

Opioid Receptors: Oligomerization and Desensitization

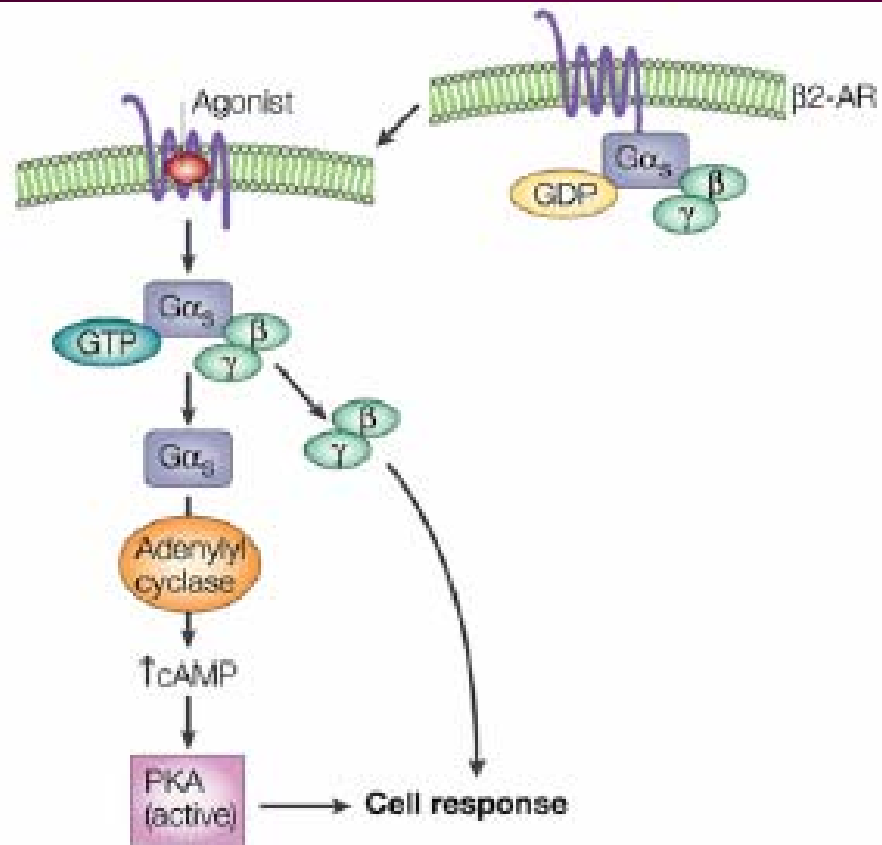
Featuring:

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Jennifer L Whistler.**

**Regulation of Opioid Receptor Trafficking and
Morphine Tolerance by Receptor Oligomerization.**

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Presented By Nadya Modyanova



Opioid receptors

- G Protein Coupled Receptors (GPCRs)
- Three main classes of opioid receptors:
 - Mu, Kappa, Delta
- Mediate physiological functions:
 - Nociceptive (pain) information, with overlapping contributions of mu, delta, kappa receptors
 - Hedonic (pleasure) homeostasis, via reinforcing properties of mu and delta agonists and aversive activity of kappa compounds
 - Stress responses with implication of mu and delta receptors.
 - Also involved in physiological responses, e.g. respiration,
 - gastrointestinal motility, endocrine and immune functions.

Opioid Receptors: ligands

- Acted on by Exogenous opiates (for 1000's of years)
 - Opium (produces euphoria, relieves pain)
 - Morphine (isolated from poppy seed, relieves pain)
 - Heroin (synthesized via morphine diacetylation, drug of abuse)
- Acted on by Naturally Occurring (endogenous) peptides (since 1978)
 - ⇒ *In 1978 , Solomon H Snyder, J Hughes, and Hans W Kosterlitz were awarded the Lasker award for their combined discovery of opiate receptors in the brain and for the discovery of the existence of endogenous ligands for opiate receptors which shared all the physiologic properties of exogenous opiates.*
 - Enkephalin - selective agonist for the delta-opioid receptors.
 - Beta-endorphin – runner's high - selective agonist for the mu-opioid receptors, also involved in glucose regulation.

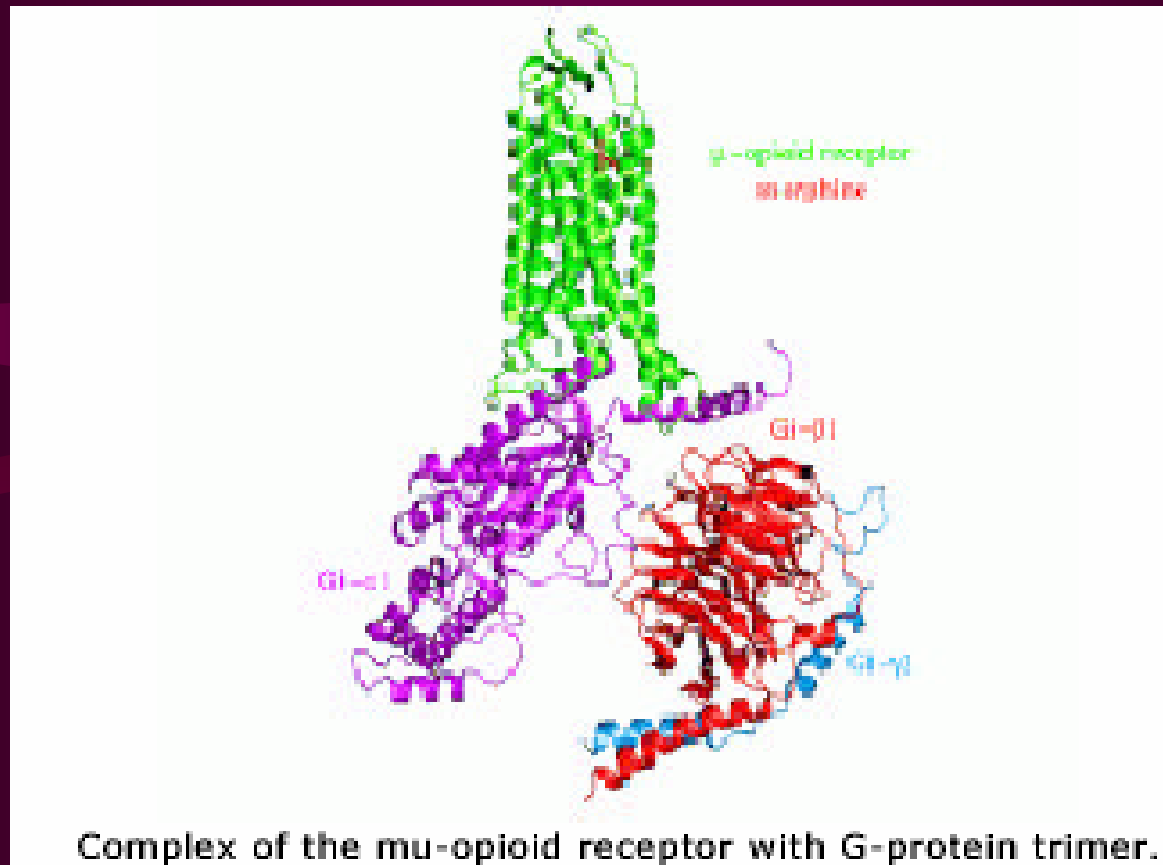
Opioid Receptors: evidence for subtypes

- Evidence for multiple receptors came from the demonstration of different profiles of pharmacological activity in the chronic spinal dog with the prototype agonists morphine, ketazocine and N-allylnormetazocine (SKF 10047).
- Genes encoding for these receptors have been cloned.
- Mice lacking every component of opioid system have been generated

Opioid Receptors: location

- Opioidergic neurons are particularly concentrated in the ventral tegmental area. The VTA is an important nerve tract in the limbic system. The VTA passes messages to clusters of nerve cells in the nucleus accumbens and the frontal cortex. This forms the brain's primary reward pathway, the mesolimbic dopamine system containing dopaminergic neurons.
- Mu-receptors are found mainly in the brainstem and the medial thalamus, in descending inhibitory pathways to the spinal cord. Descending fibers are disinhibited by MOR by MOR's inhibiting spontaneous GABA release for local interneurons, which activates voltage sensitive potassium conductance

Complex of the mu-opioid receptor with G-protein trimer .



interaction surface for G protein coupling to GPCRs is composed of multiple contacts with transmembrane helices 3, 5, 6, and intracellular loops 2, 3, and 4.

Opioid Receptors: action

- Opioid receptors presynaptically inhibit transmission of excitatory pathways.
- All of the cloned opioid receptor types belong to the Gi (inhibitory)-coupled superfamily of receptors
- Effector mechanisms:
 - inhibition of adenylyl cyclase
 - Inhibition of neurotransmitter release
 - activation of an inwardly rectifying potassium channel by κ -receptors in some cell types
 - Decreased neuronal excitability
 - inhibition of voltage operated calcium channels
- In contrast Beta adrenergic receptors are coupled to Gs (stimulatory G protein)

Other Consequences of opioid-evoked changes in other effector pathways

- increases in neuronal firing rate
 - inhibition of inhibitory transmitter release - disinhibition
- changes in gene expression
 - long-term changes in adenylyl cyclase activity
 - elevation of intracellular calcium levels,
 - activation of cAMP response element binding protein (CREB))

Opioid dependency...

- Tolerance represents a reduced effect upon repeated exposure to a drug at a constant dose, or the need for an increased dose to maintain the same effect.
- Dependence is defined as the need for continued exposure to a drug so as to avoid a withdrawal syndrome (physical or psychological signs) when the drug is withdrawn.
- Addiction is defined as the compulsive use of a drug despite its adverse consequences.

Opioid dependency...

- Opiate drugs, e.g. heroin, are readily self-administered intravenously by animals
- When provided an opiate for a few hours per day, rats and primates maintain stable daily opiate intake without major signs of physical dependence when not taking the drug
- Both systemic administration and central administration of competitive opiate antagonists into the nucleus accumbens and ventral tegmental area decrease intravenous opiate self administration.
- The reinforcing actions of heroin and morphine appear to be mediated largely by the mu-opiate receptor subtype.
 - Mu-opioid antagonists produce dose-dependent decreases in heroin reinforcement
 - mu-knockout mice show loss of morphine-induced reward and loss of morphine induced analgesia.

