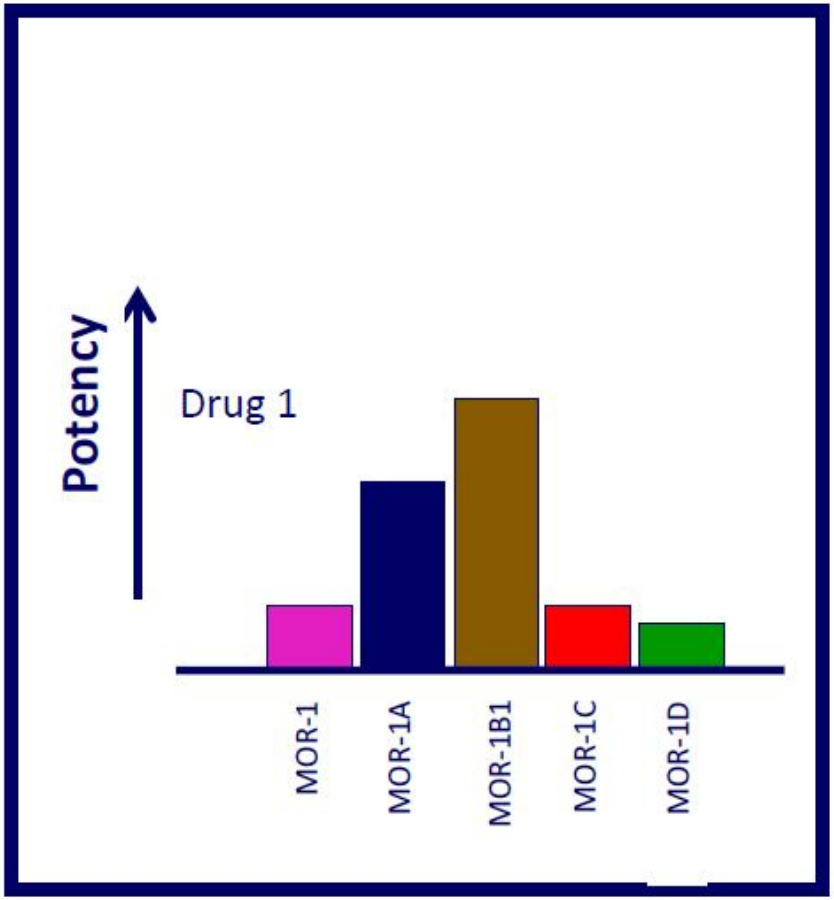
	MOR-1	MOR-1A	MOR-1B1	MOR-1E	MOR-1O
DAMGO	1.0	1.0	1.0	1.0	1.0
Morphine	-1.5	1.2	-1.2	-11.9	2.1
β-Endorphin	-2.1	1.0	1.4	1.1	1.1
Methadone	2.1	2.5	-1.9	1.8	1.7
Fentanyl	-4.4	-2.5	-6.0	-3.0	-4.3
Levorphanol	-2.6	1.6	-1.3	-1.3	95.9

Compares a specific drug against multiple variant

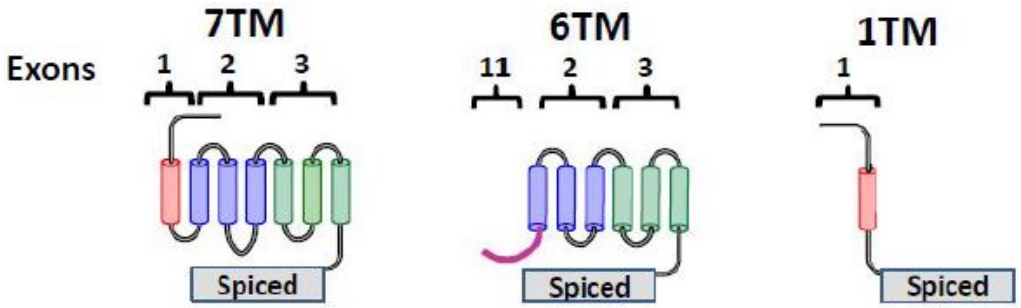
	MOR-1	MOR-1A	MOR-1B1	MOR-1E	MOR-1O
DAMGO	1.0	-1.9	-3.5	1.6	-10.2
Morphine	1.0	-1.1	-2.8	-5.0	-3.3
β-Endorphin	1.0	1.1	-1.1	3.7	-4.3
Methadone	1.0	-1.6	-13.4	1.4	-12.4
Fentanyl	1.0	-1.1	-4.7	2.3	-10.0
Levorphanol	1.0	2.1	-1.7	3.1	24.4

