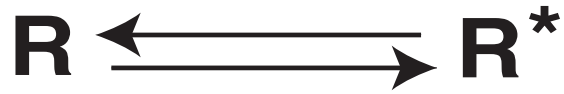
Receptor pharmacology in neuroscience practice
Lecture 2: things that confound our newfound knowledge
Of pharmacology…..

Neuroscience 201A, October 23rd, 2015



A ligand cannot have an effect unless it occupies the receptor

- **agonist**
- **antagonist**
- **inverse agonist**

The degree of occupancy is determined by affinity (and PK in vivo)

Once it has occupied a receptor, the magnitude of the effect is determined by efficacy

The potency of a ligand is therefore a function of both affinity and efficacy

- **High affinity, low efficacy**
- **Low affinity, high efficacy**

= Where potency doesn't match affinity

With receptor classes with more than one member one also must be cognizant of specificity, especially when more than one member of the class is in your system

So what can confound our new knowledge of pharmacology?

1. Allosteric modulators

2. Receptor oligomerization

3. Receptor desensitization/downregulation

4. Biased agonism

1. Allosteric modulators: definition

A ligand that binds to a site other than that for the endogenous ligand

There are two kinds of allosteric modulators

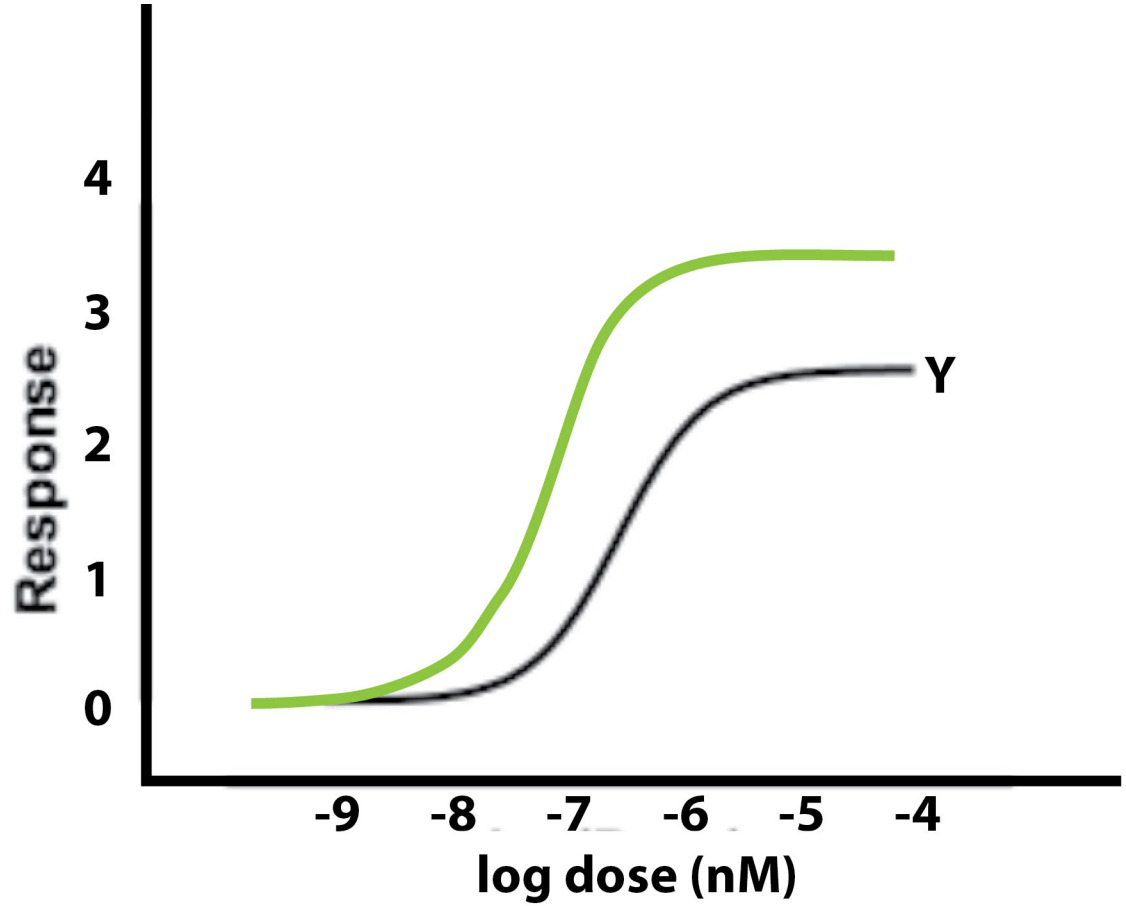
A) positive modulators enhance the effects of agonists
e.g. benzodiazepines and ethanol at the GABA-A receptor

B) negative modulators inhibit the effects of agonists
e.g. strychnine at the Glycine receptor

There are two distinct ways in which an allosteric modulator can change the effects of an agonist

1. By changing affinity of the agonist

2. By changing the efficacy of the agonist



So what can confound our new knowledge of pharmacology?

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2. Receptor oligomerization

3. Receptor desensitization/downregulation

4. Biased agonism

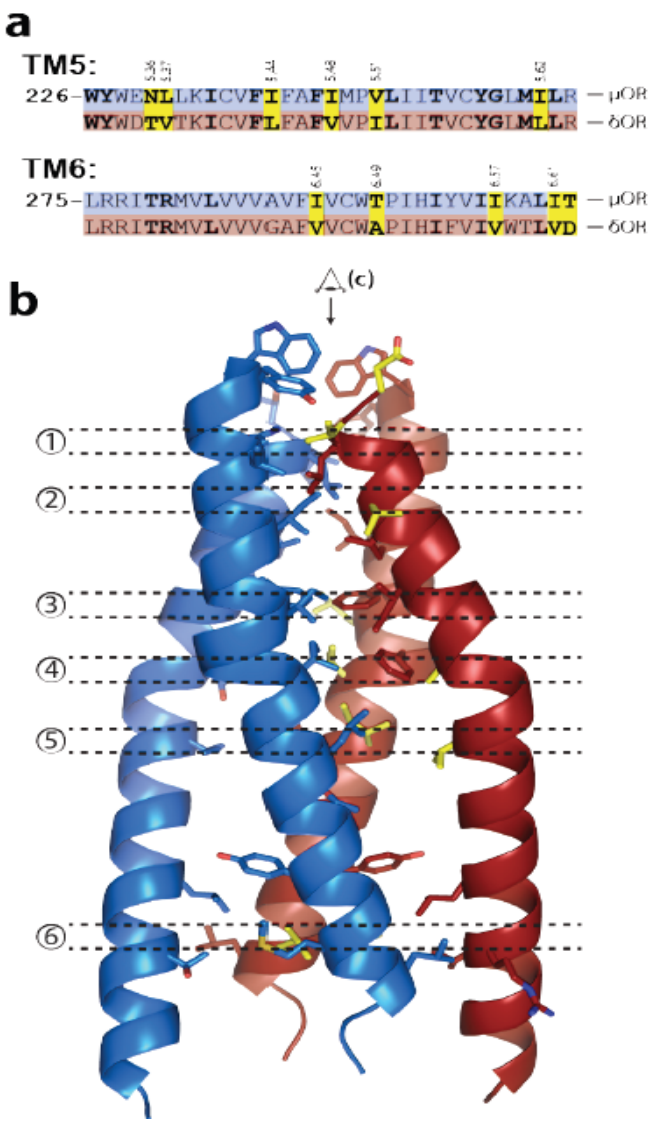
Some receptors are obligate dimers/oligomers

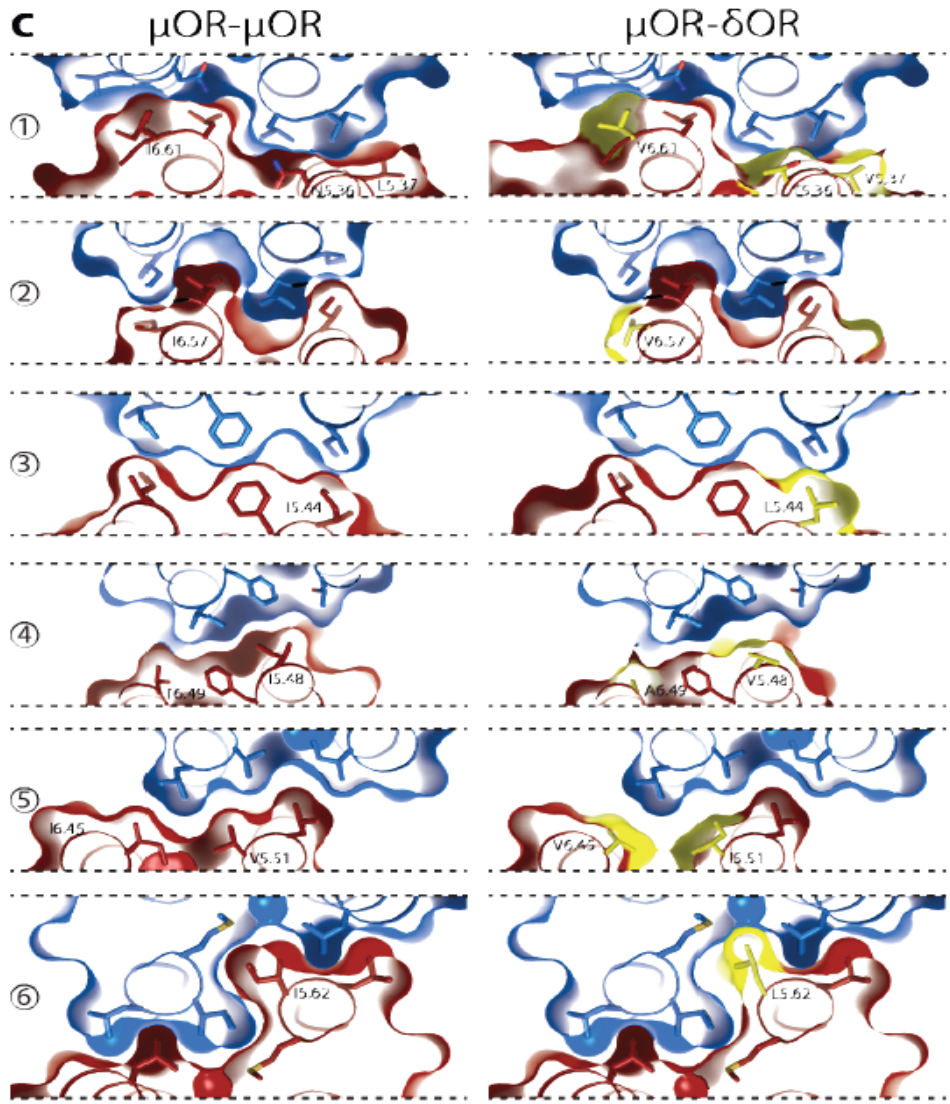
All ion channels—subunit composition can dramatically change pharmacology
- **important for "plasticity" (LTP, LTD)**
- **important for "selectivity"**

Among GPCRs obligate dimers include mGluRs, GABA-B, and taste receptors

Mounting evidence that many GPCRs are dimers/oligomers, both homomers and heteromers

The MOR crystallizes as a dimer

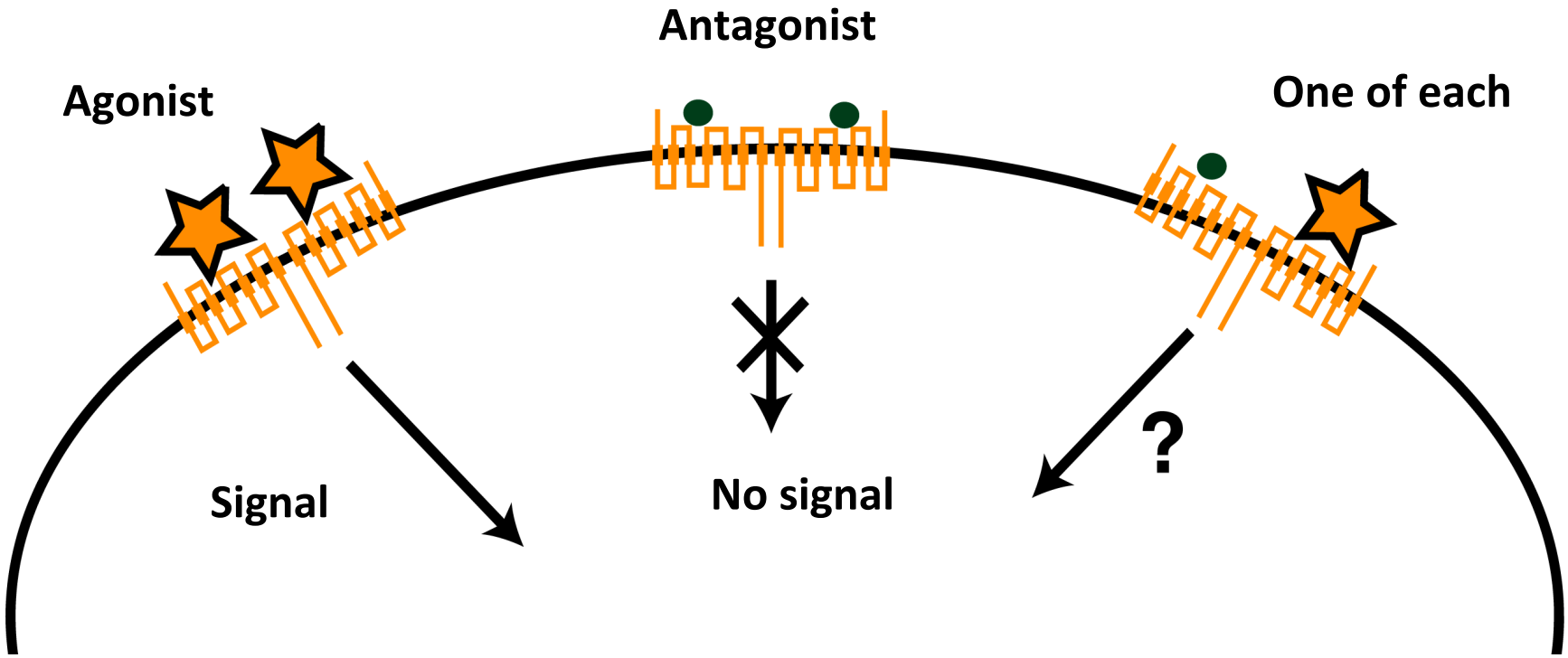




TM5 – TM6

TM6 – TM5

Manglik et al, 2012, Nature



1. Allosteric effects of one ligand on the others' affinity
2. Generation of binding pockets with altered affinity
3. Heteromers that couple to different G proteins than their homomers
4. Receptor dimers with different desensitization/downregulation properties

So what can confound our new knowledge of pharmacology?