Gene knockouts

(Kieffer & Gaveriaux-Ruff 2002)

- Mice lacking every component of opioid system have been generated, all viable, fertile and no overt anatomical deficits.
- Opioid peptides and receptors show overlapping distributions (redundancy possible)
- in all mutants, anatomical distribution of remaining opioid receptor sites was unchanged
- Therefore the absence of one opioid receptor does not drastically alter expression of other ones
- However absence of one component of opioid system during development would induce adaptive changes to maintain homeostasis of opioid system or activity of other functionally associated neurotransmitter systems.
- Possible adaptation at level of receptor coupling efficiency, rather than ligand binding.

MOR-/-

- Mice lacking mu receptors all normal responses to morphine abolished
 - No binding of agonists (DAMGO)
 - Unchanged or slightly reduced locomotion
 - Increased sensitivity in three different responses to heat, suggesting existence of MOR mediated tone in thermal nociception
 - E.g. tail immersion, tail flick, hot plate
 - Sensitivity to mechanical pain, as determined by response to pressure, was unaltered
 - Decreased pain perception in response to acetic acid
 - Recover quicker from induced hyperalgesia.
 - Decreased LTP in dentate gyrus of hippocampus but did not induce any change in CA1 region.

DOR -/-

- Reduced responses to thermal pain
- Decreased mechanical pain
- However full effects only in MOR & DOR deficient mice
- Therefore Mu receptors are required for full responses to delta agonists...

In vivo responses to mu-opioid agonists in mice lacking MORs (in Keiffer & Gaveriaux-Ruff 2002)

Opioid	Responses	MOR-/-
Morphine	Analgesia	Abolished (Matthes et al., 1996; Sora et al., 1997; Loh et al., 1998; Schuller et al., 1999; Sora et al., 1999; Fuchs et al., 1999)
	Tolerance to analgesia	
	Hyperlocomotion	Abolished (Tian et al., 1997; Sora et al., 2001; Becker et al., 2000)
	Reward	Abolished (Matthes et al., 1996; Sora et al., 2001)
	Self-administration	Abolished (Sora et al., 2001; Becker et al., 2000)
	Withdrawal	Abolished (Matthes et al., 1996; Sora et al., 2001)
	Respiratory depression	Abolished (Matthes et al., 1998; Dahan et al., 2001)
	Inhibition of GI transit	Abolished (Roy et al., 1998a)
	Inhibition of VD twitch	Abolished (Maldonado et al., 2001)
	Immunosuppression	Abolished (Gaveriaux-Ruff et al., 1998; Roy et al., 1998b)*
	Increase in ACTH and corticosterone	Abolished (Roy et al., 2001)
Heroin	Potentiation of pentobarbital and apomorphine responses	Abolished (Park et al., 2001; Jung et al., 2000)
MSG	Analgesia	Abolished (Kitanaka et al., 1998) Maintained (Schuller et al., 1999)
Methadone	Analgesia	Abolished (Schuller et al., 1999)
Endomorphins	Analgesia	Abolished (Loh et al., 1998; Mizoguchi et al., 1999)
DAMGO	Analgesia	Abolished (Schuller et al., 1999; Qin et al., 2000)
	Inhibition of VD twitch	Abolished (Maldonado et al., 2001)
	Respiratory depression	Abolished (Morin-Surun et al., 2001)

Biochemical basis of opiate dependency

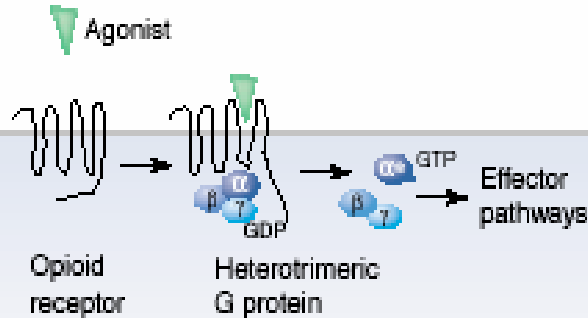
- The discovery of endogenous opiate receptors raised the possibility that opiate addiction might be mediated by changes in these receptors. A decade of research has failed to identify changes in the number of opioid receptors and also changes in levels of endogenous opioid peptides.*
- Pharmacological and biochemical studies have indicated that modulation of LC neuronal firing rates contributes to the physical aspects of opiate addiction, namely physical dependence and withdrawal in several species.*
- Chronic administration of opiate leads to upregulation of the cAMP system, which increases the intrinsic excitability of LC neurons, and thereby accounts, at least in part, for opiate tolerance, dependence, and withdrawal exhibited by these neurons. In the opiate-tolerant/dependent state, the combined presence of the opiate and the upregulated cAMP system would return LC firing rates toward pretreatment levels, whereas removal of the opiates would leave the upregulated cAMP system unopposed to withdrawal activation of the neurons.*

Desensetization

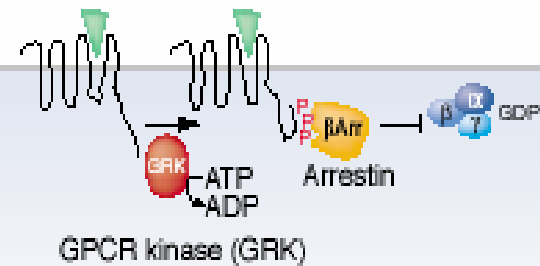
- E.g. when a given receptor is phosphorylated, it can still bind its ligand, but cannot activate adenylyl cyclase or increase the cAMP level
- Removal from cell surface - endocytosis
 - Via phosphorylation by GPK (GPCR kinase), binding of beta-arrestin, and initiation of clathrin vessicle formation by Phospholipase D2 (Koch et al 2004)
 - Once the receptors are concentrated in clathrin-coated pits, dynamin causes fission from plasma membrane and fusion with endosomes.
 - *PLD2 – a widely distributed phospholipid-specific diesterase that hydrolyses phosphatidylcholine to phosphatidic acid and choline and is assumed to play an important function in cell regulation and receptor trafficking*
 - *Activation of phospholipase D 2 is required for agonist-induced mu-opioid receptor endocytosis*

Series of events occurring after agonist-induced activation of opioid receptors.

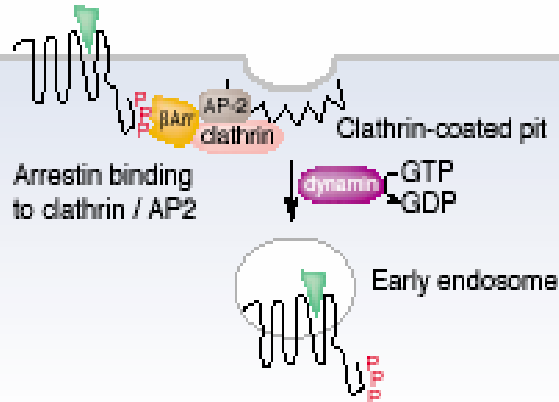
(a) Acute signaling



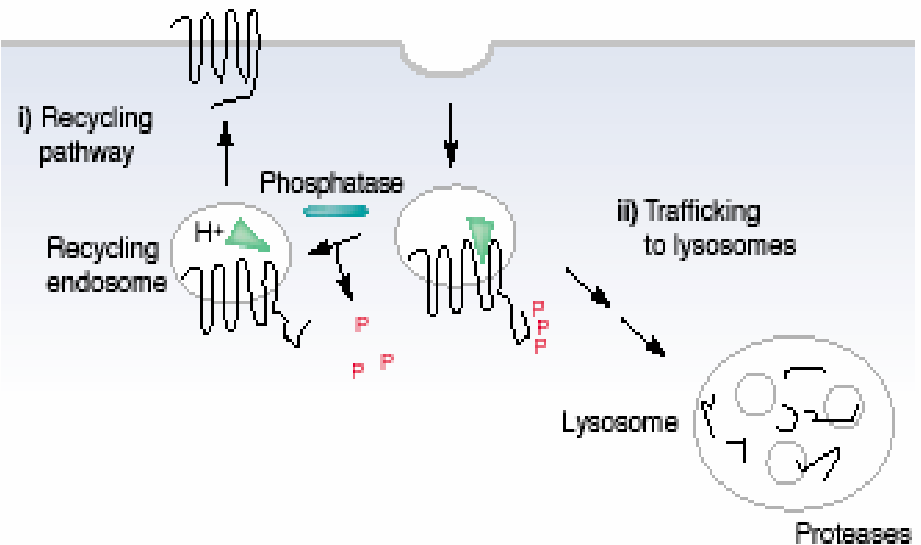
(b) Rapid desensitization



(c) Endocytosis



(d) Post-endocytic sorting



Series of events occurring after agonist-induced activation of opioid receptors

- (a) Acute signaling: receptor-mediated activation of heterotrimeric G proteins. The triangular agonist could be either an opioid peptide or a drug. The heterotrimeric G protein could be either G_i or G_o . The effector pathways could be ion channels, adenylyl cyclase or kinase cascades. (b) Rapid desensitization: phosphorylation and arrestin binding prevent activation of G proteins. (c) Endocytosis: arrestin promoted concentration of receptors in a clathrin coated pit and dynamin dependent formation of endocytic vesicles. (d) Post-endocytic sorting (i) Recycling to the plasma membrane termed 'resensitization'. (ii) Trafficking to lysosomes termed 'down-regulation'. Zastrow et al 2003.