^{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

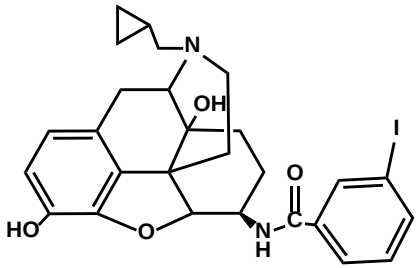
Sensitivity of mu opioids to loss of 6TM variants

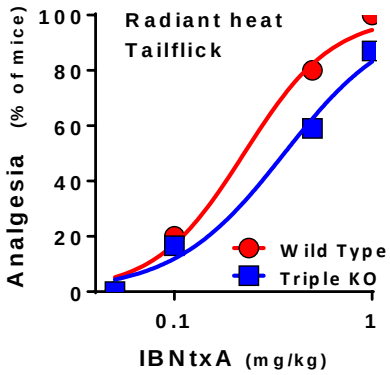
		ED_{50} (mg/kg, s.c.)		Shift
		WT	Exon 11 KO	
7TM	Morphine	1.6	2.6	1.6
7TM	Methadone	1.5	1.8	1.2
7TM + 6TM	Fentanyl	0.6	3.2	5
7TM + 6TM	Levorphanol	5	30	6
7TM + 6TM	Butorphanol	12.4	200	16
7TM + 6TM	Buprenorphine	0.2	>10	>50
6TM	IBNtxA	0.53	> 20	>35-fold

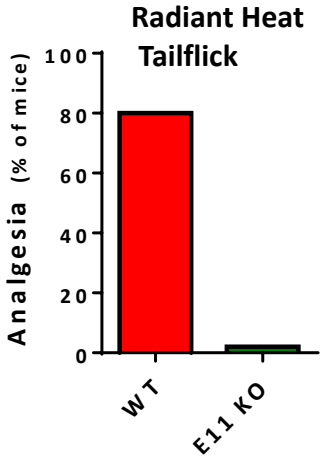
Knockout models indicate:

- 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

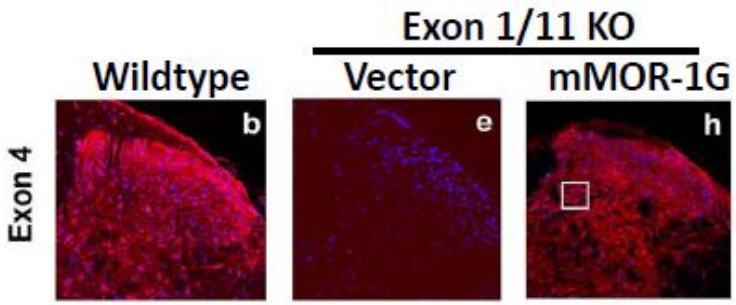
Assay	ED_{50} mg/kg
Tail-Withdrawal (49°C)	0.78
Hot-Plate (54°C)	0.53
Paw-Withdrawal (15%)	0.27
Formalin (late phase)	0.32
Complete Feund's Adjuvant (mechanical allodynia)	0.35
Spared Nerve Injury (mechanical allodynia)	0.35