1. Allosteric modulators

2. Receptor oligomerization

3. Receptor desensitization/downregulation

4. Biased agonism

Desensitization:

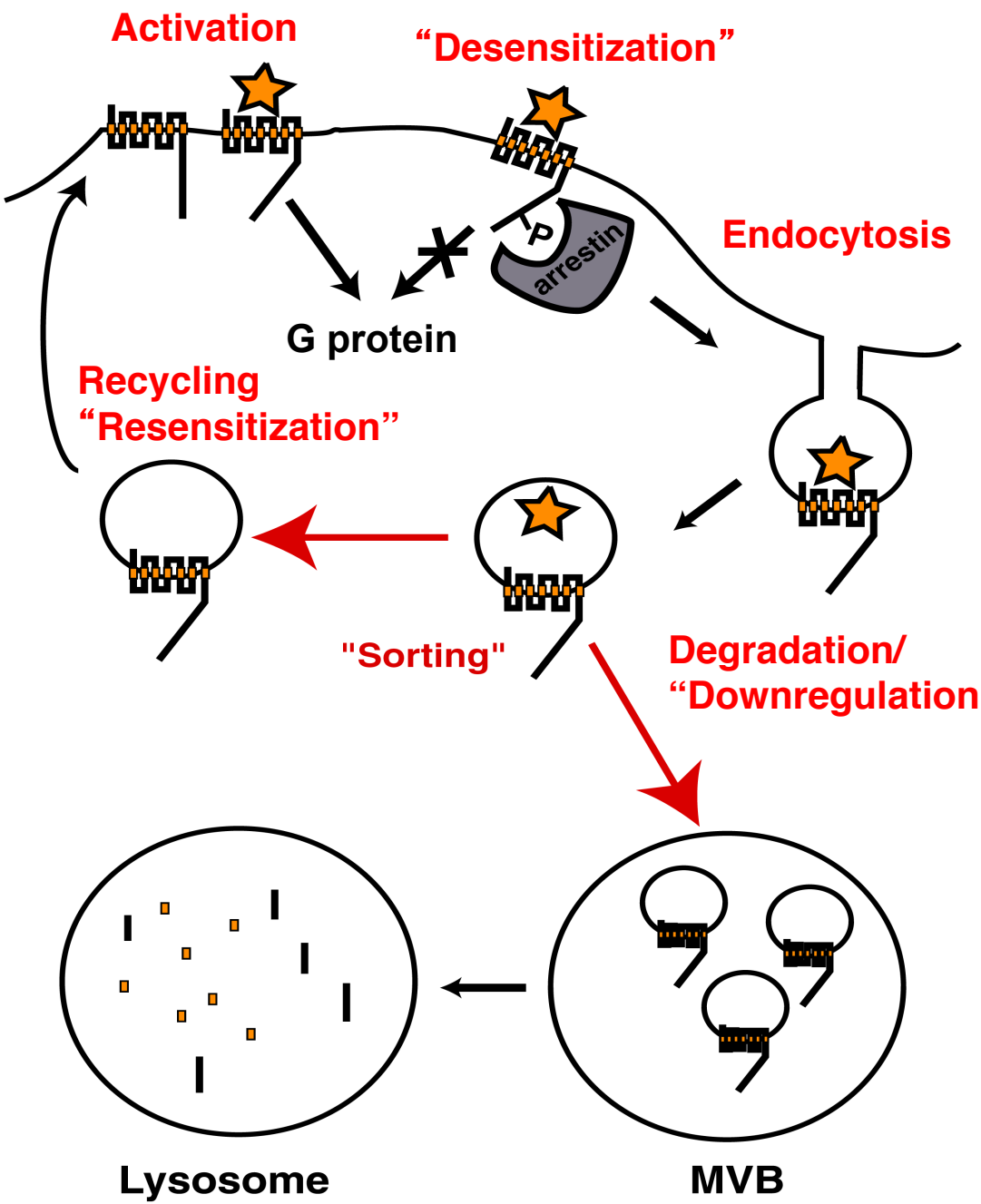
Receptors are present (and bind ligand) but no longer transduce signal to their downstream effector(s)

Downregulation:

Actual loss of receptor number

How would you distinguish between these possibilities?
Will a functional dose response tell you?

Consider how the amount of "receptor reserve", or "spare receptor" would influence your ability to detect either of these phenomena

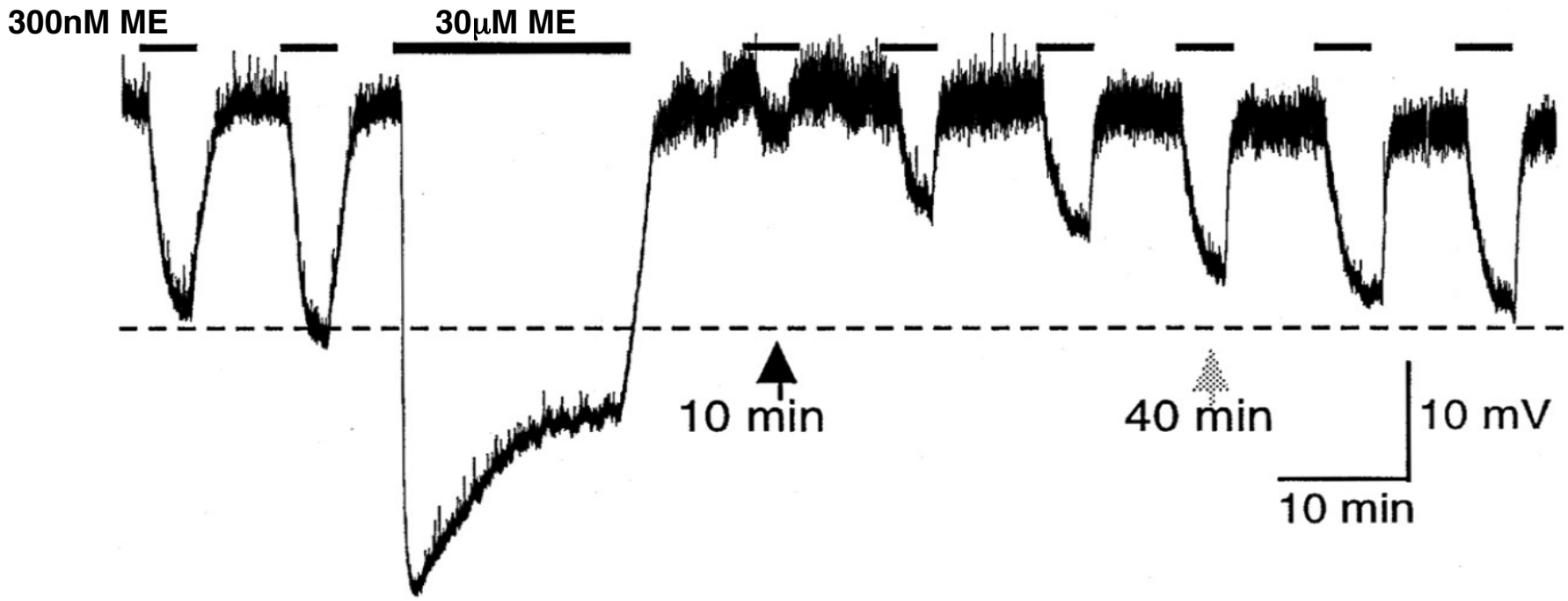


Most GPCRs are desensitized (and endocytosed) after activation

Most are also rapidly recycled/resensitized

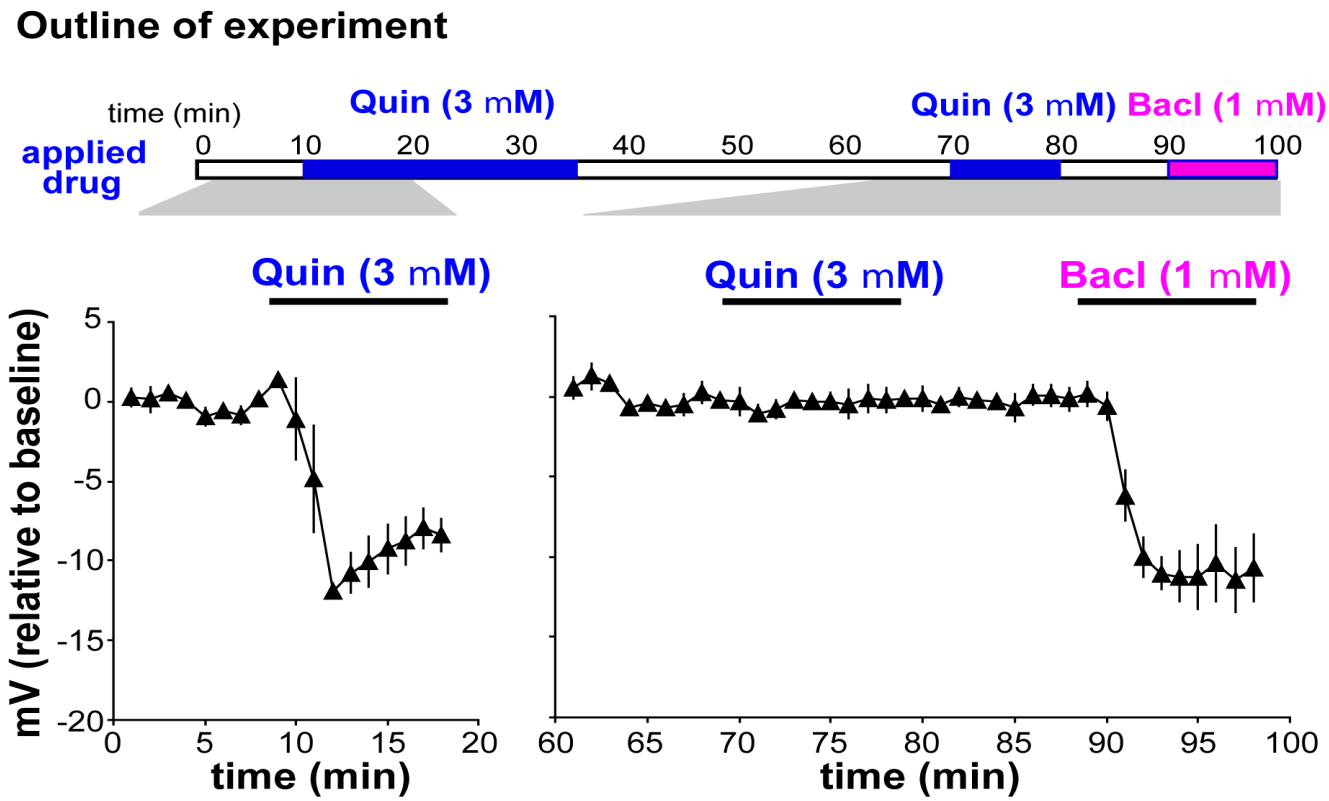
Some are not recycled/resensitized but instead are degraded/downregulated