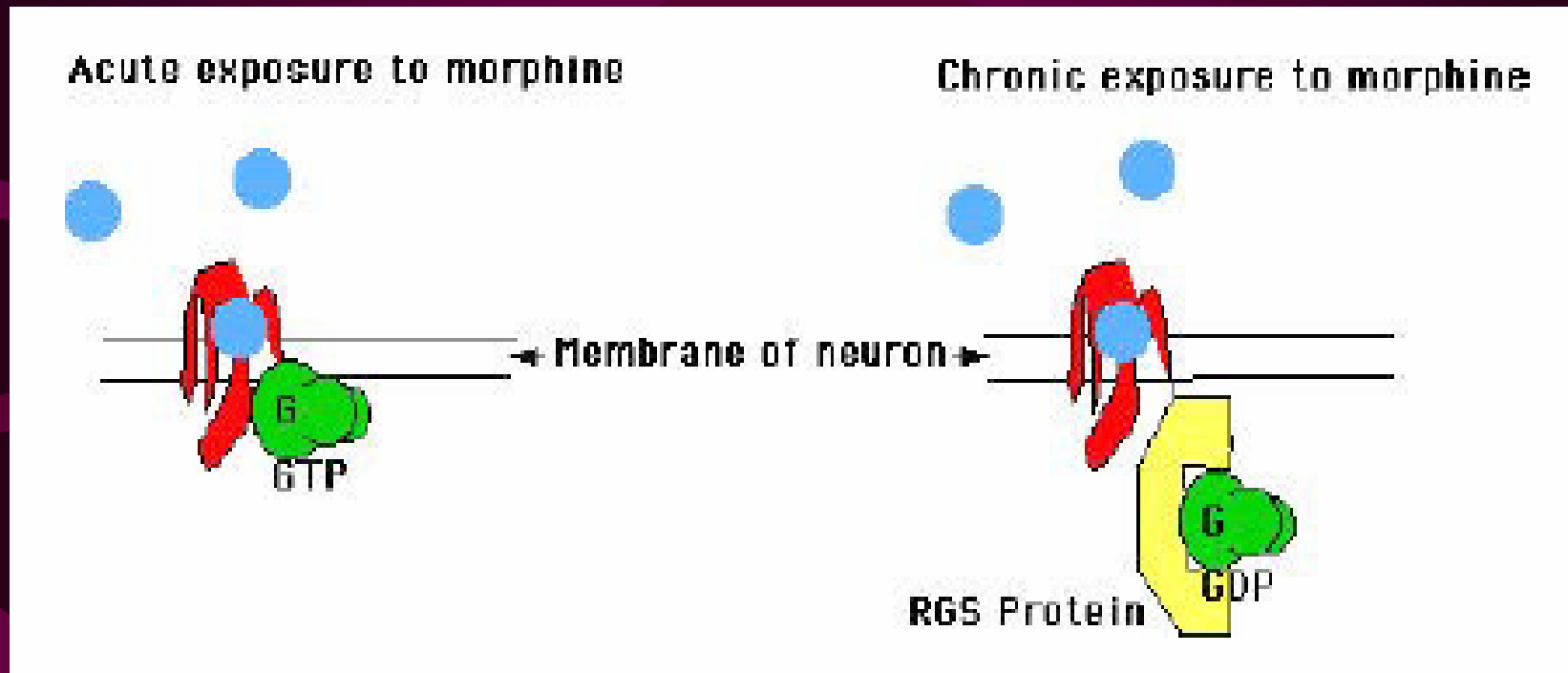
How might endocytosis (I.e. desensitization) inhibit the development of tolerance?

- Traditional view on opioid tolerance:
- Desensitization and endocytosis contributes to physiological tolerance by reducing number of functional receptors

Morphine

- Morphine, highly addictive opioid, can activate mu-opioid receptors, but does not induce receptor internalization
- Morphine does not cause endocytosis because:
- Morphine restrains the receptor in a conformation that is a poor substrate for GPCR kinases and subsequent beta-arrestin binding.

Morphine does not induce endocytosis



Morphine fails to fit the traditional view

- Does not promote endocytosis of MOR
 - Does not cause downregulation of MOR
 - Does cause tolerance
- Why does morphine cause tolerance then???

Morphine tolerance - abuse of natural feedback mechanism

- Can occur due to superactivation of adenylyl cyclase signaling pathway, which masks morphine's effect by altering the homeostatic baseline of MOR expressing cells.
- Resulting cellular changes:
 - Increased expression of adenylyl cyclases, PKA, CREB
- Changes try to compensate for continued inhibition of adenylyl cyclase, serve to increase amount of signaling through the cAMP pathway and thus subvert the effect of morphine.
- Superactivation after drug withdrawal indicates that MORs are still coupled to second messenger cascades when drug is present

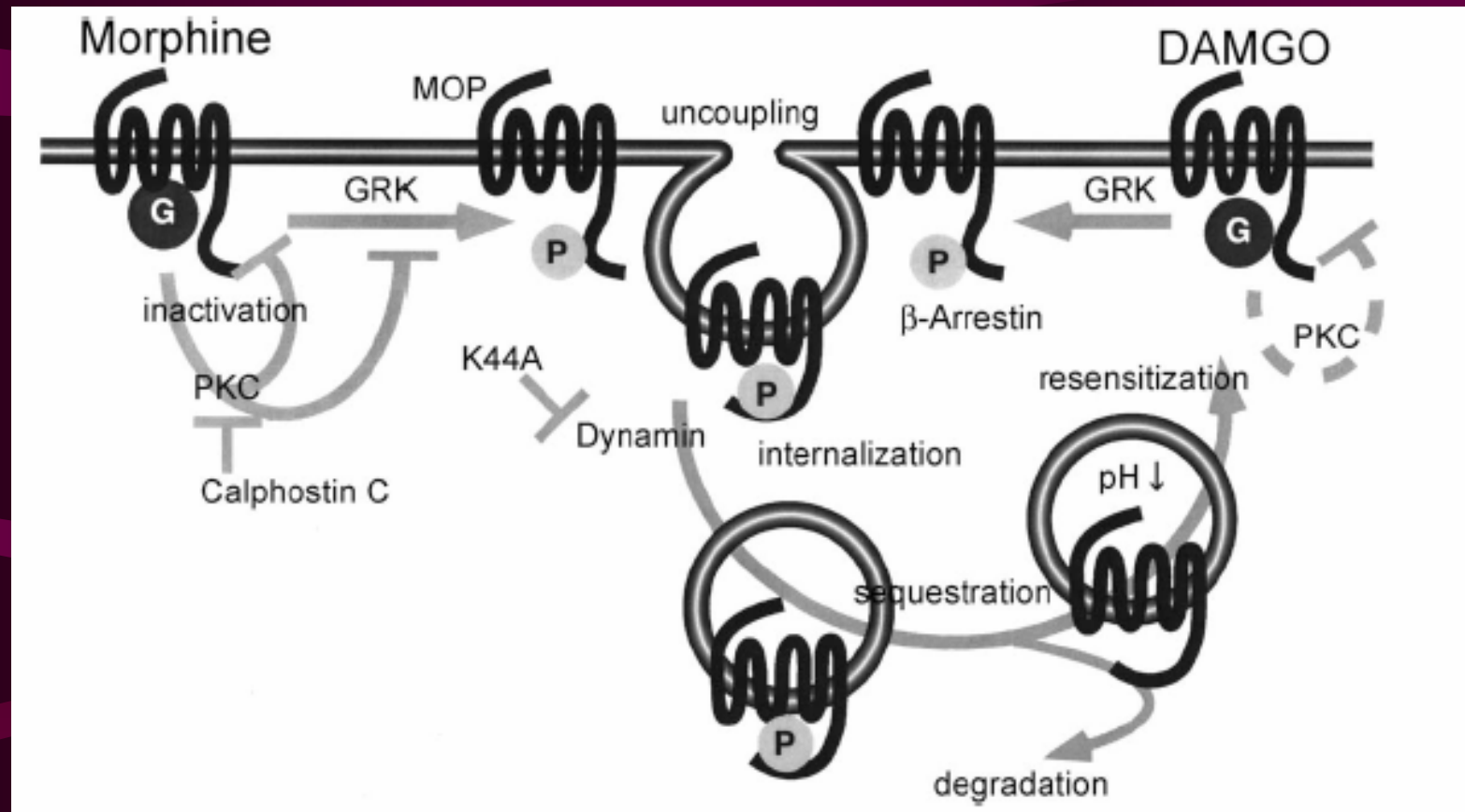
New hypothesis (He et al 2002)

- Endocytosis actually plays a protective role, in recycling receptors, protecting them from continuous activation/overstimulation, prevent adaptive processes eventually leading to dependence
- RAVE = relative activity versus endocytosis
 - Net amount of signal transmitted to the cell as a result of agonist activity and receptor endocytosis.
- Morphine = high RAVE value, therefore can't cause endocytosis
- Endocytosis indeed contributes to functional desensitization of signal transduction by reduction the number of receptors present in the plasma membrane
- But at the same time Contributes to functional resensitization of signal transduction by promoting dephosphorylation and recycling of receptors to the plasma membrane.

DAMGO

- Hydrolysis-resistant derivative (analog) of enkephalin
- selectively binds to the mu opioid receptor
- used as a model for drug permeability experiments.
- Independently known to promote endocytosis of MOR
- Affinity for MOR similar to that of Morphine

Schematic model for acute morphine tolerance based on PKC and RAVE hypotheses.