Weiskopf et al., Pain 155:2063, 2014

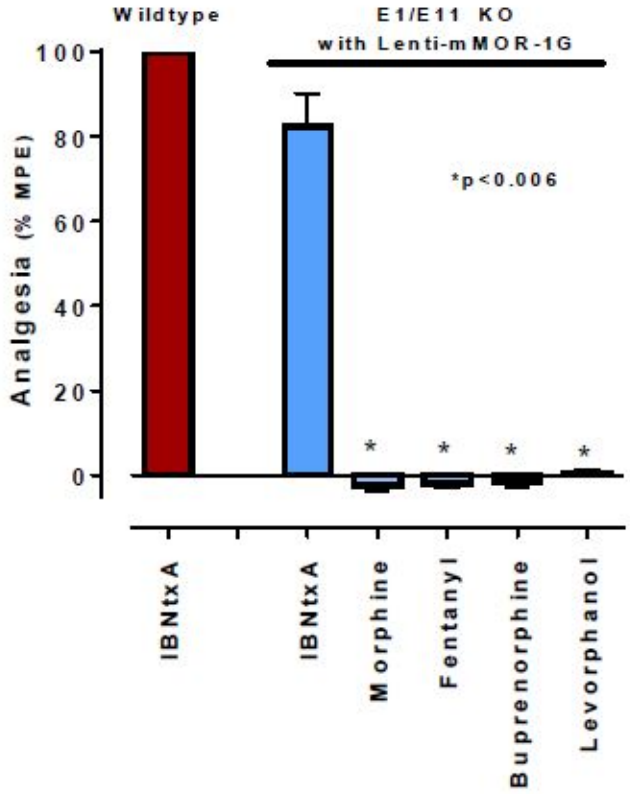
IBNtxA analgesia

- 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

Dorsal Horn of Spinal Core



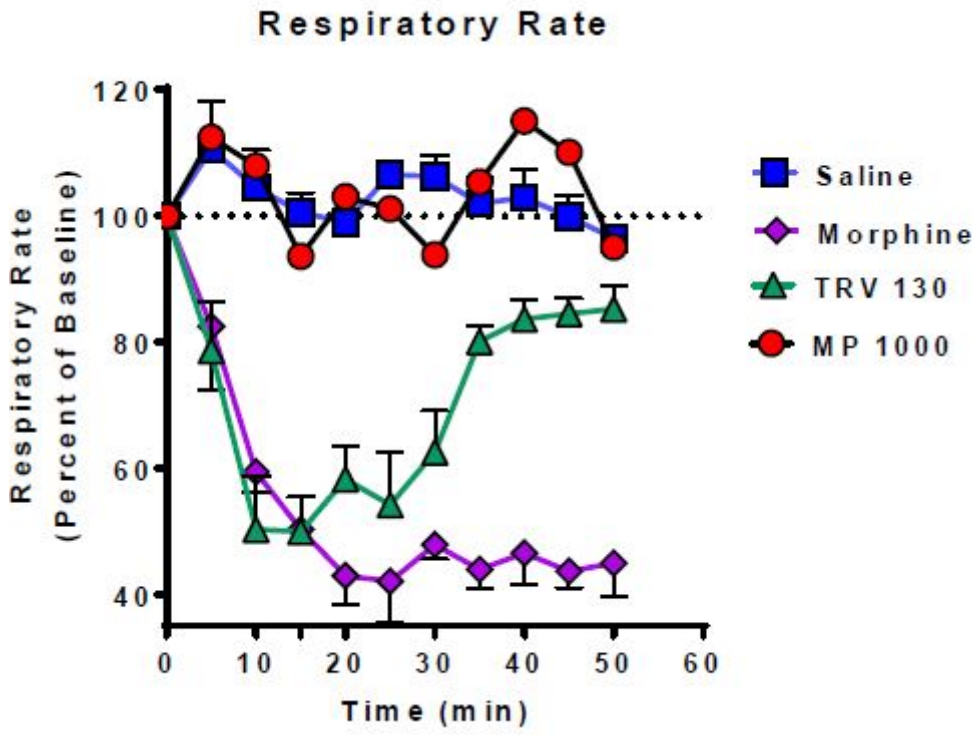
Lentivirus/mMOR-1G vector restores expression



Lentivirus/mMOR-1G vector restores only IBNtxA analgesia

J Clin Invest 125:2626, 2015

IBNtxA Effects on Respiratory Rate



Pharmacological Profiles of 7TM and 6TM Compounds

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

Summary

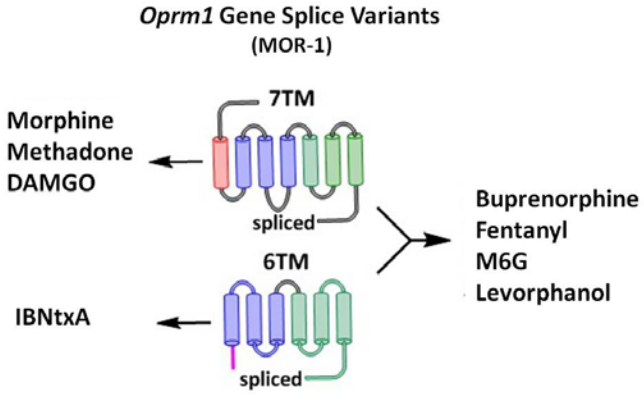
Cloning the opioid receptors has permitted the transition to a molecular classification of receptors and their subtypes

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**

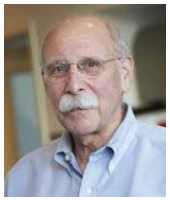
Mu opioids can be classified into three categories:

- **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

Gavril Pasternak, MD PhD



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