The mu opioid receptor resensitizes

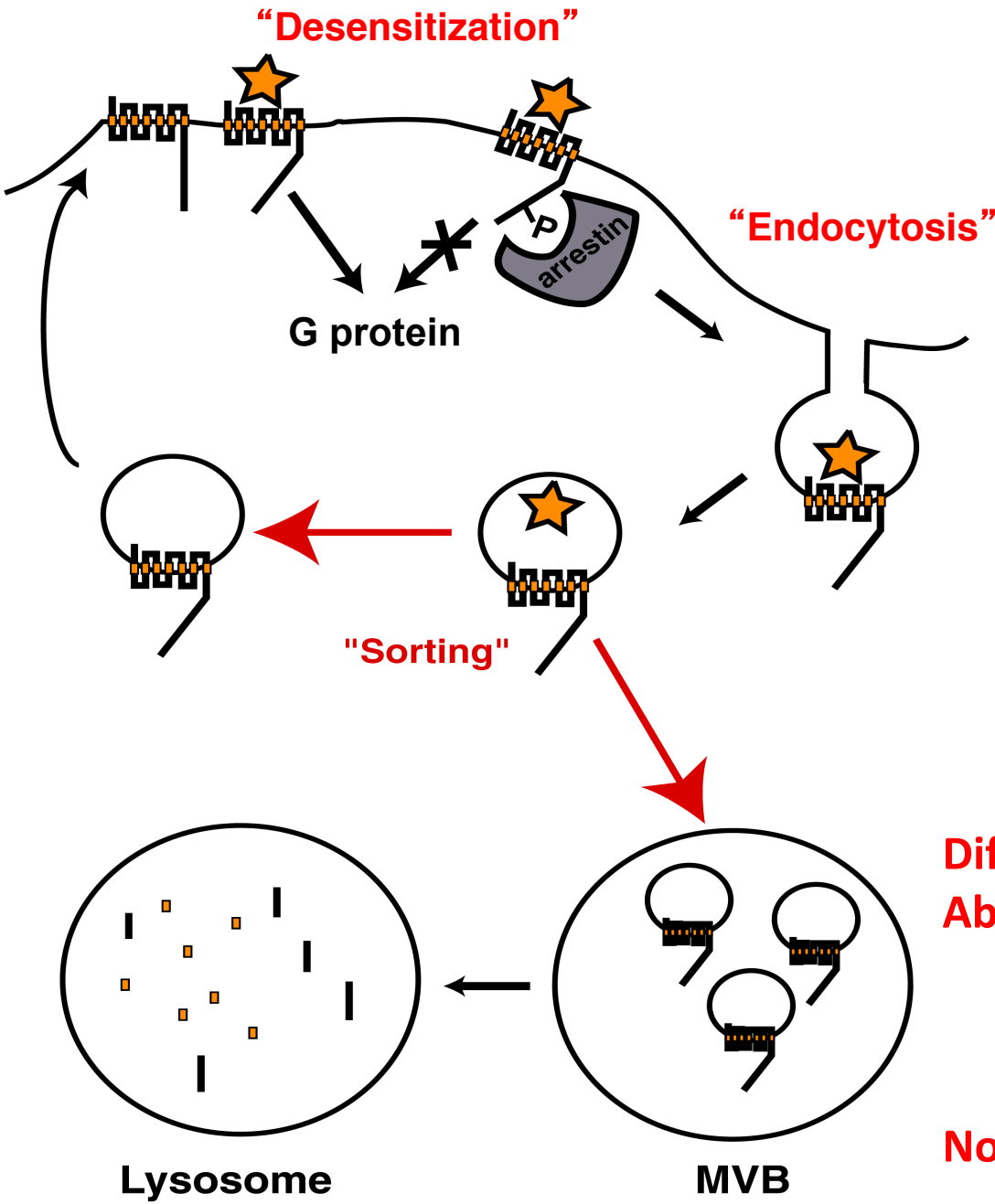


Alvarez et. Al J.Neurosci, 2002

On the other hand, D2 responses do not resensitize



Bartlett et al, 2005



Most GPCRs desensitized (and endocytosed) after activation

Most are also rapidly recycled/resensitized

Some are not recycled/resensitized but instead are degraded/downregulated

Different ligands have different intrinsic Abllities to drive:

- desensitization
- endocytosis
- downregulation

Not always linearly related to efficacy at G protein

So what can confound our new knowledge of pharmacology?

1. Allosteric modulators

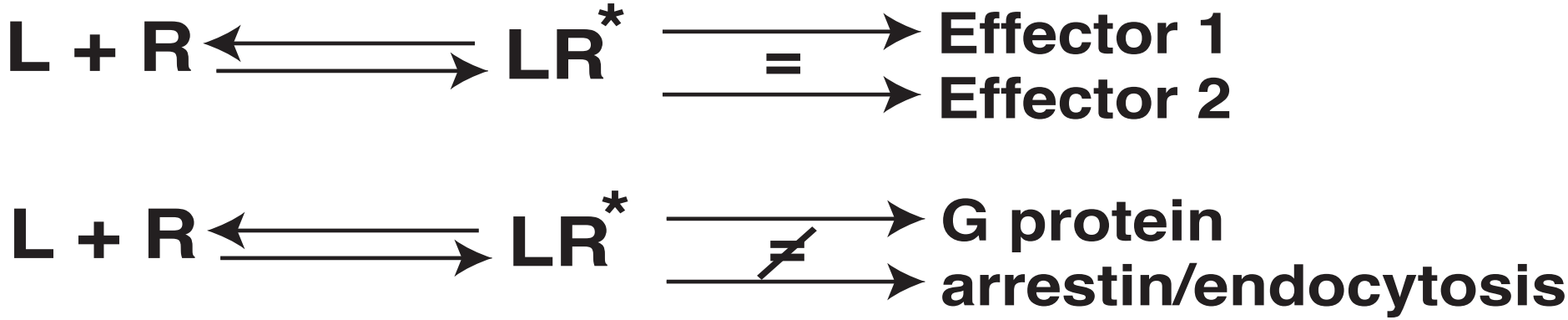
2. Receptor oligomerization

3. Receptor desensitization/downregulation

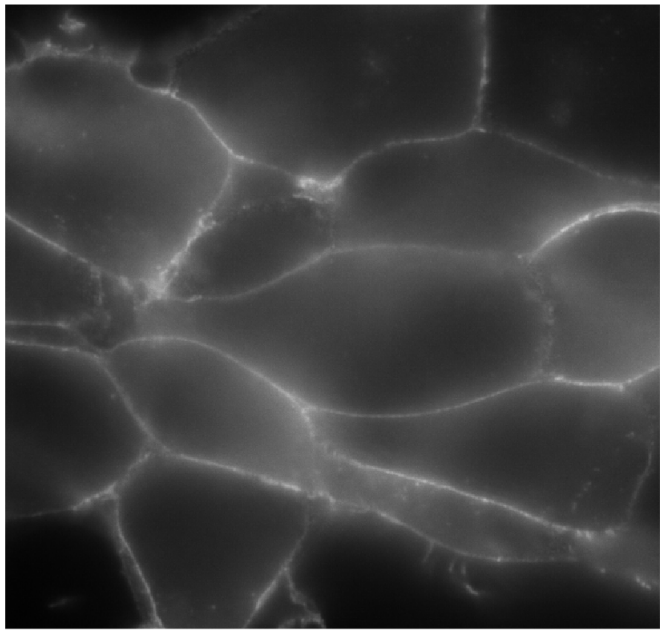
4. Biased agonism

Functional Selectivity/Biased Agonism

A single ligand can be an agonist, partial agonist, antagonist or inverse agonist Depending on the effector that is being measured.



The mu opioid receptor shows “biased agonism”



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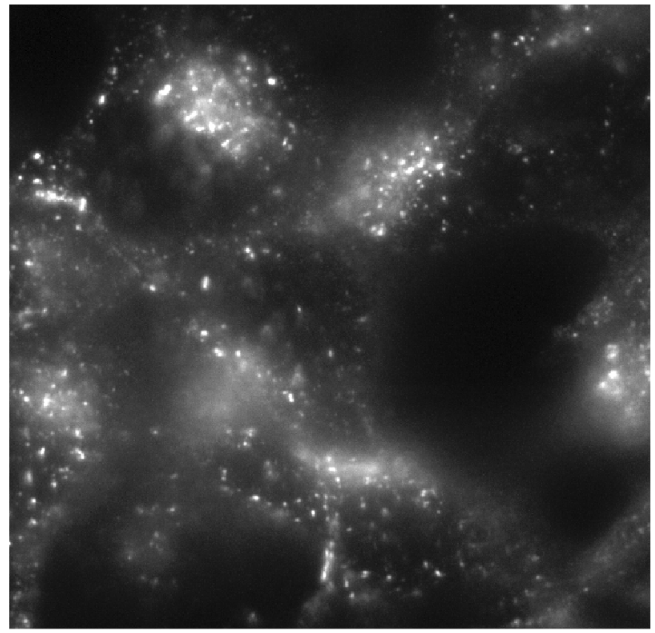
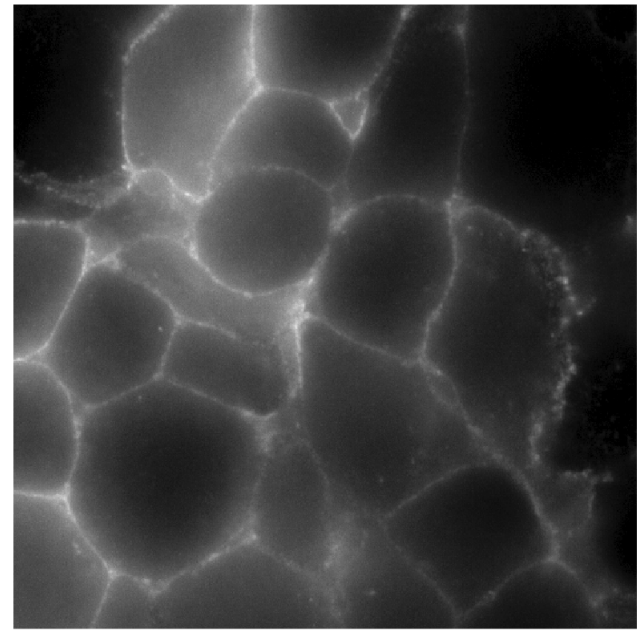


Enkephalin (or methadone)

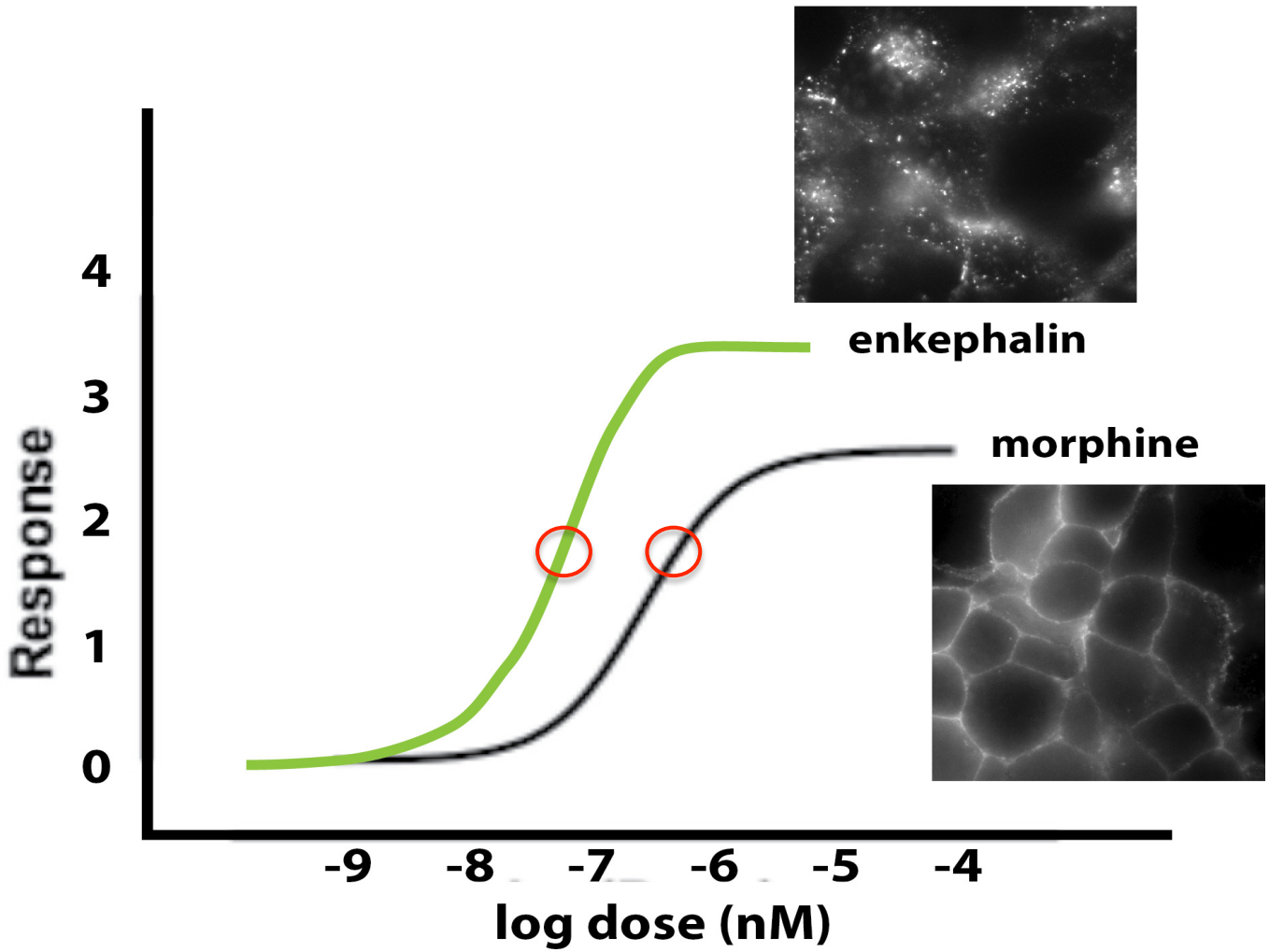


Morphine

$$L + R \rightleftharpoons LR^{*} \overset{\neq}{\rightarrow} \begin{matrix} \text{G protein} \\ \text{arrestin/endocytosis} \end{matrix}$$

How to distinguish “biased agonism” from partial agonism?

Keith et al, JBC 1996



How to distinguish “biased agonism” from partial agonism?