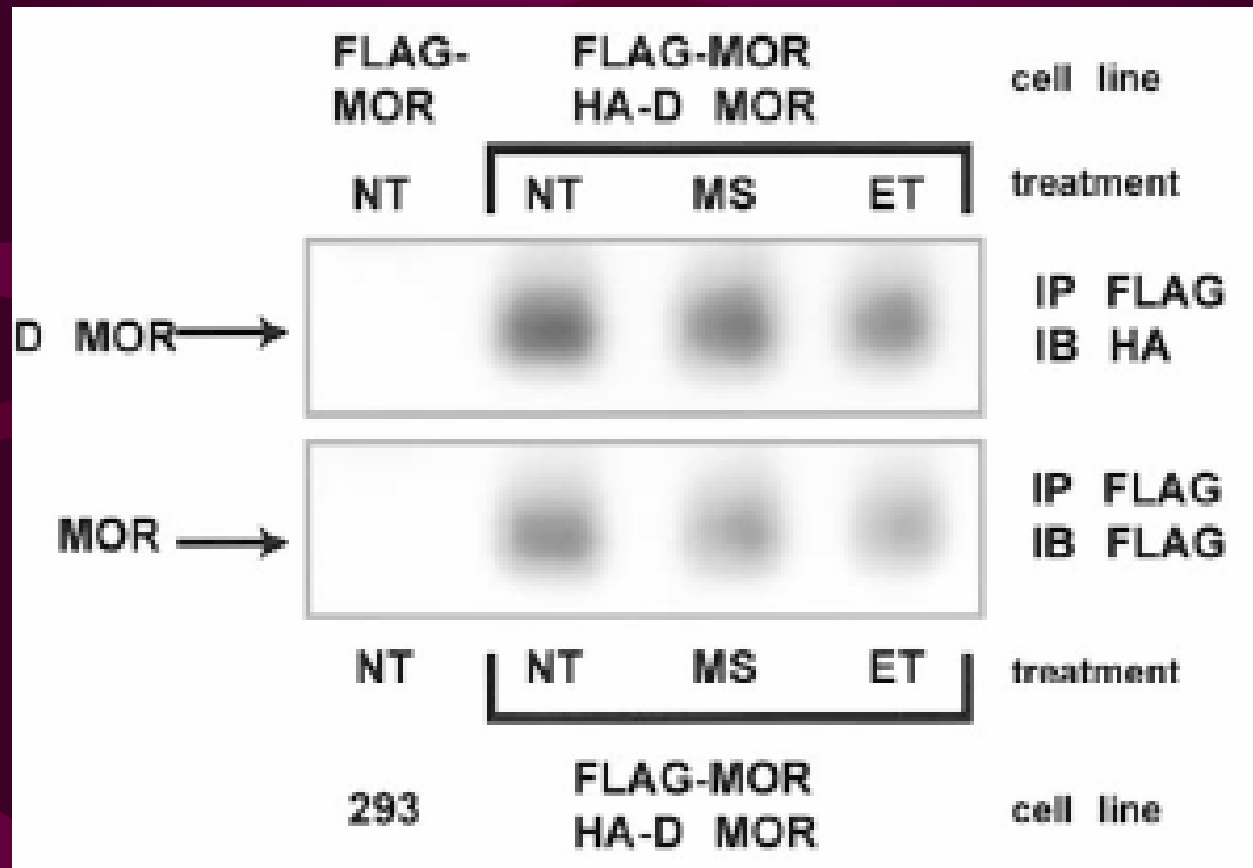


The addition of DAMGO, which has low relative activation versus endocytosis (RAVE) activity causes MOP stimulation, Gi activation and MOP phosphorylation by GRK. The GRK-phosphorylated MOP is then attacked by beta-arrestin, clathrin and dynamin to be endocytosed. During the period of endosomal stage, MOP is dephosphorylated, followed by sequestration into the membrane as a functional receptor. On the other hand, the addition of morphine causes a PKC-phosphorylation of MOP, and prevents GRK phosphorylation and endocytosis. MOP-function will be desensitized by PKC-phosphorylation. When endocytosis mechanism is inhibited by introducing dynamin negative mutant, DAMGO-stimulated MOP receptor remains in the membrane, and followed by phosphorylation and desensitization by PKC. Ueda et al 2003

Presence of flags in HEK293 cells

DMOR = chimera capable of endocytosis with morphine.

MOR = wild type, incapable of endocytosis.



DIFFERENT FLAGS DISTINGUISH DIFFERENT RECEPTORS.
TOP = DETECT BOTH; BOTTOM = DETECT MOR

- How quantitative is this coIP assay?
- Can one assess how much of one subunit is associated with the other?
- How can this experiment be improved?

Human Embryo Kidney cells

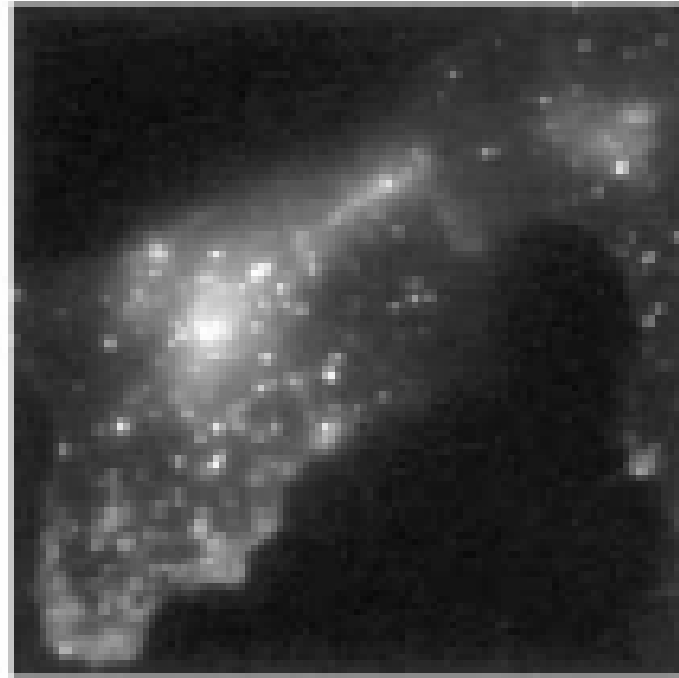
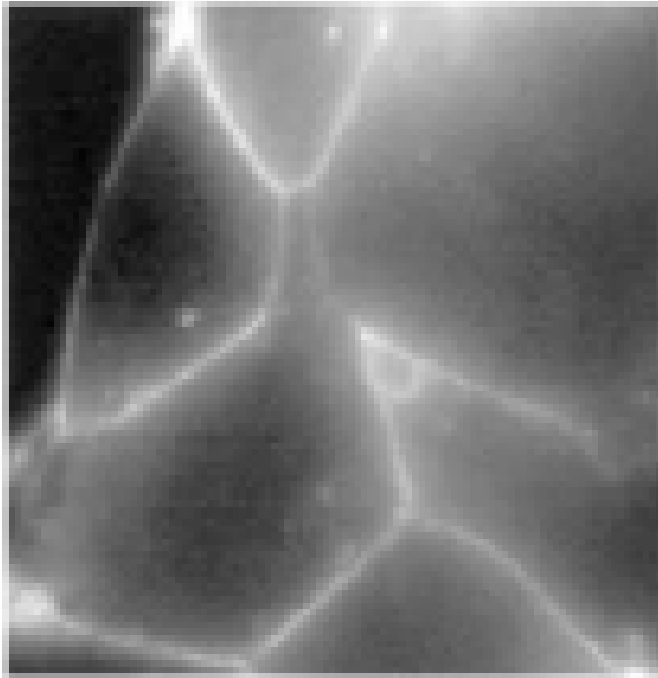
with 5 μM morphine treatment