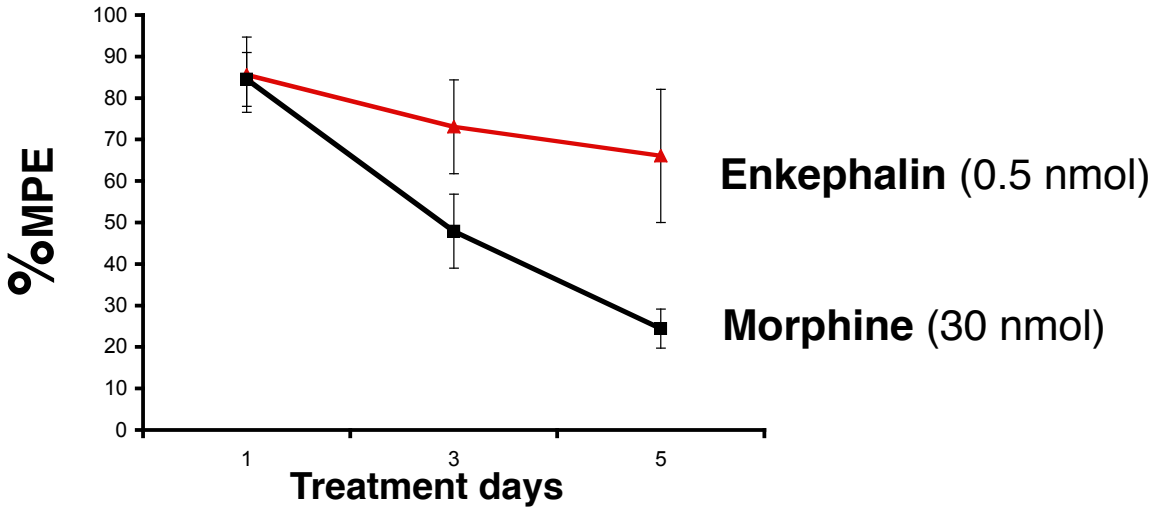
Does a change in bias matter?

What are the key caveats to these experiments?

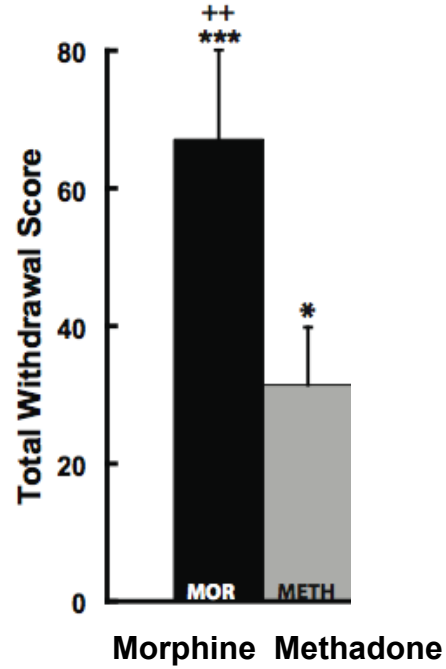
Morphine
Methadone
Enkephalin

All mu opioid receptor agonists for G protein
And all are analgesics

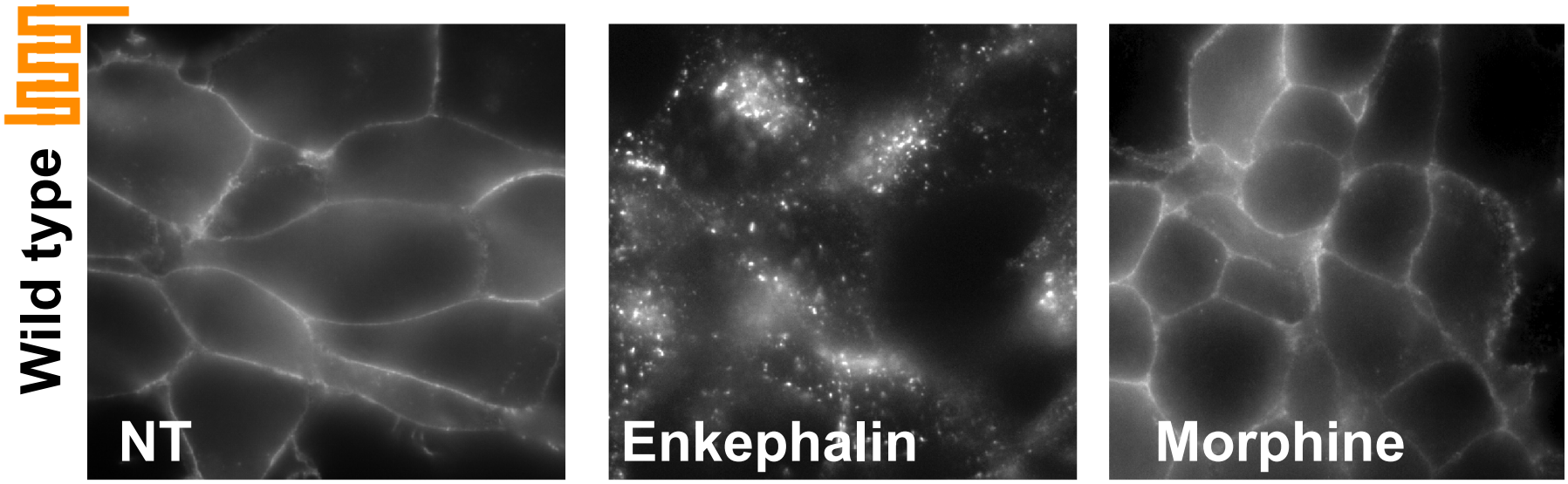
Analgesic Tolerance



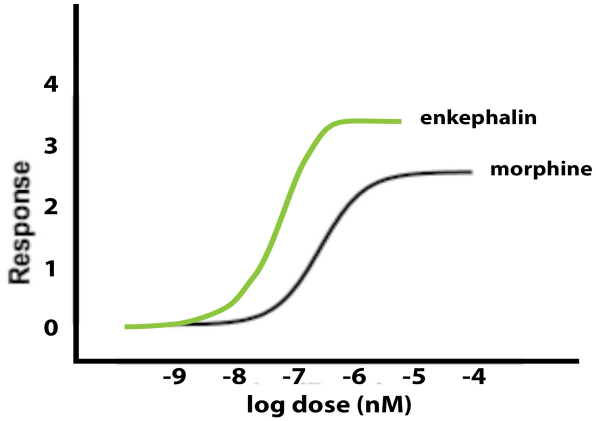
Physical Dependence/ Withdrawal

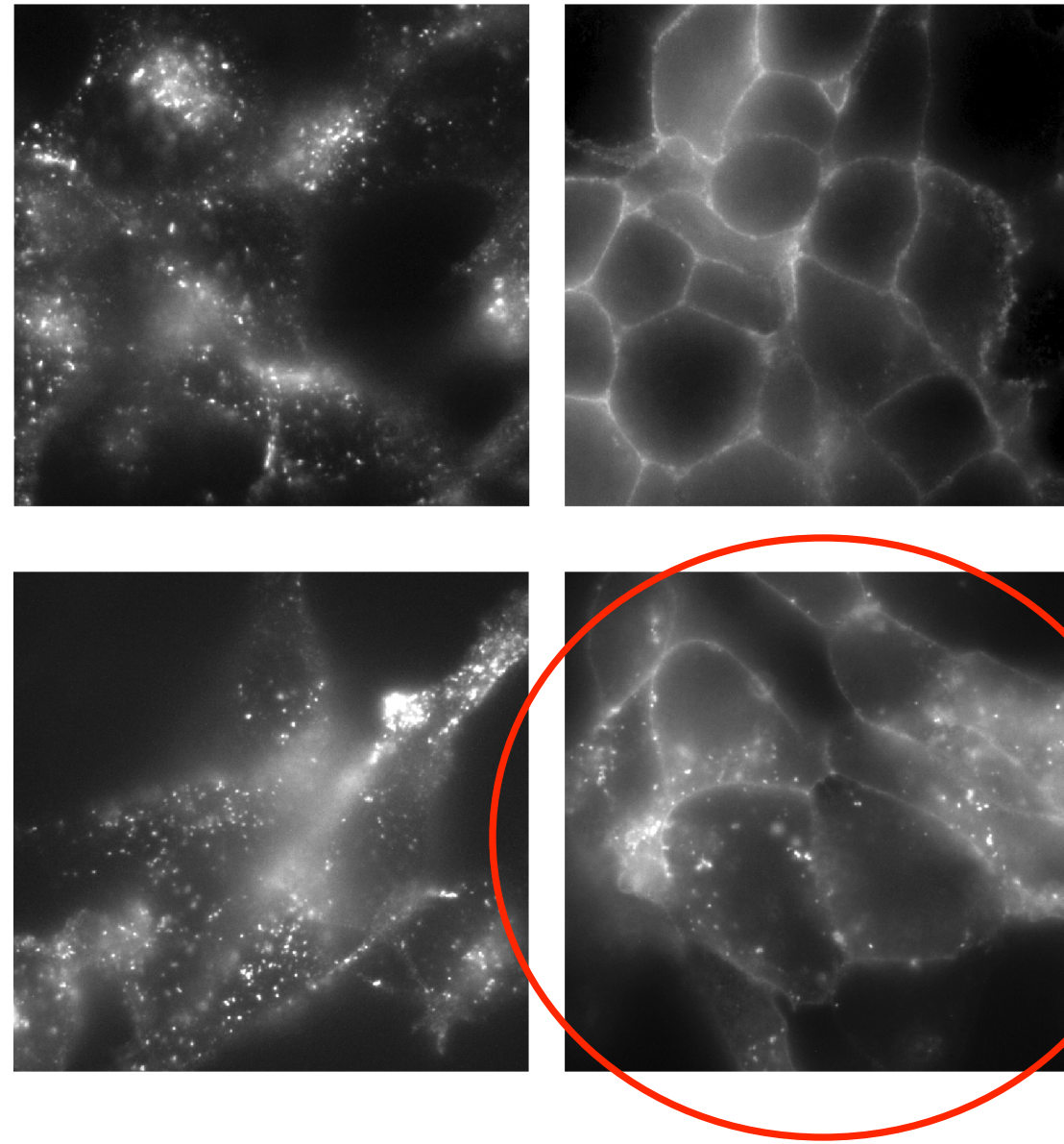


The “cleanest” way to examine the importance of biased agonism is to selectively change only the receptor and only in response to the biased drug

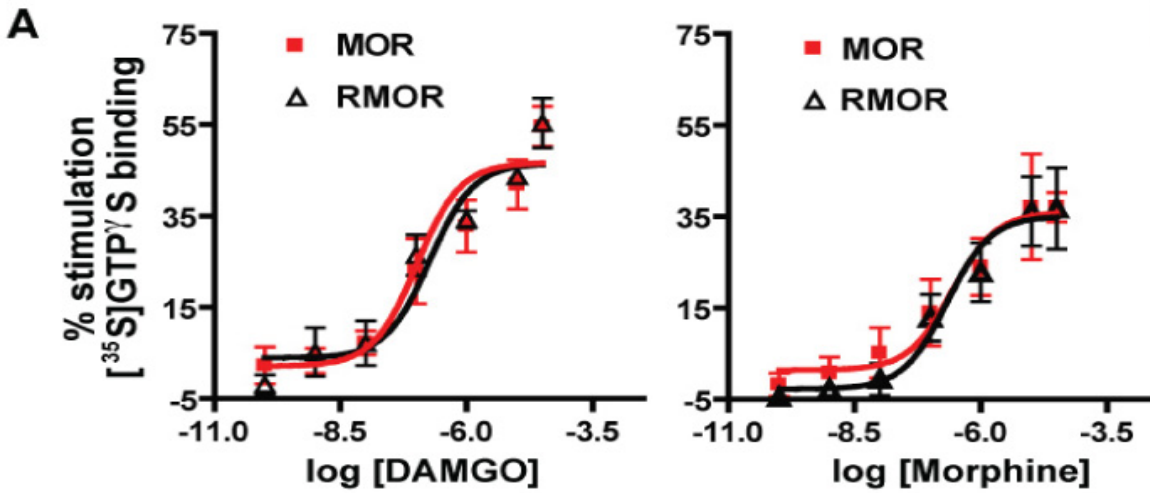


What else might have changed?



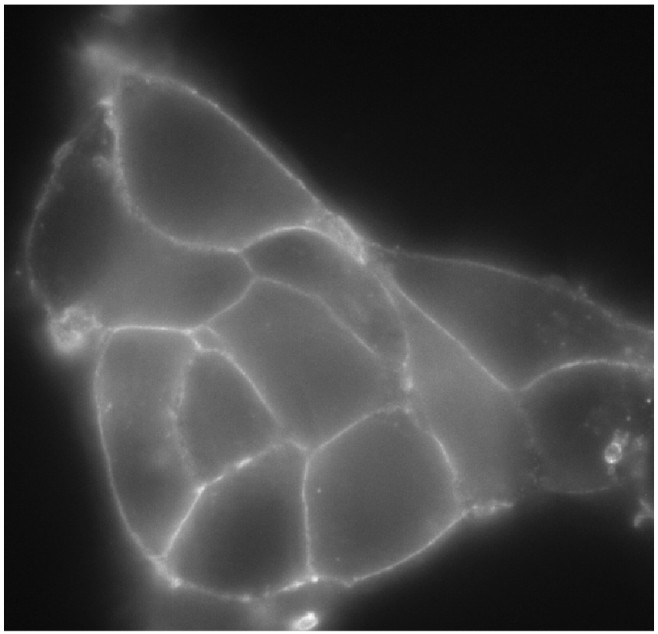


There is no difference in affinity or potency in wild type and mutant



Kim et al. Curr.Biol, 2008
Madhavan et al. J. Neuro 2010

The mu opioid receptor shows biased agonism



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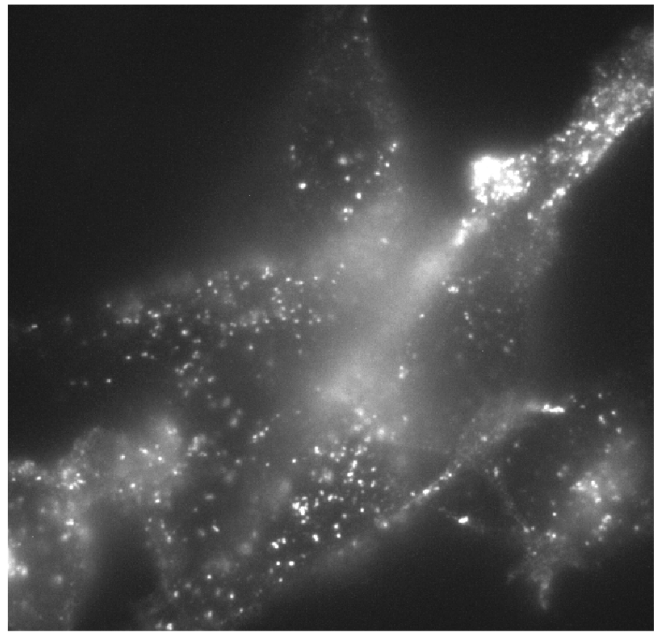
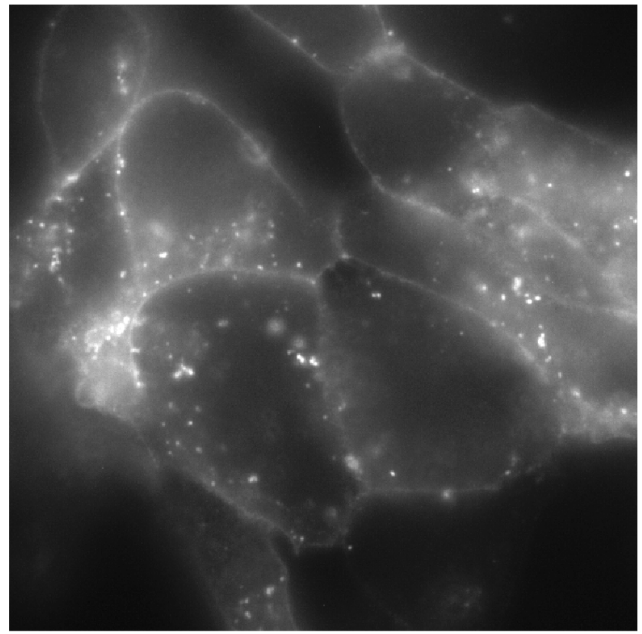
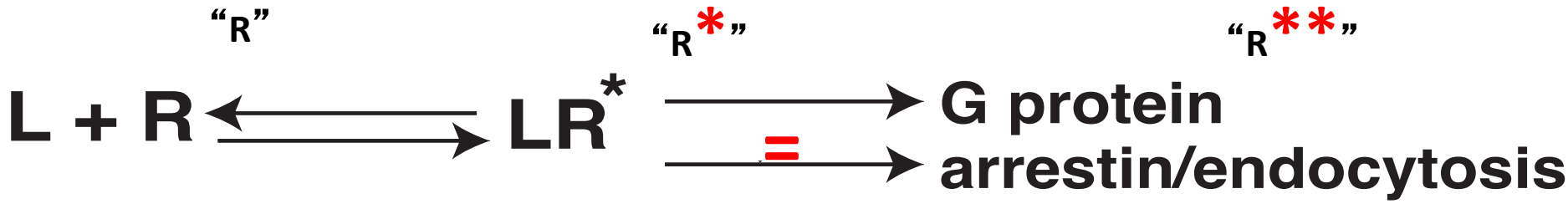


Enkephalin



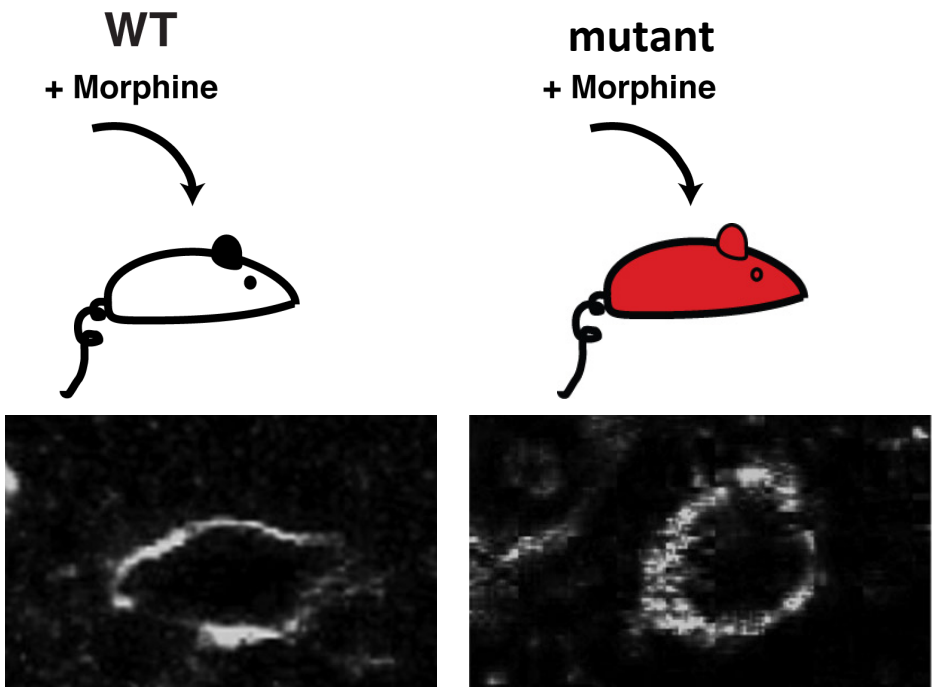
Morphine



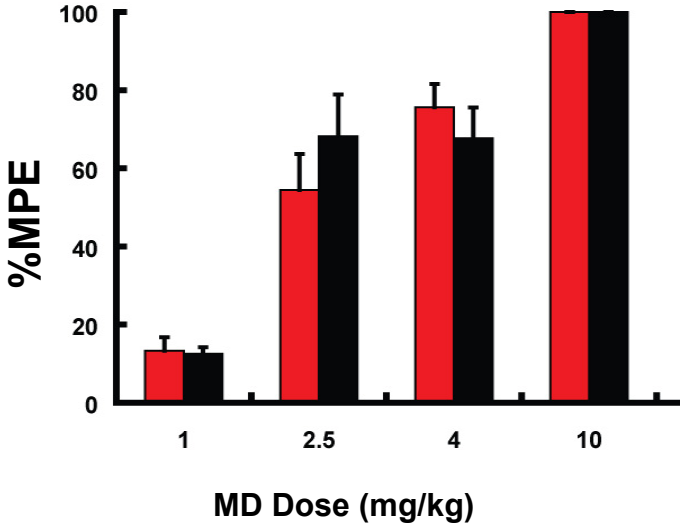
What are the functional consequences?

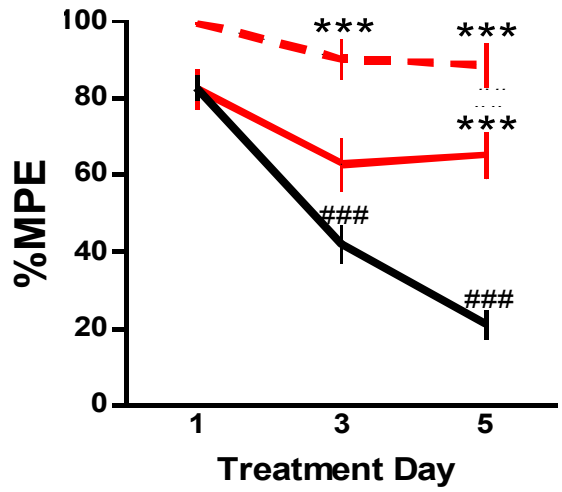
Keith et al, JBC 1996

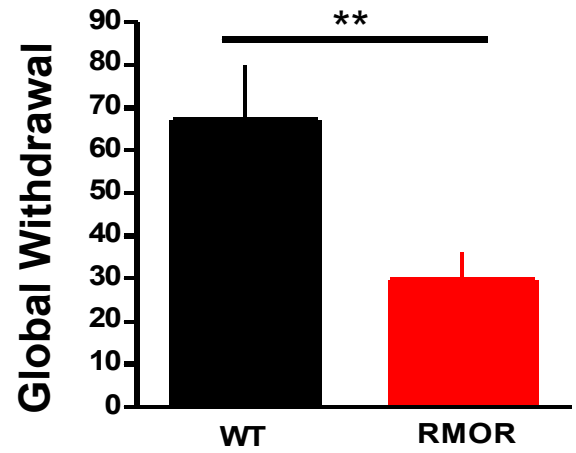
Does a change in bias matter?



Analgesia --improved (morphine)
Tolerance --reduced
Dependence --reduced







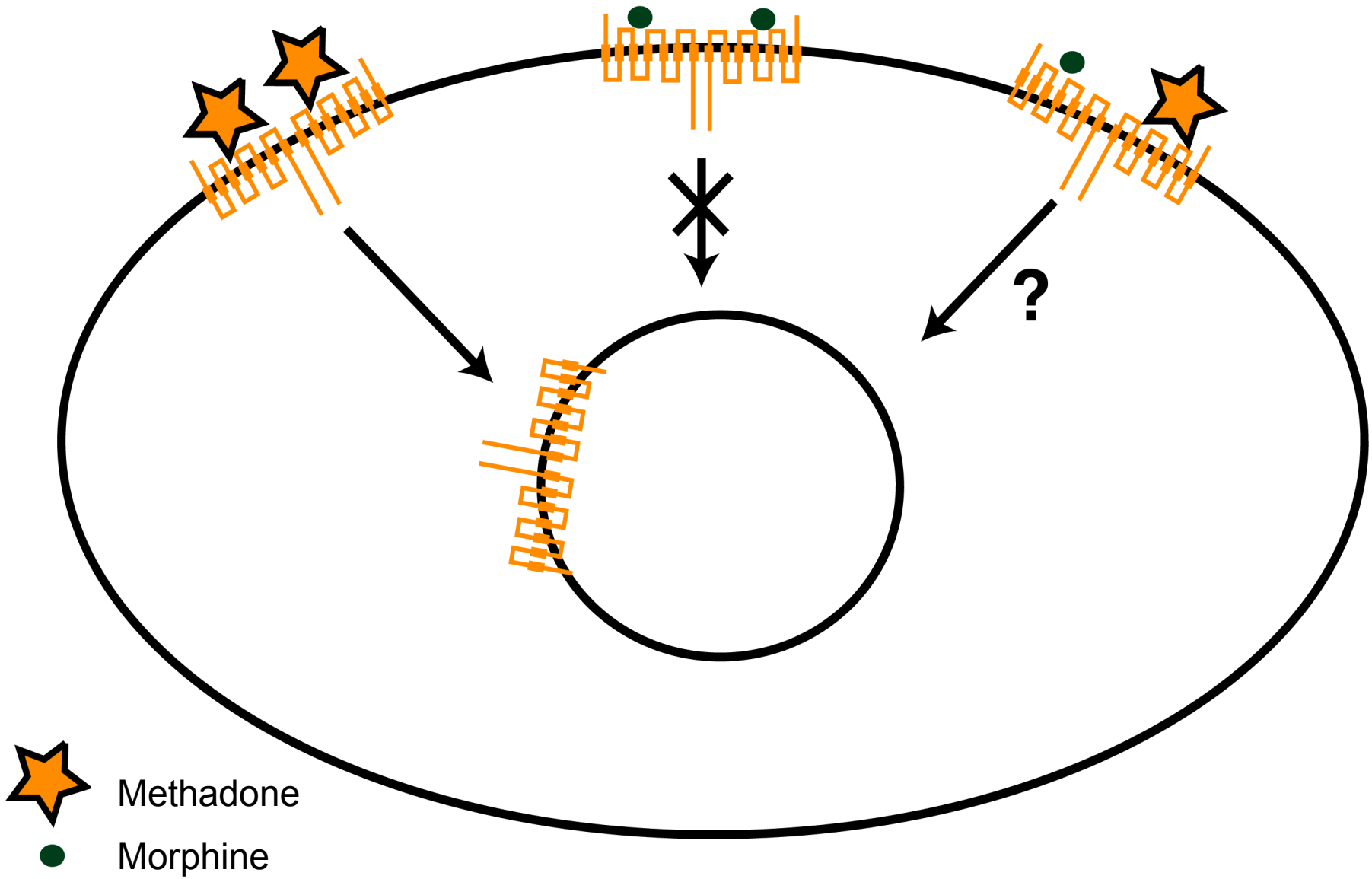
Kim et al. Curr. Biol, 2008

Can we leverage this information to make better analgesics?