Only receptors initially on the cell surface were detected

F-MOR

HA-D MOR

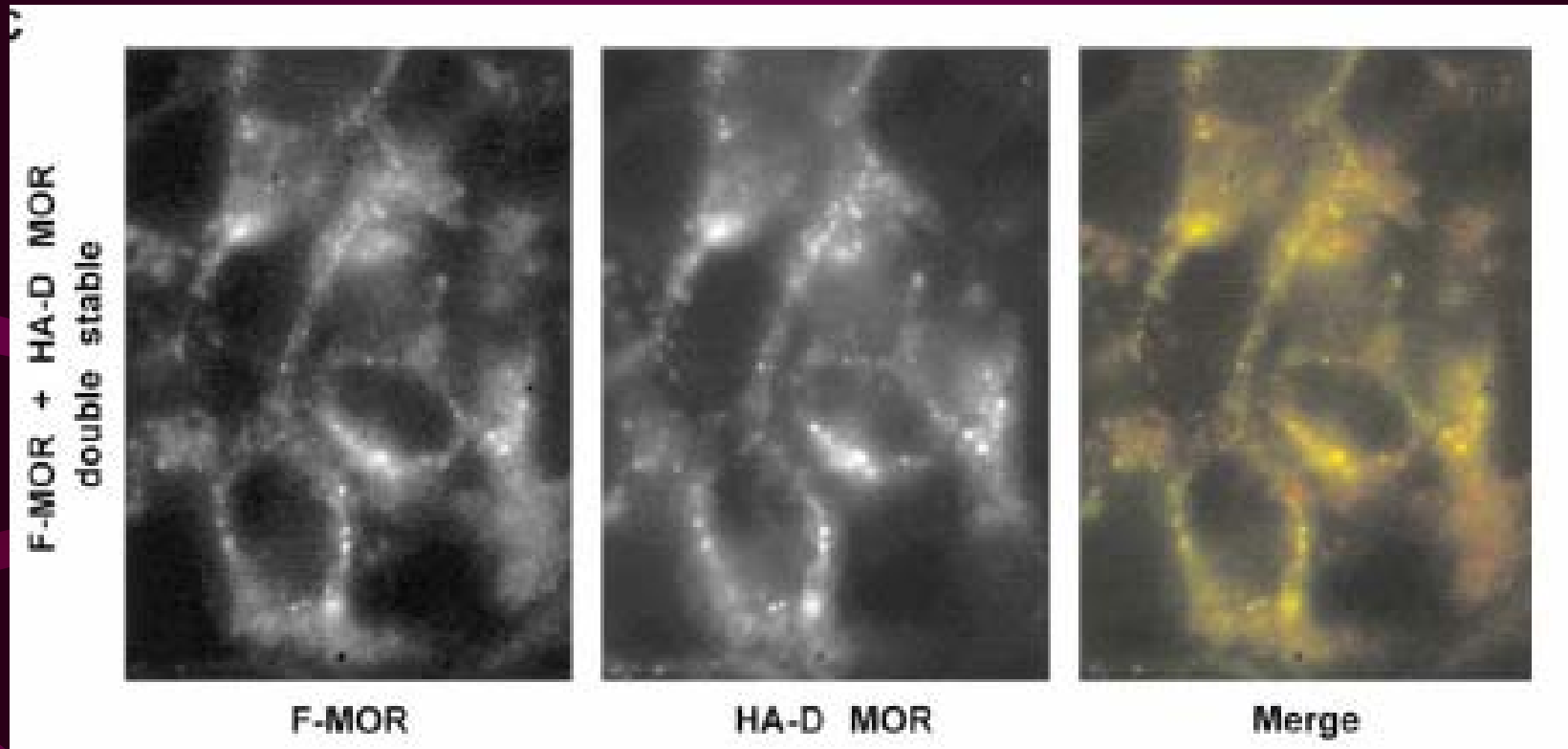
single stables



Cell surface

In endocytic vesicles

Human Emrbyo Kidney cells with 5 microM morphine treatment



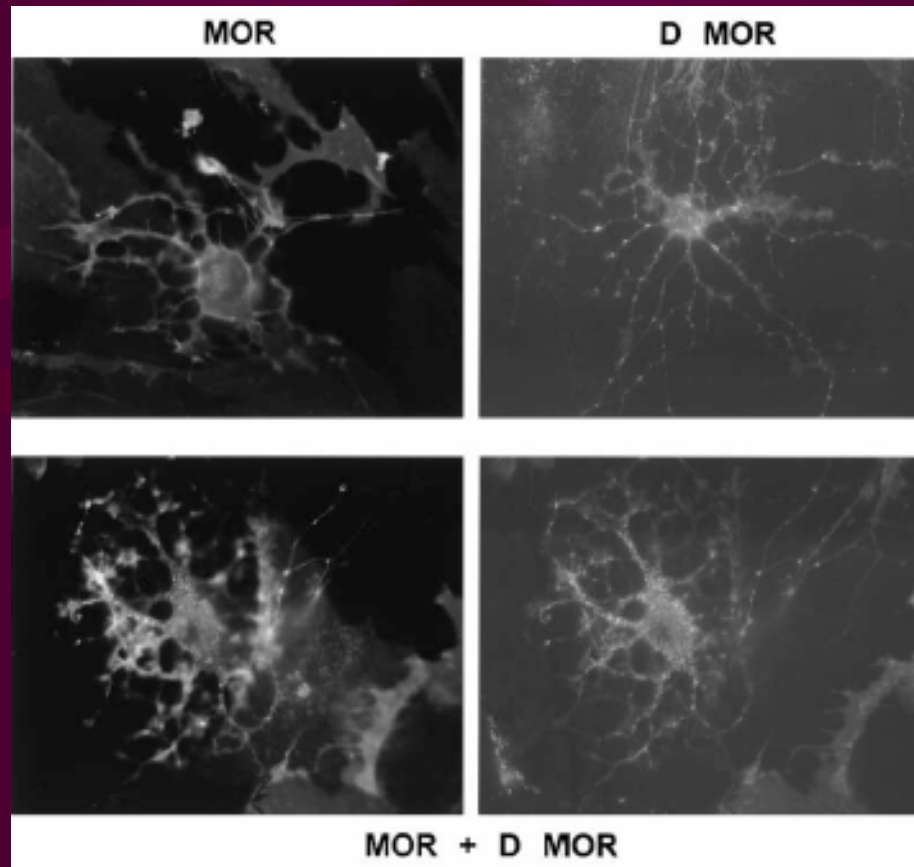
In cells expressing both DMOR and MOR, both receptors were redistributed to endocytic vesicles. Colocalization in yellow

- Why would expression of both receptors in the same cells effect endocytosis?
- This can happen only if receptors somehow bind together - oligomerize, such that activation of one receptor is 'enough' to cause endocytosis of all receptors attached.
⇒ 'dragging'

- What is the antibody feeding assay?
- Why epitope tag the receptors?

Rat hippocampal cells
transfected with MOR, DMOR, or both,
with 5 microM Morphine treatment.

On Cell
surface

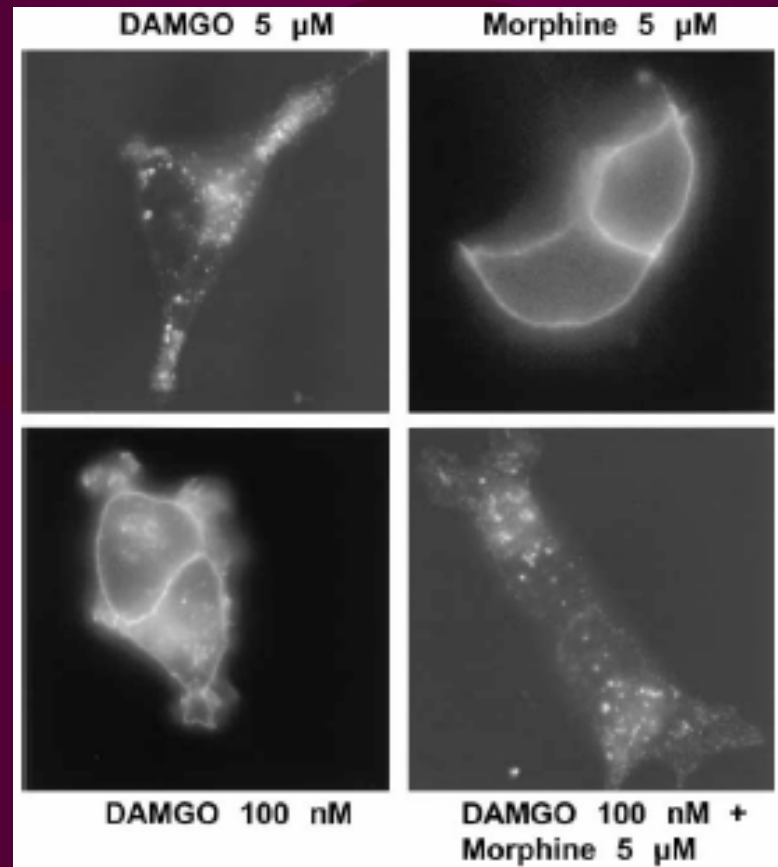


In Endocytic
vesicles

Both receptors redistributed to endocytic vesicles

- It is not clear whether rat hippocampal cells have endogenous MOR.
- Poor quality of experiment, culture quality was suboptimal

Human Embryo Kidney cells, expressing MOR with 5 microM morphine or DAMGO treatment



Saturating dose -
endocytosis

Subsaturating dose
- reduced
endocytosis

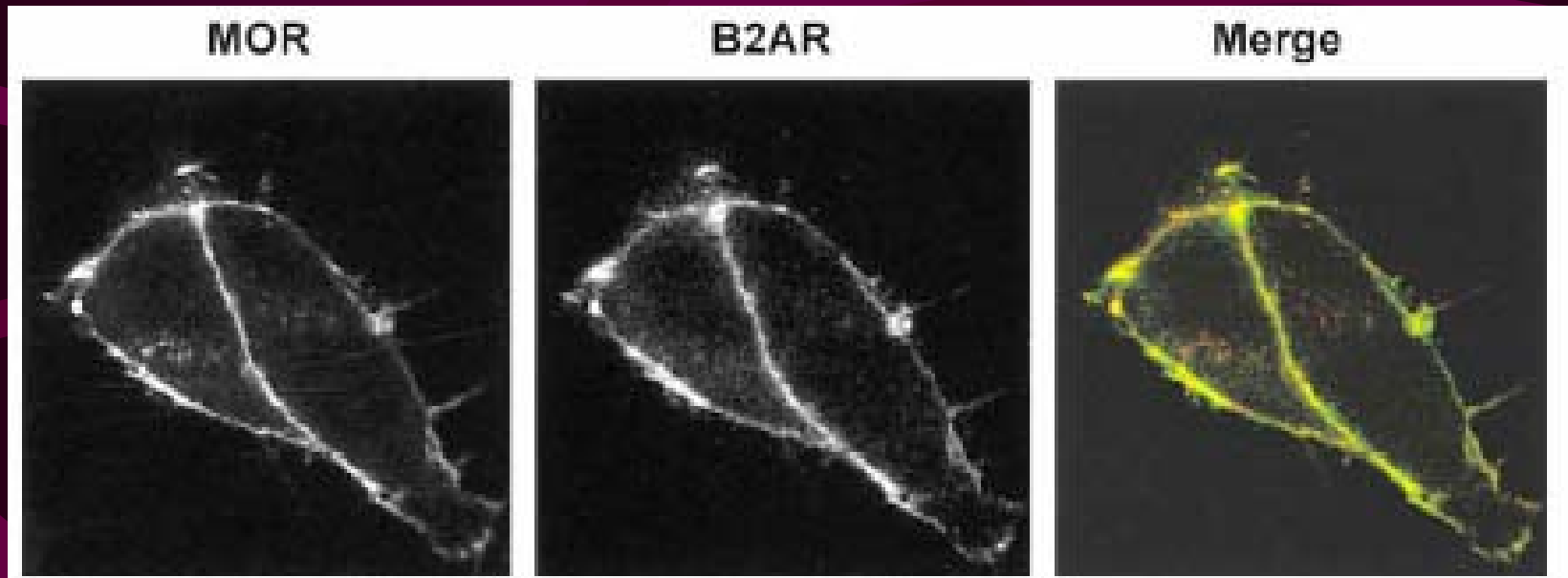
On cell surface

Subsaturating dose
DAMGO + saturating
dose Morphine
facilitates endocytosis

Previous slide can be explained in two ways

- DAMGO is required to be part of the oligomer complex to help endocytosis
 - merging MOR with a different receptor WILL disrupt DAMGO-facilitated endocytosis
- DAMGO can just hang around, as a helper molecule, but does not have to bind
 - Merging MOR with a different receptor WILL NOT disrupt DAMGO-facilitated endocytosis

Human Embryo Kidney cells, expressing MOR or B2AR,
no treatment, receptors on cell surface