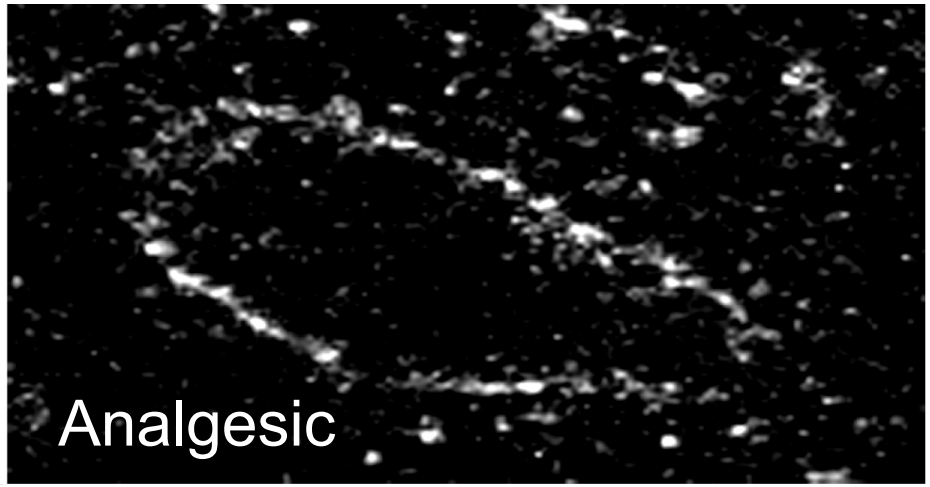
Opioid drugs with potency/efficacy/PK of morphine AND the ability to engage arrestin/promote receptor endocytosis will have excellent analgesic efficacy and reduced liability for the side effects of tolerance and dependence.

None of the current opioid drugs meet these criteria….

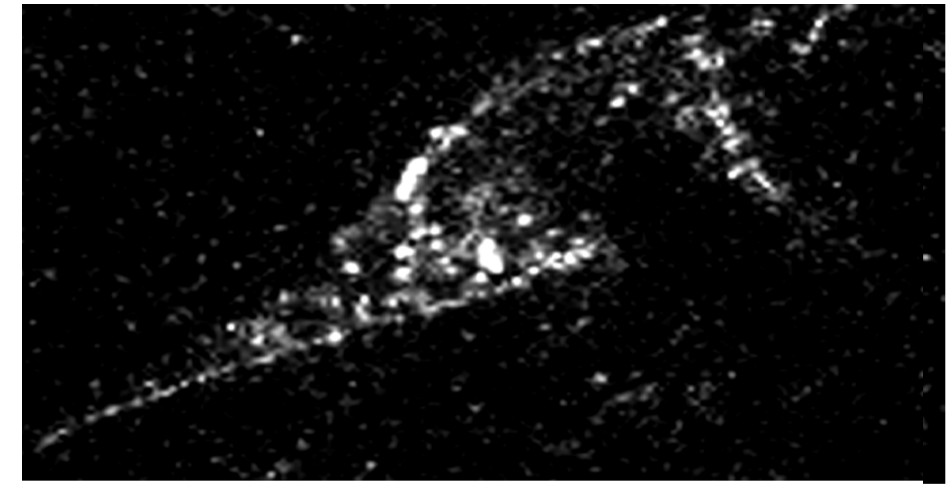
Can we use the dimeric nature of the MOR to alter morphine-induced endocytosis?



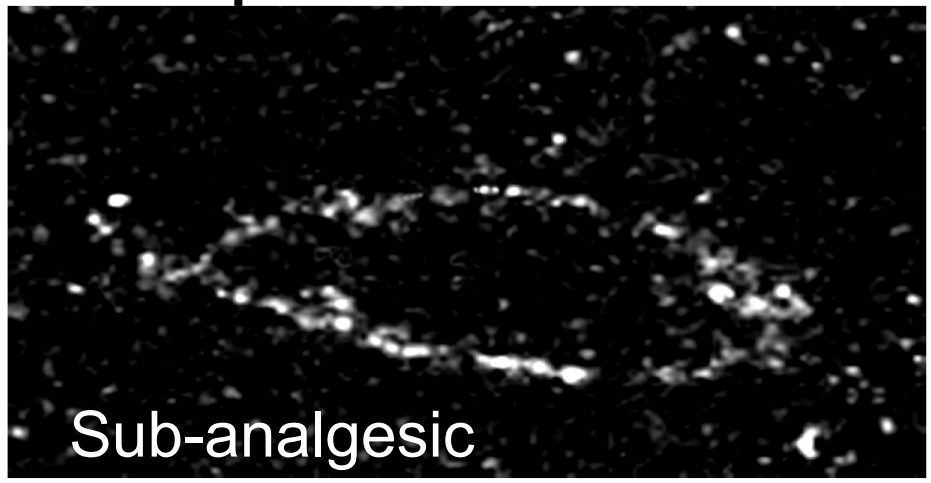
Methadone promotes morphine-induced endocytosis