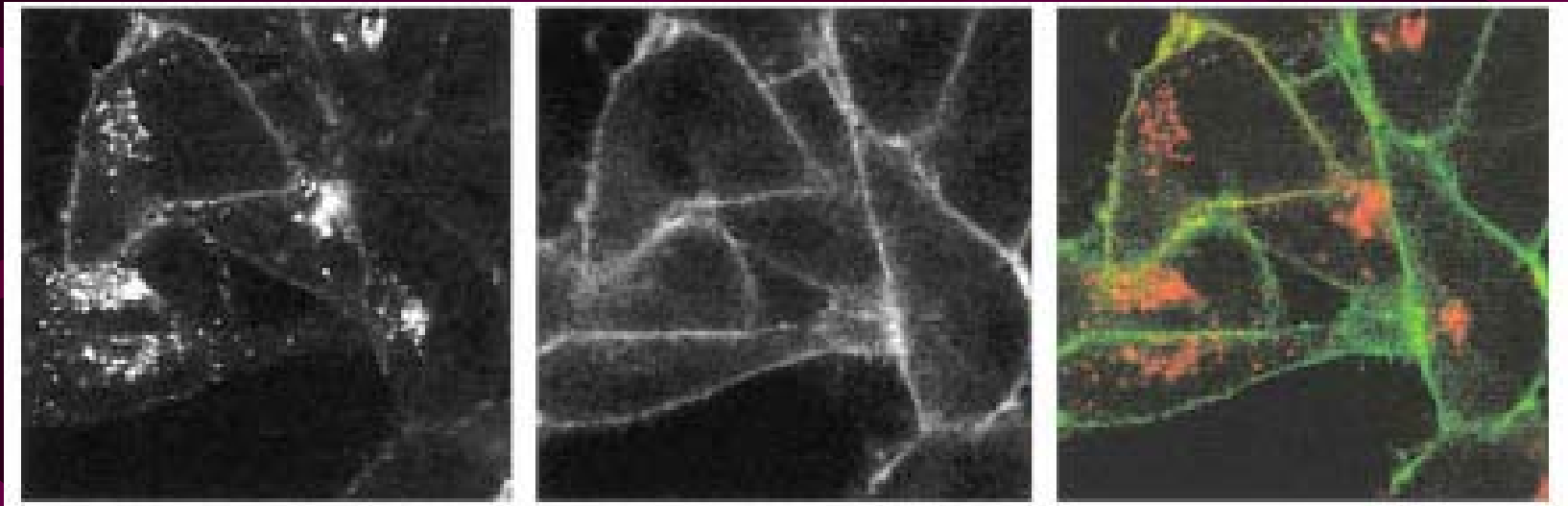


Human Emrbyo Kidney cells, expressing MOR or B2AR,
treated with DAMGO
only MOR expressing cells show endocytosis

MOR

B2AR

Merge

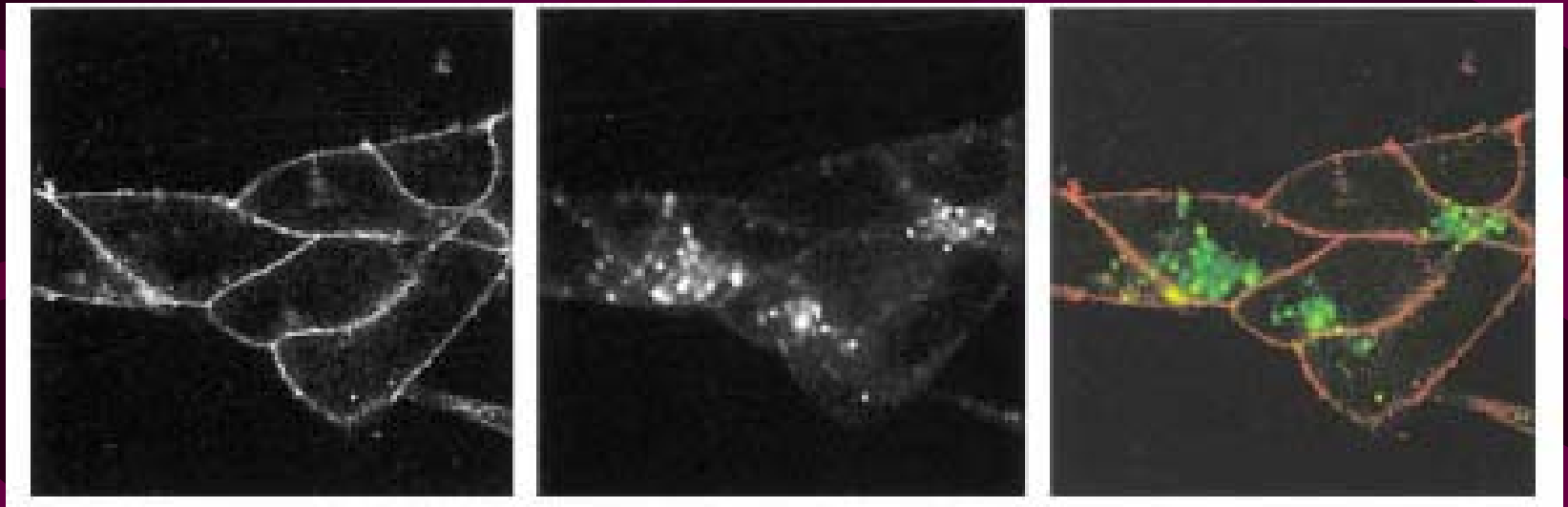


Human Embryo Kidney cells, expressing MOR or B2AR,
treated with Isoproterenol (promotes B2AR endocytosis)
only B2AR expressing cells show endocytosis

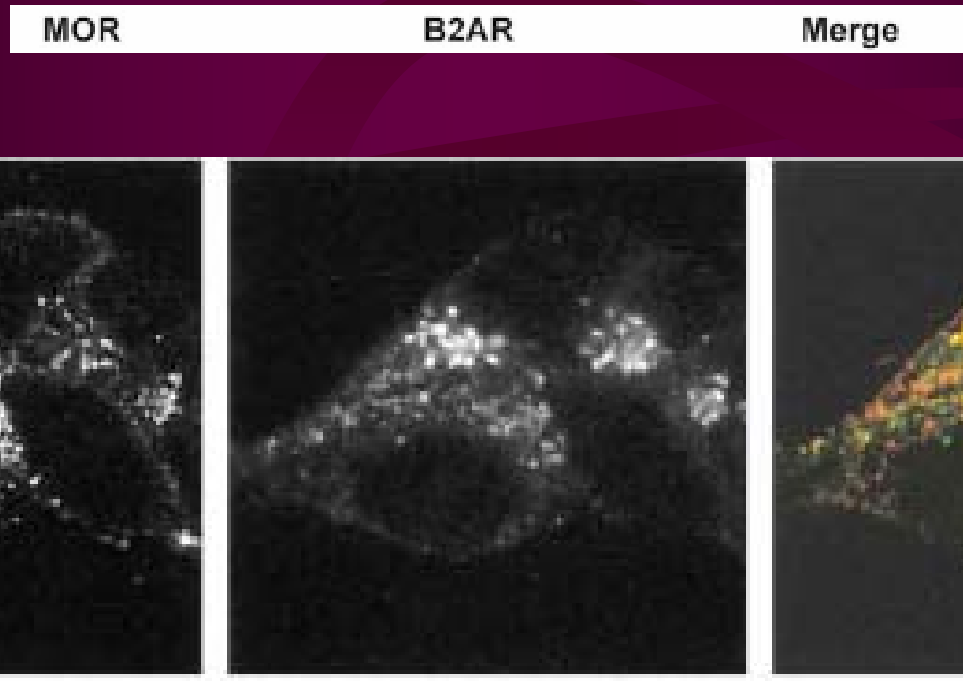
MOR

B2AR

Merge



Human Emrbyo Kidney cells, expressing MOR or B2AR,
treated with Isoproterenol and DAMGO
all cells show endocytosis

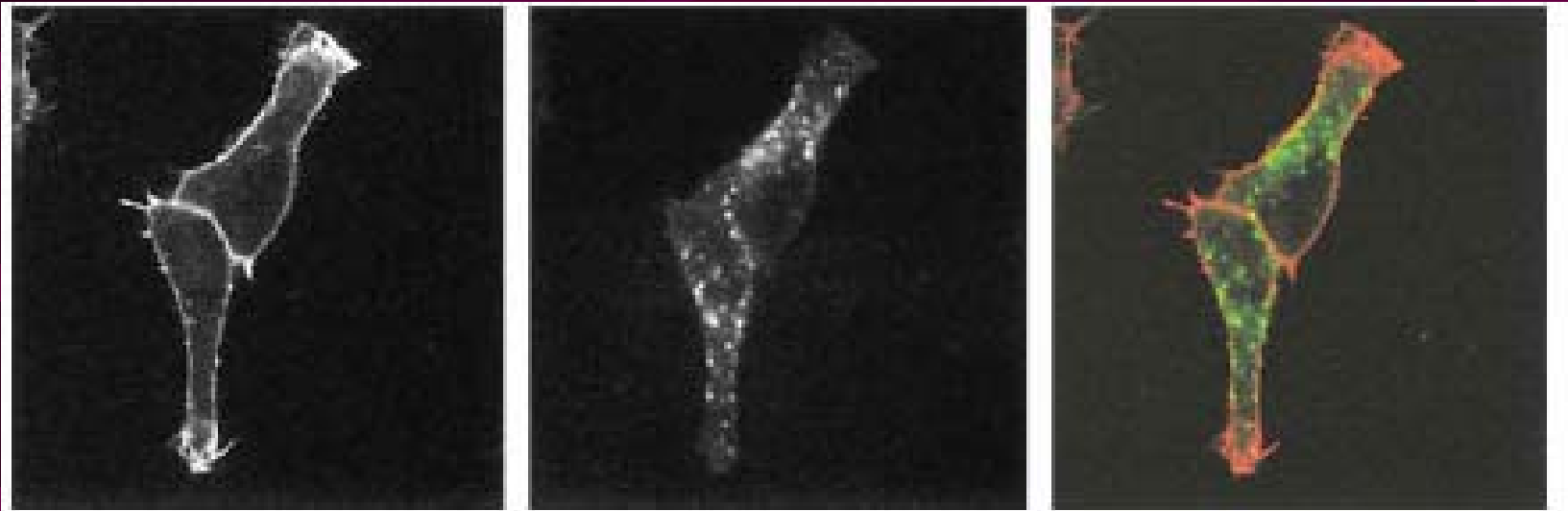


Human Embryo Kidney cells, expressing MOR or B2AR,
treated with Isoproterenol and Morphine
B2AR expressing cells fail to drag in MOR

MOR

B2AR

Merge



B2AR vs MOR tests

- Activating B2AR receptor and consequent membrane recruitment of arrestin is insufficient to promote endocytosis of nearby MORs
- Therefore, MORs must be in oligomeric complex, in order to house both Morphine and DAMGO, for dragging to be efficient

DAMGO facilitates morphine-induced endocytosis of MOR....