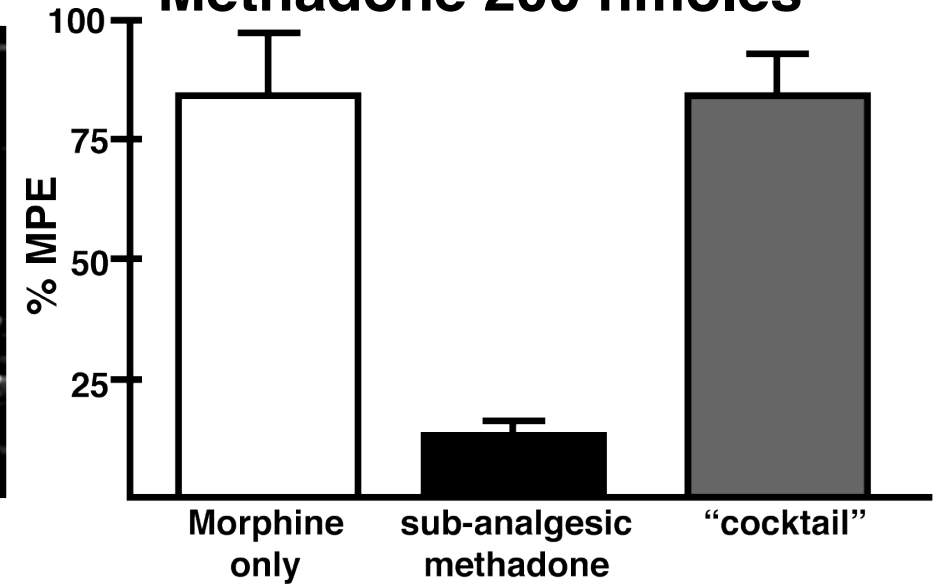
Morphine 30 nmoles



Methadone 200 nmoles



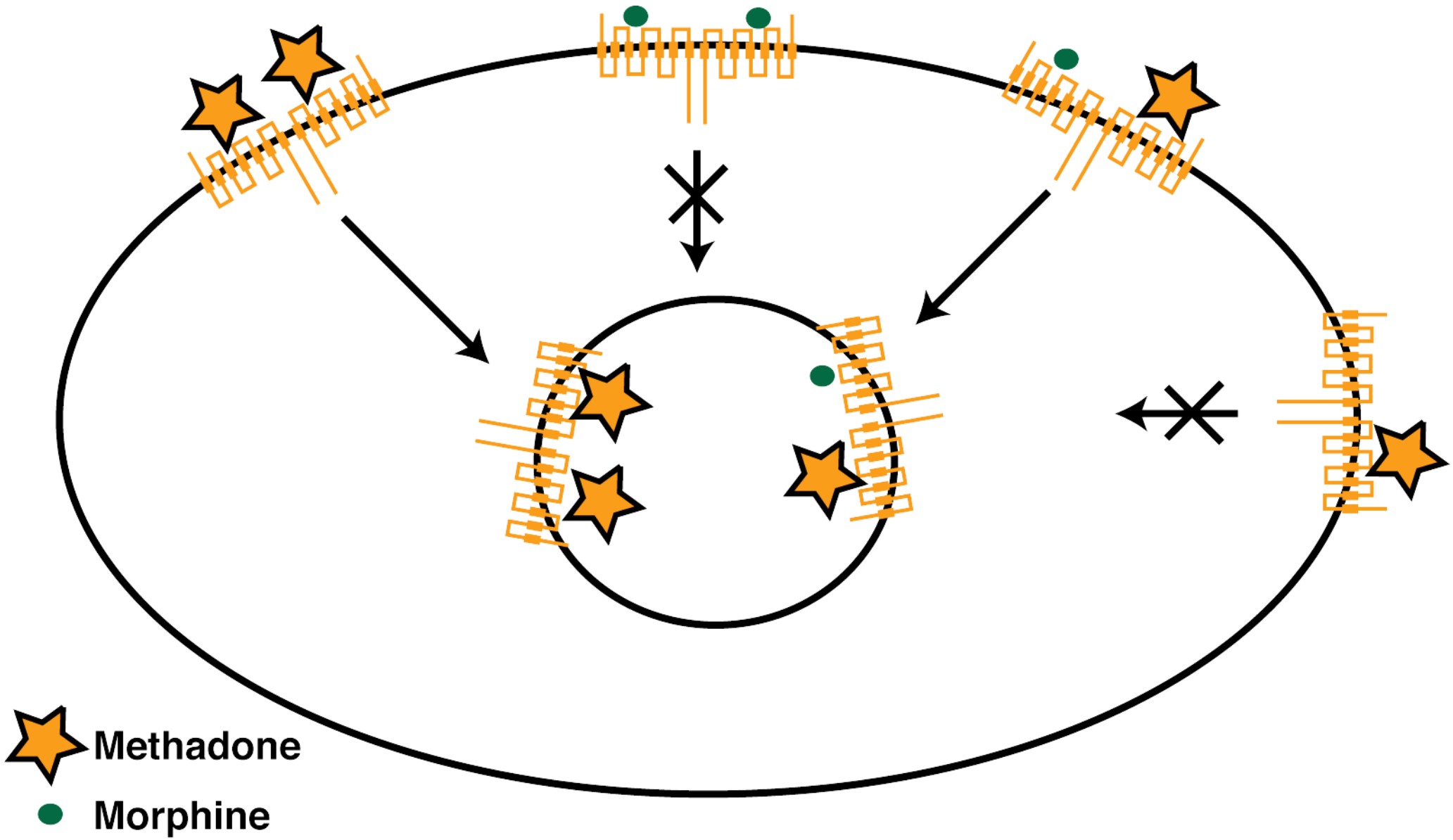
Methadone 2 nmoles



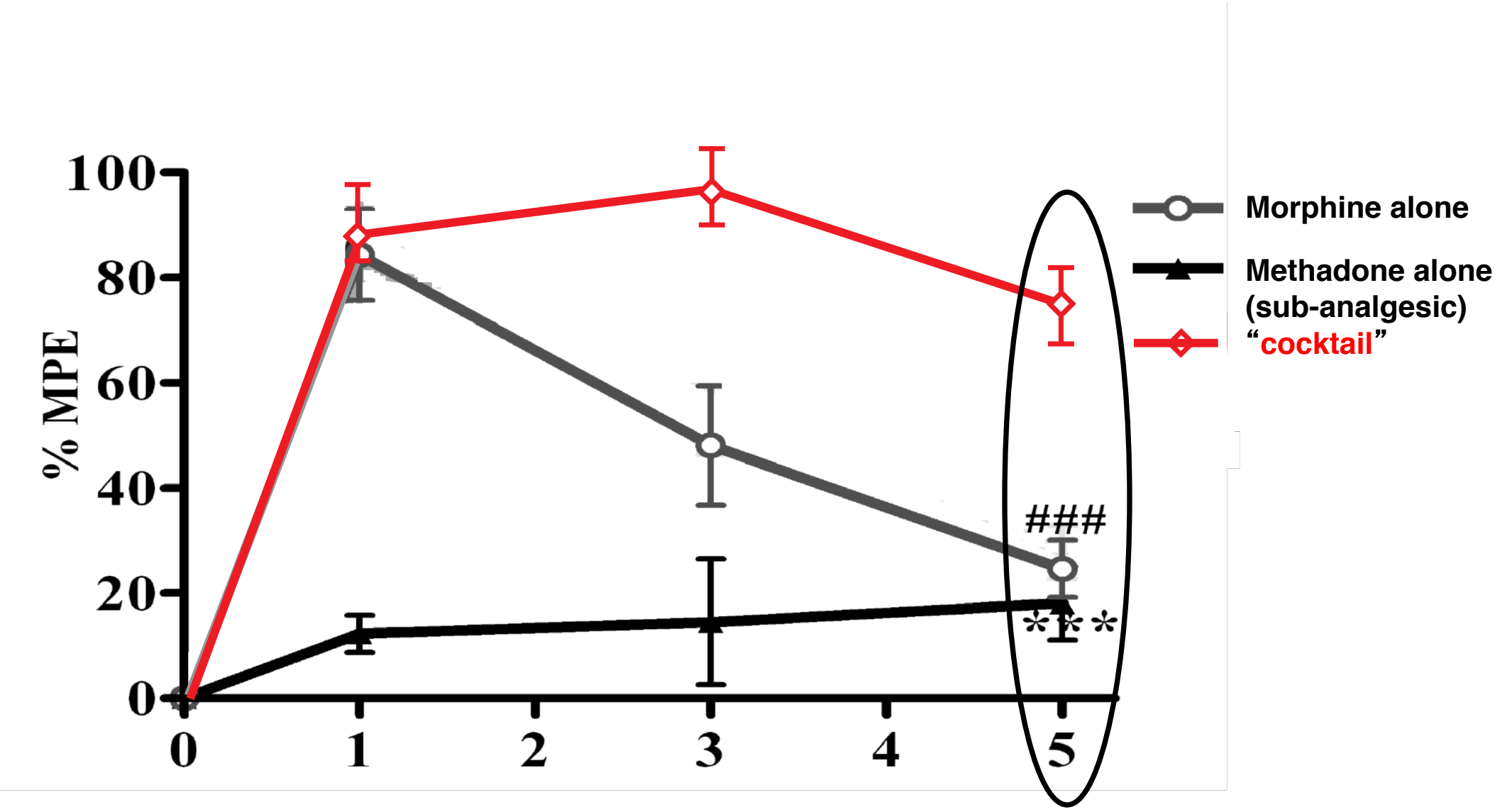
He et al, Curr Biol 2005

Methadone, in trans, promotes morphine-induced endocytosis of MOR

Occupancy of “all” protomers by agonist is required

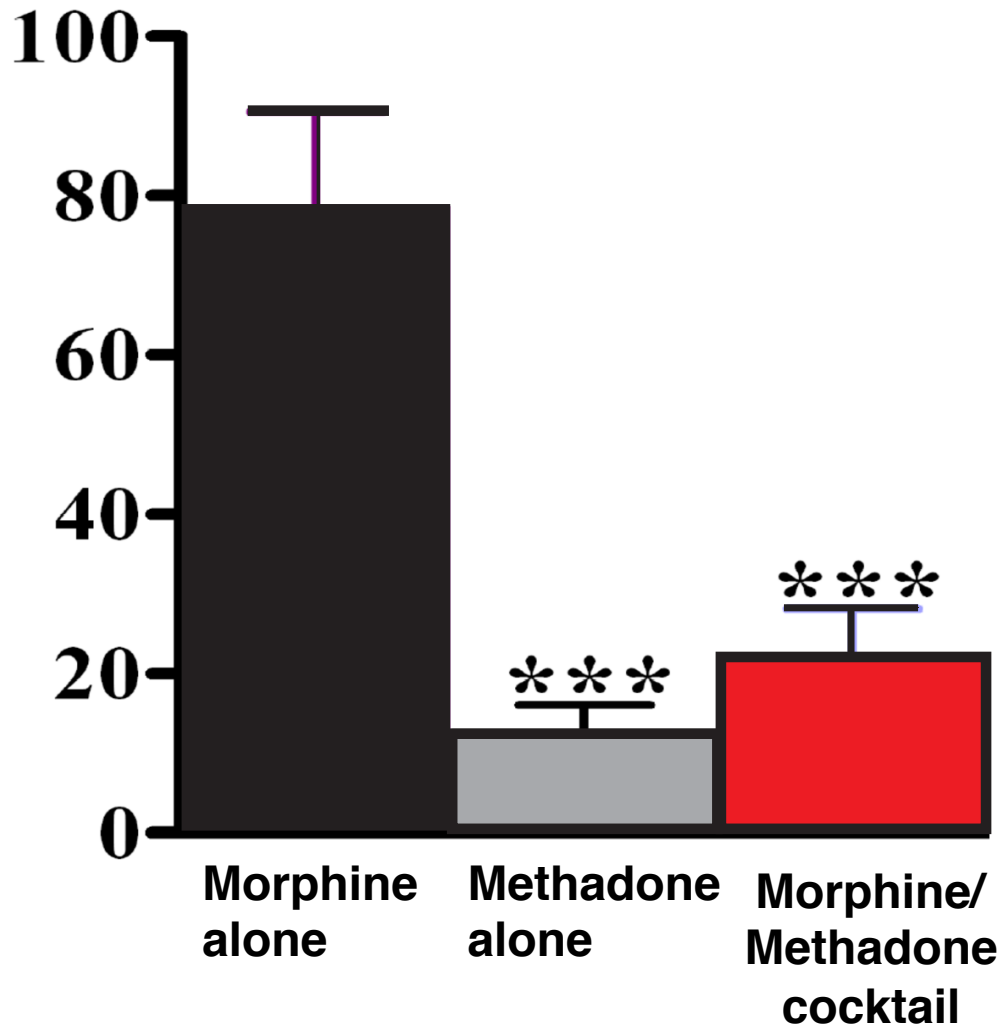


A sub-analgesic dose of Methadone prevents morphine tolerance



He et al, Curr. Biol 2005

Sub-analgesic methadone reduces morphine withdrawal



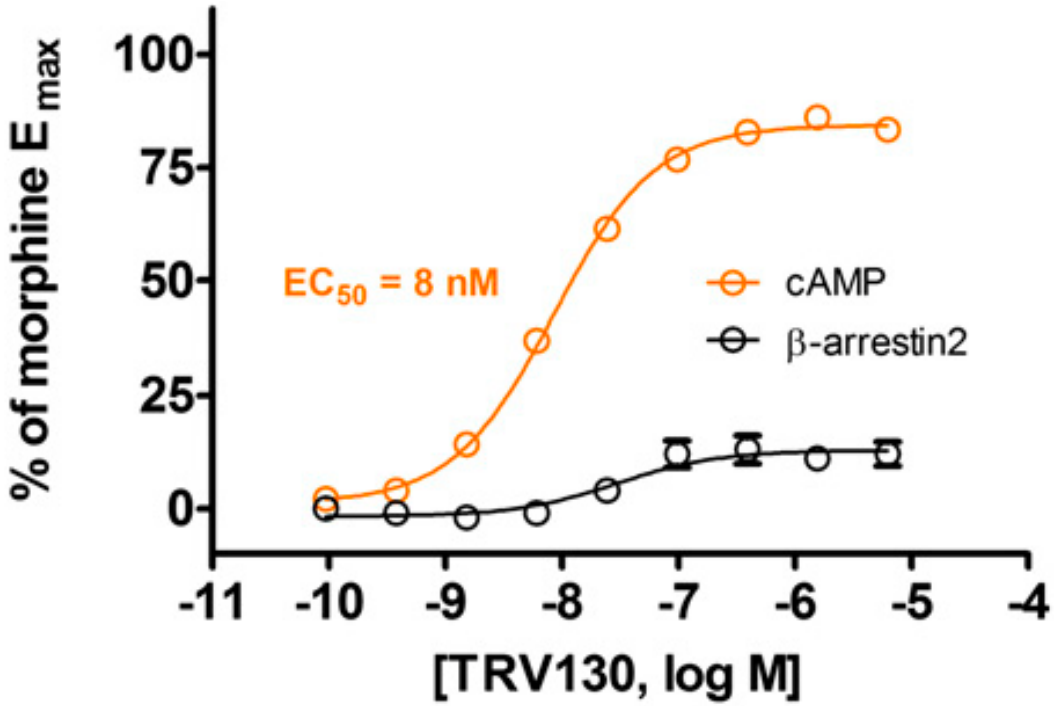
He et al, Curr. Biol 2005

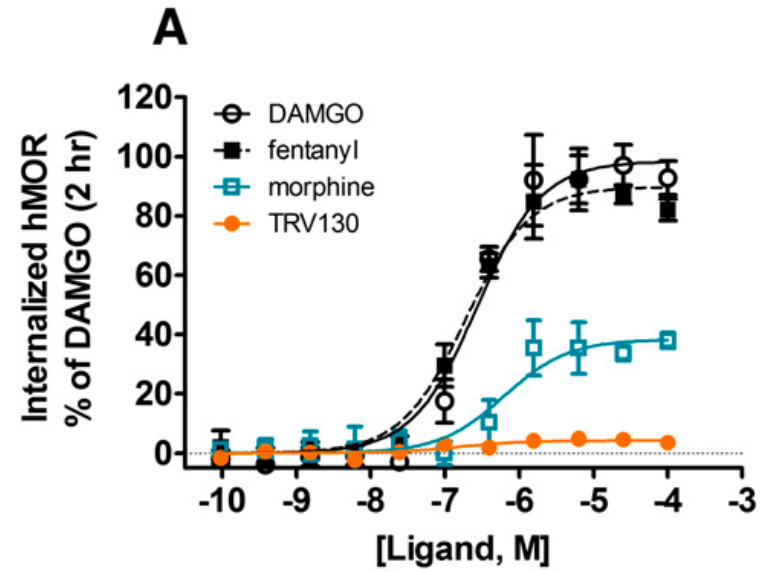
When the bias is changed, morphine retains its “beneficial effects”: Analgesia
But not its “side effects”: Tolerance, Dependence

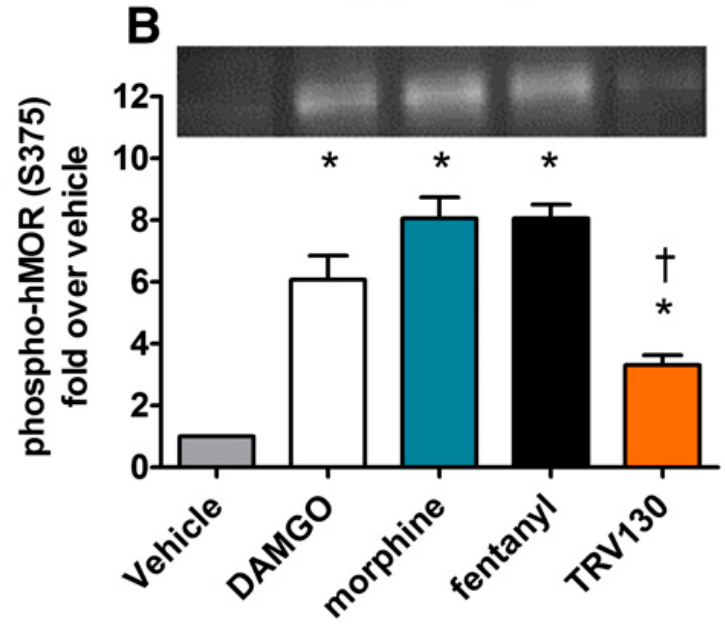
***Are there other new chemical entity opioids with the “right” profile?**

The NCEs in development actually have a bias AGAINST arrestin recruitment

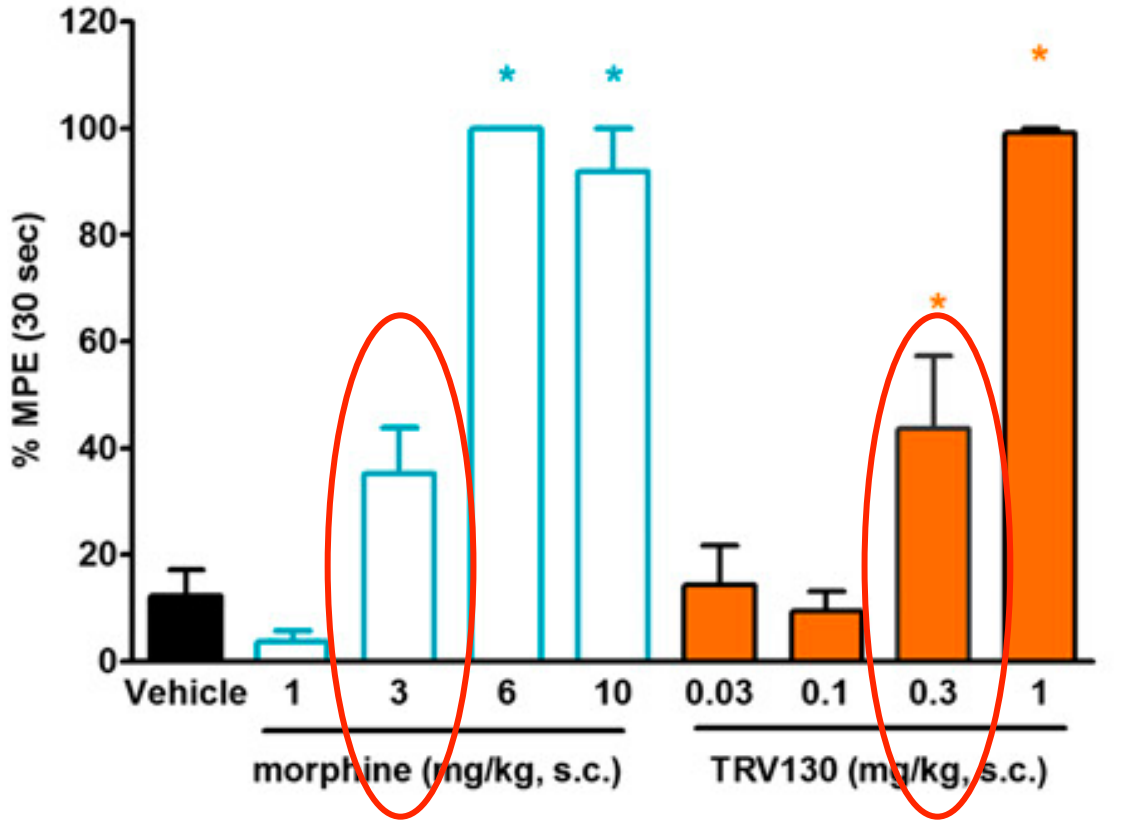
TRV130 is a G protein biased MOR selective agonist