- How do authors envisage this working with subsaturating concentrations of DAMGO???
- Is the beta2-adrenoreceptor negative control convincing?
- Are there any other mechanisms of dragging independent of receptor oligomerization.?

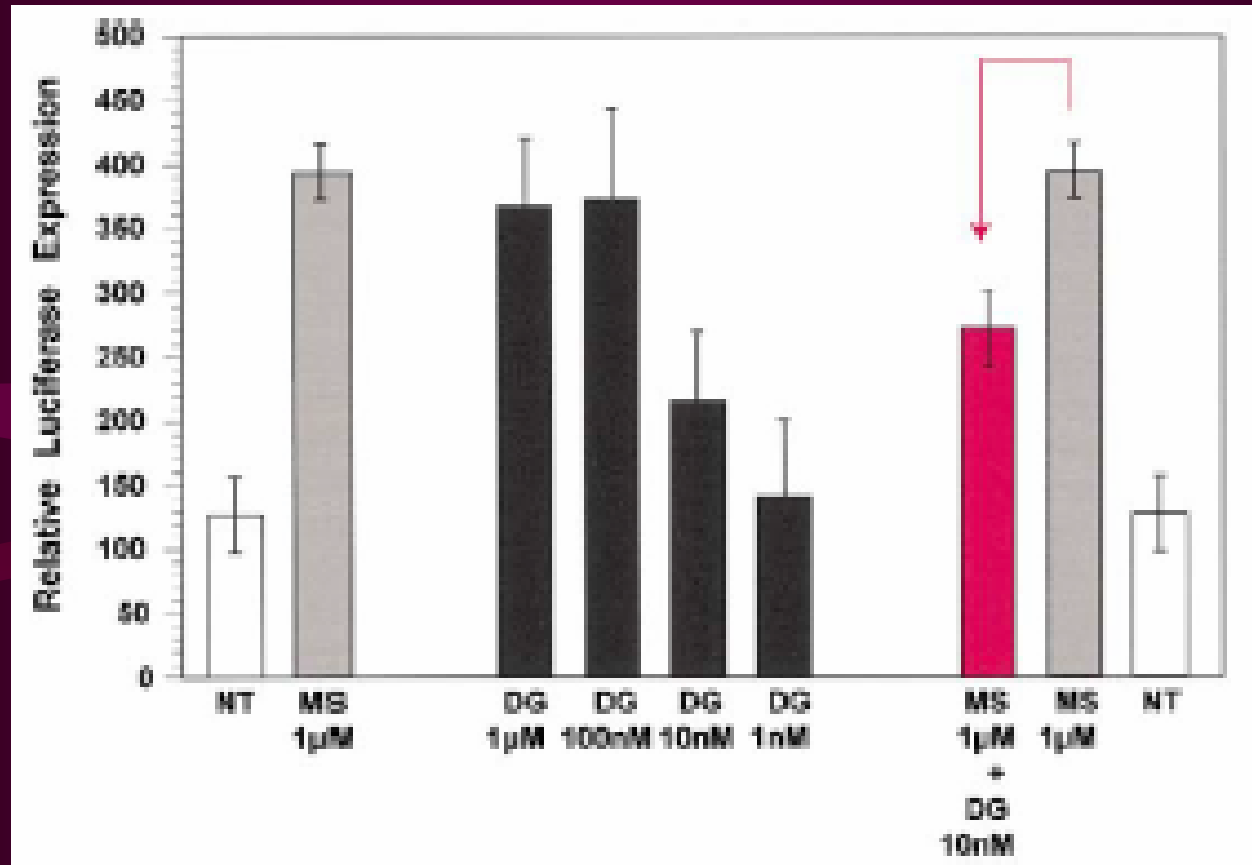
Cellular changes

- Chronic morphine treatment produces compensatory upregulation of cAMP pathway.
- Receptor dragging could reduce superactivation in cell culture model
- Use cells Expressing MOR and CRE-luciferase reporter (expression of which is increased with increased activation of cAMP)

Morphine tolerance - abuse of natural feedback mechanism

- Can occur due to superactivation of adenylyl cyclase signaling pathway, which masks morphine's effect by altering the homeostatic baseline of MOR expressing cells.
- Resulting cellular changes:
 - Increased expression of adenylyl cyclases, PKA, CREB
- Changes try to compensate for continued inhibition of adenylyl cyclase, serve to increase amount of signaling through the cAMP pathway and thus subvert the effect of morphine.
- Superactivation after drug withdrawal indicates that MORs are still coupled to second messenger cascades when drug is present

DAMGO reduced morphine-induced cAMP Superactivation



Grey = morphine causes superactivation

Black = DAMGO causes dose dependent superactivation

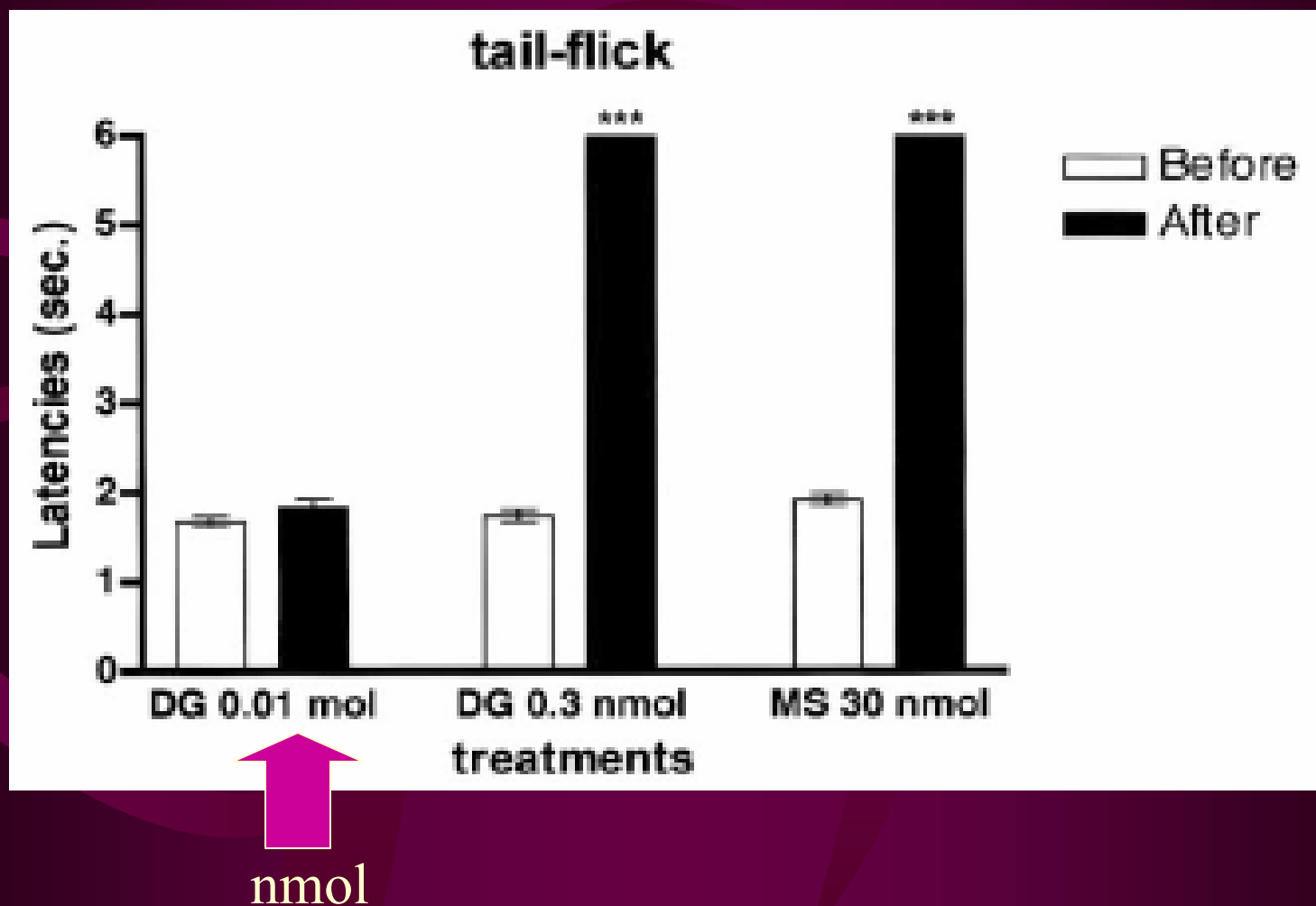
Red = morphine + small DAMGO dose --> reduced superactivation

Behavioral Studies

- Rats implanted with intrathecal catheter
- Examined effects of single injections on analgesia (tail flick) and endocytosis

QuickTime™ and a
GIF decompressor
are needed to see this picture.