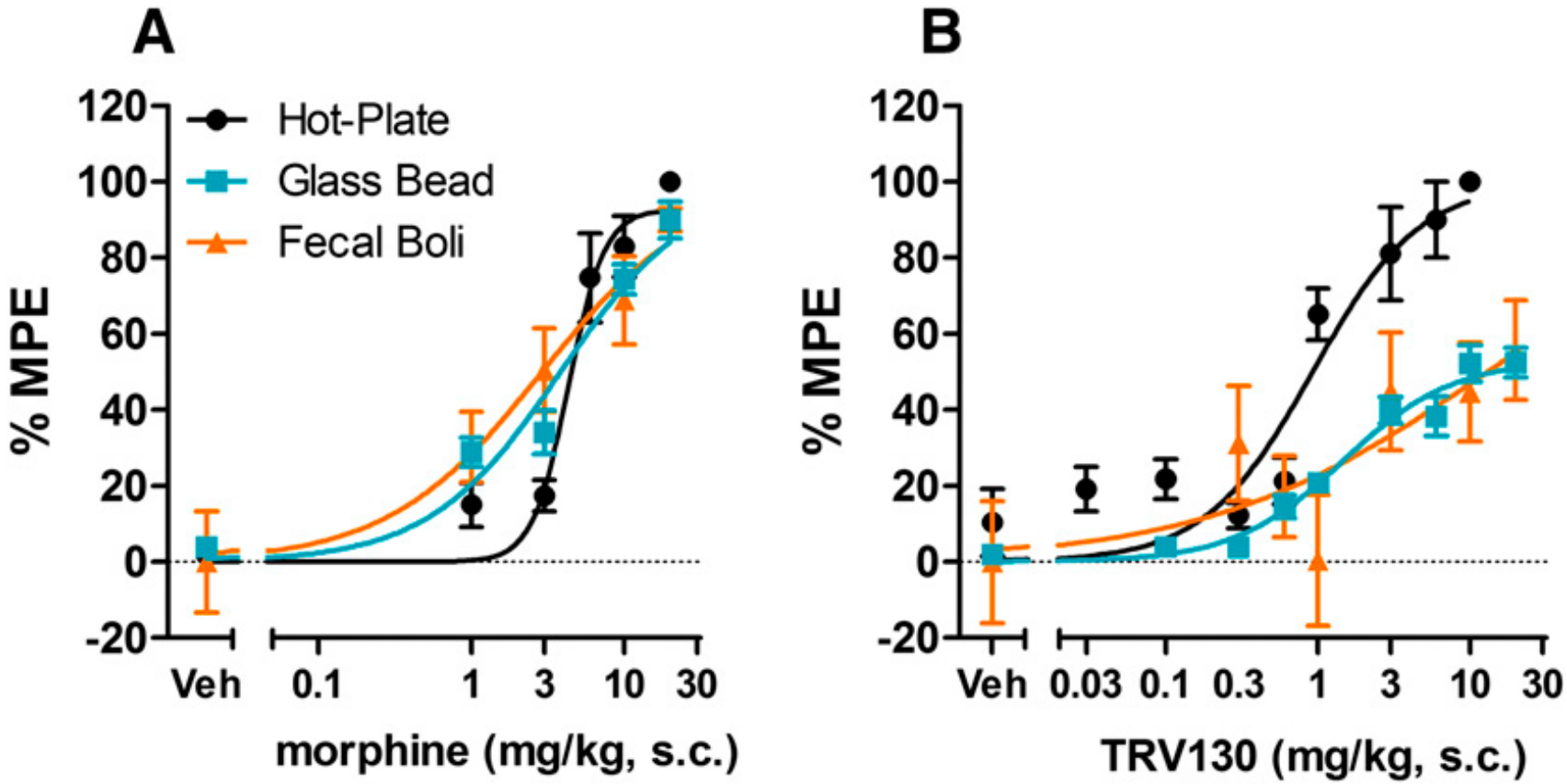


TRV130 is a potent analgesic (more potent than morphine) with a similar affinity as morphine

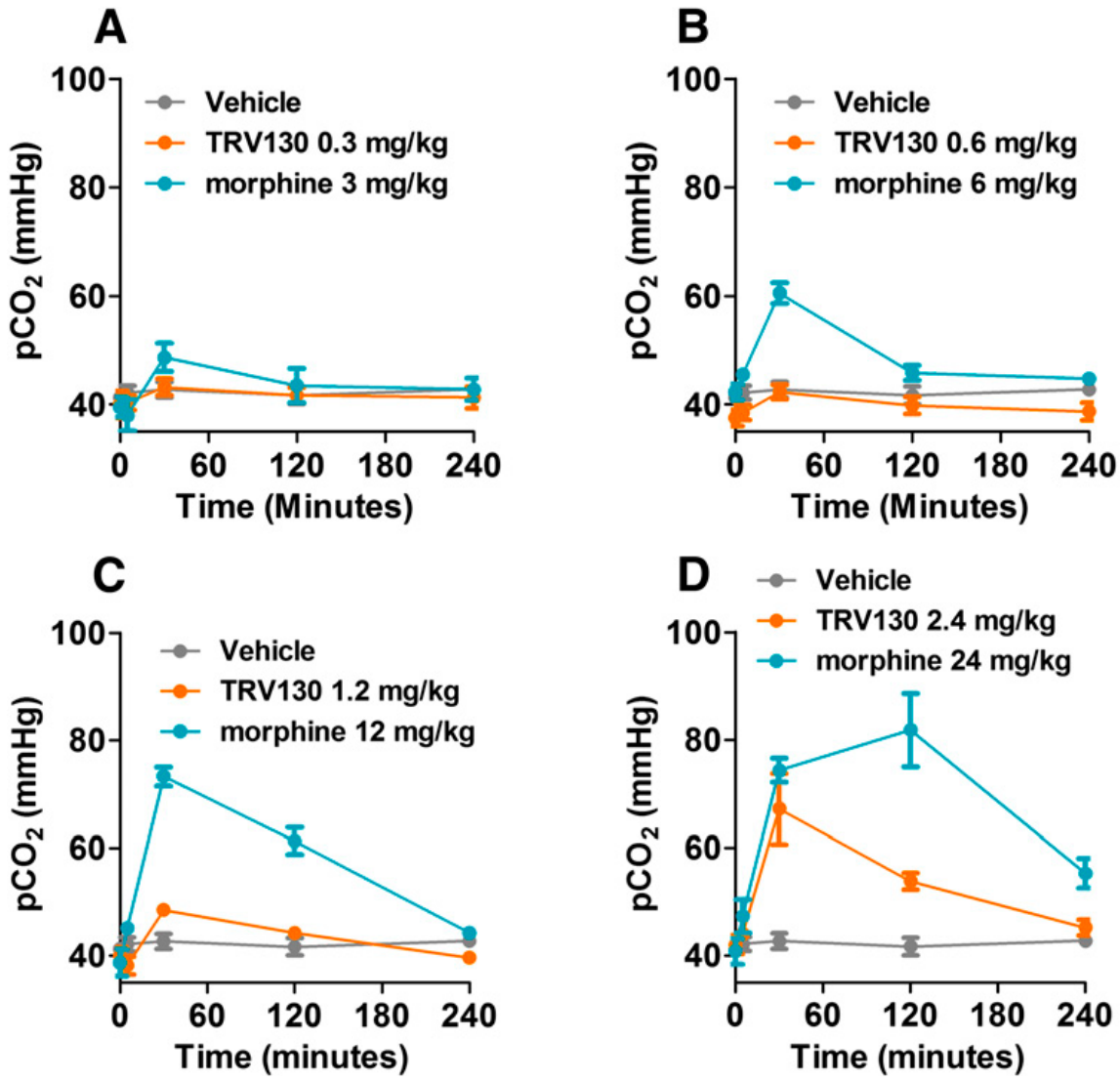


What does this tell us about the intrinsic efficacy of TRV130?
What about receptor occupancy at therapeutically relevant doses?

TRV130 is a G protein biased MOR selective agonist with reduced gastrointestinal dysfunction



…And reduced respiratory dysfunction at equi-analgesic doses

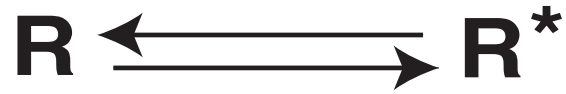


What is the difference in receptor occupancy under these conditions?

The best “ligand bias” may depend on the indication

For chronic pain, the primary liabilities are analgesic tolerance and dependence

For post surgical pain, the primary liabilities are constipation and respiratory suppression



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- **antagonist**
- **inverse agonist**

The degree of occupancy is determined by affinity (and PK in vivo)

Once it has occupied a receptor, the magnitude of the effect is determined by efficacy

The potency of a ligand is therefore a function of both affinity and efficacy

High affinity, low efficacy
Low affinity, high efficacy **= Where potency doesn't match affinity**

With receptor classes with more than one member one also must be cognizant of specificity, especially when more than one member of the class is in your system

When in doubt, think about occupancy--who is bound, to what target(s)?
Then hypothesize functional consequences

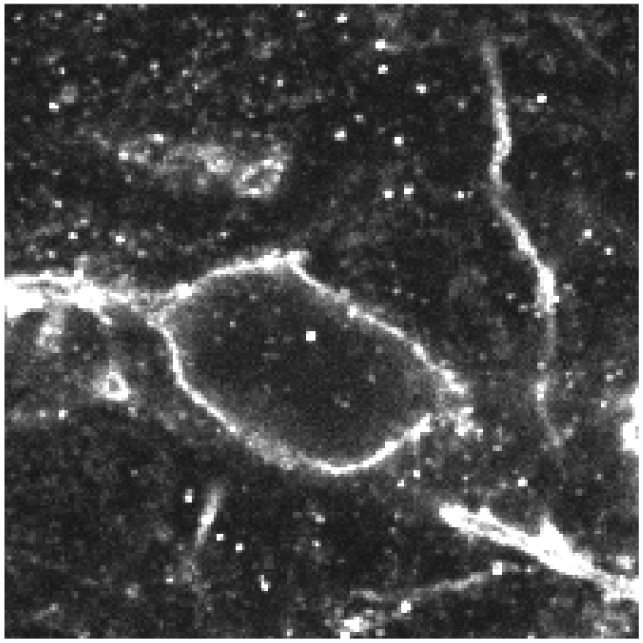
When there is more than one possibility, think about the experiment(s) you would do to discriminate between them.

Paper for Monday:

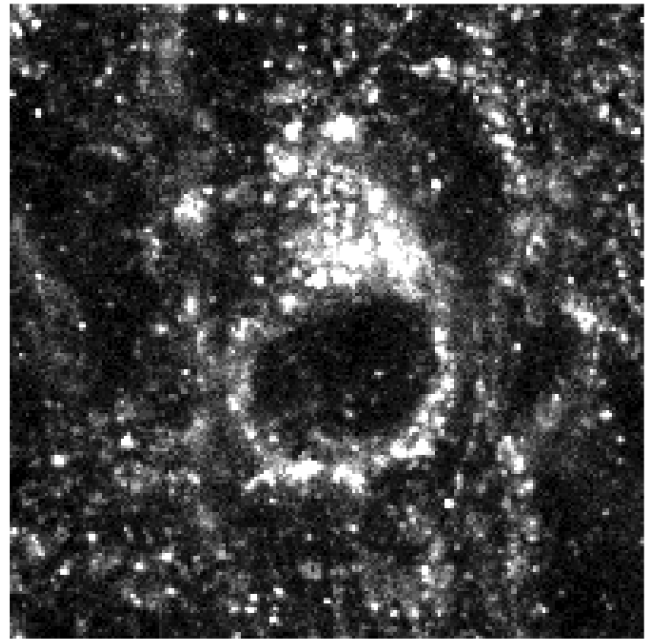
Fribourg et. al
Decoding the Signaling of a GPCR Heteromeric Complex Reveals a Unifying Mechanism of Action of Antipsychotic Drugs
Cell 147, 1011–1023, 2011

I'll also post some papers related to this story,
a review on biased agonism,
and a recent paper from my lab that combines biased agonism, heteromerization and behavioral plasticity

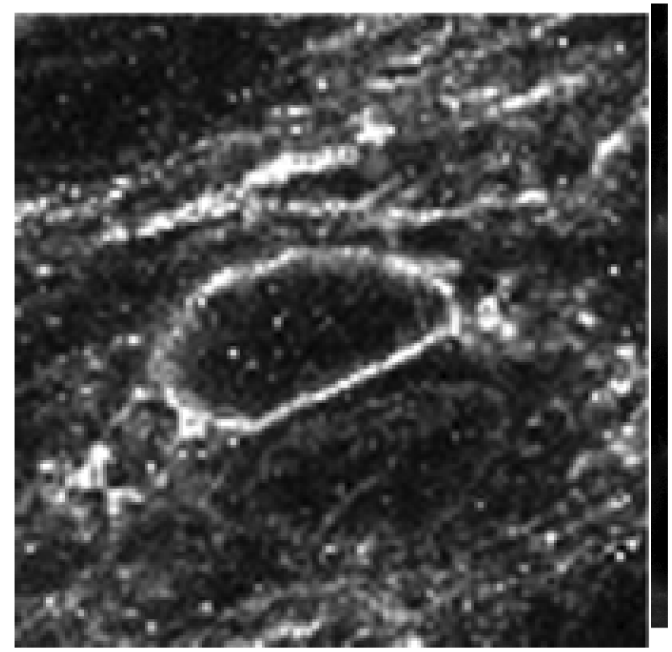
The MOR shows agonist-specific endocytosis
Or "biased agonism" "functional selectivity"



No treatment



Enkephalin



Morphine

"R" "R*" "R**"

$$L + R \rightleftharpoons LR^* \xrightarrow{\neq} \begin{matrix} \text{G protein} \\ \text{arrestin/endocytosis} \end{matrix}$$

How to distinguish "biased agonism" from partial agonism?

Keith et al, JBC 1996