DAMGO facilitated morphine-induced MOR endocytosis in rat spinal cord : analgesia

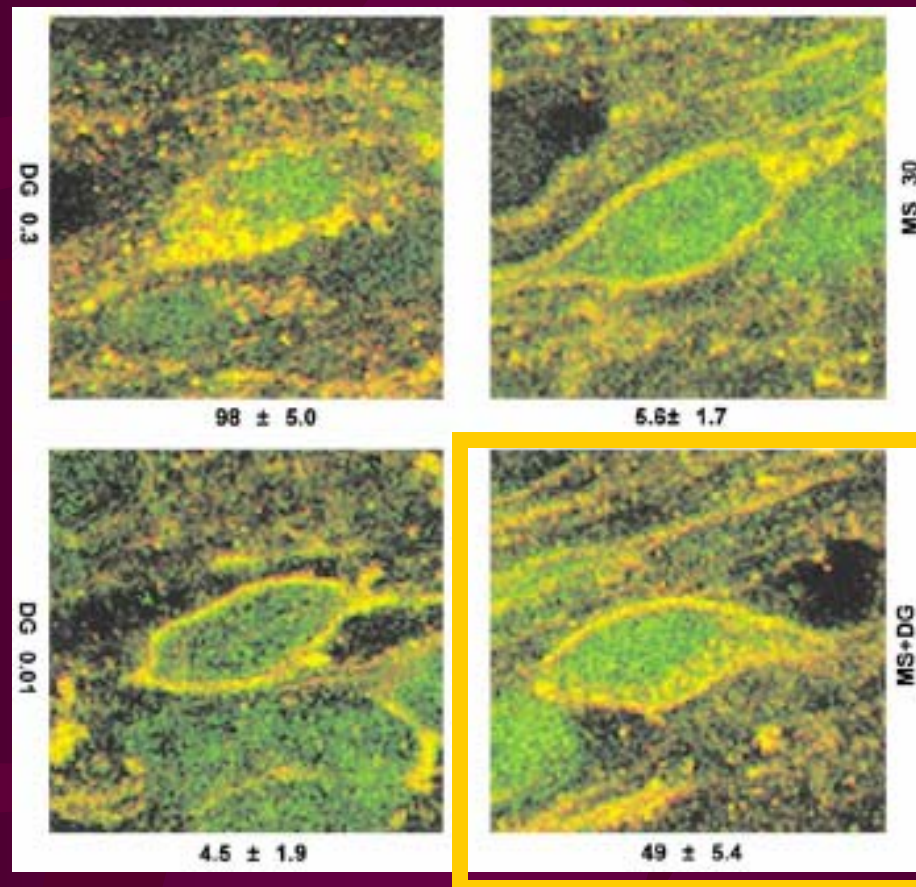


endocytosis immediately following behavioral test/ neurons from lamina II of spinal cord dorsal horn - play important role in pain transmission

GREEN = CELLS, YELLOW = MORS

High dose DAMGO,
MOR in endosomes
= endocytosis

Small dose DAMGO,
No MOR endocytosis



High dose morphine,
MOR in cell
membrane
= no endocytosis

Small dose
DAMGO +
dose Morphine =
Remarkable
endocytosis

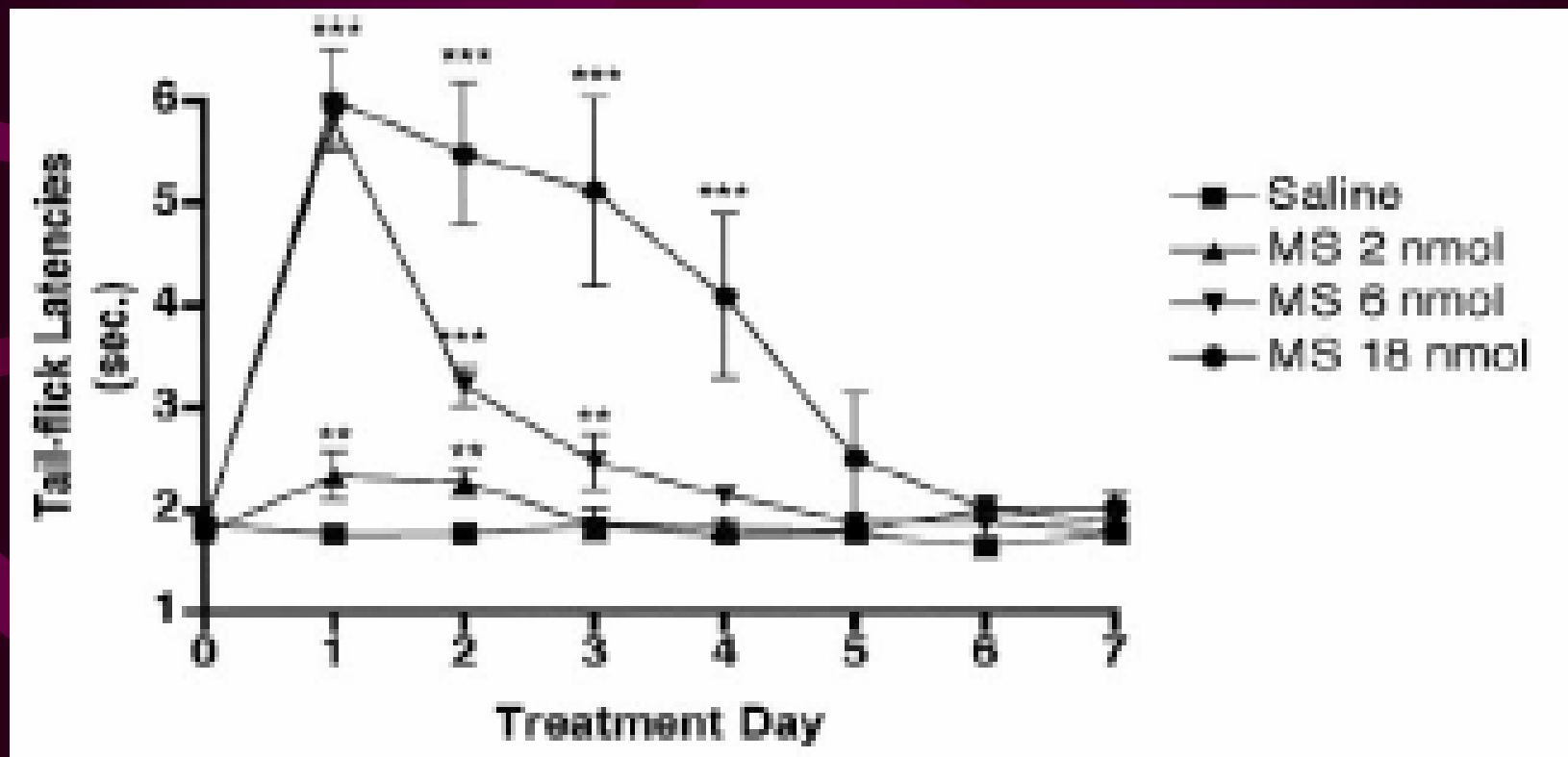
This indicates that

- DAMGO and morphine differentially regulate MOR trafficking in spinal cord
- DAMGO can facilitate morphine-induced endocytosis in vivo

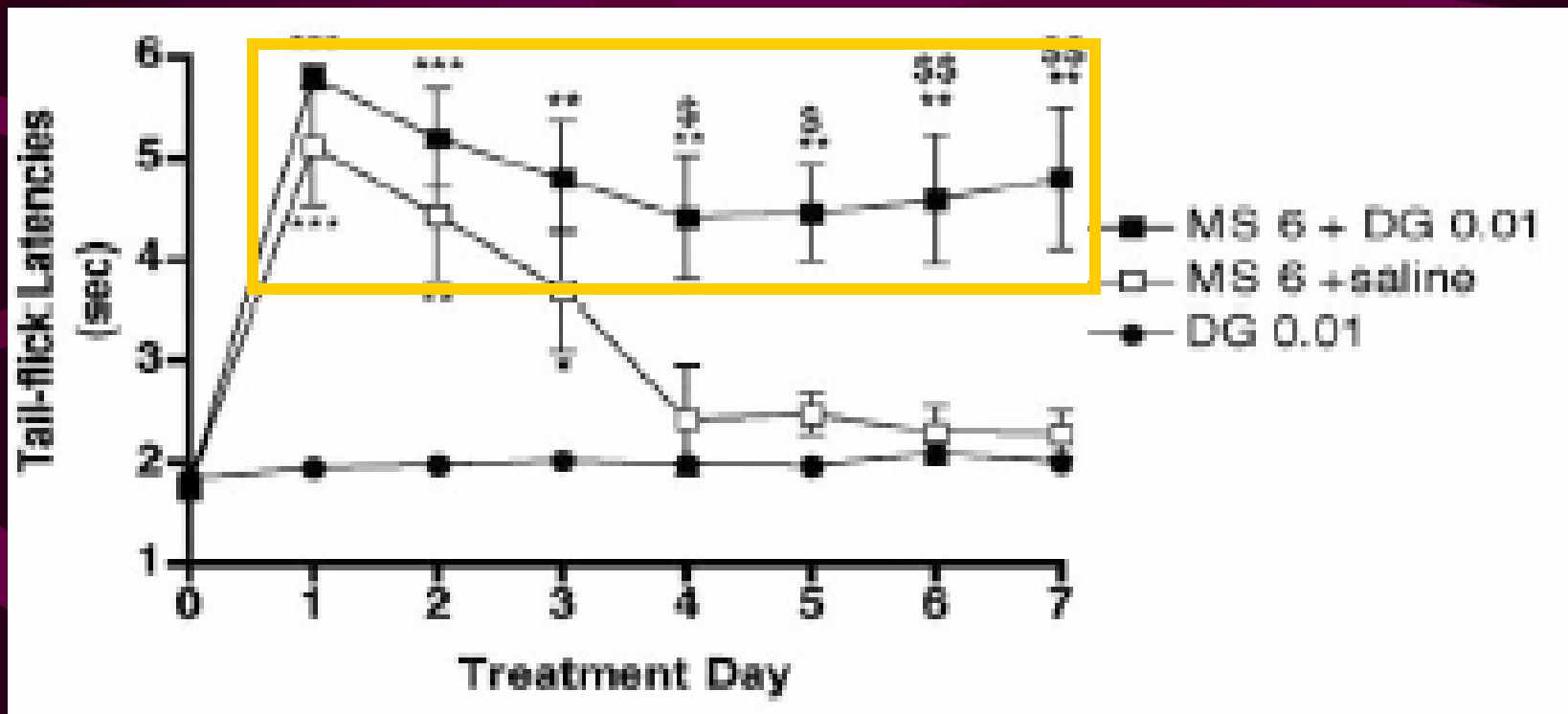
More behavioral studies

- Would increasing endocytosis reduce morphine tolerance?
- Rats implanted with intrathecal catheter
- Examined effects of chronic morphine administration
- And of subanalgesic dose of DAMGO

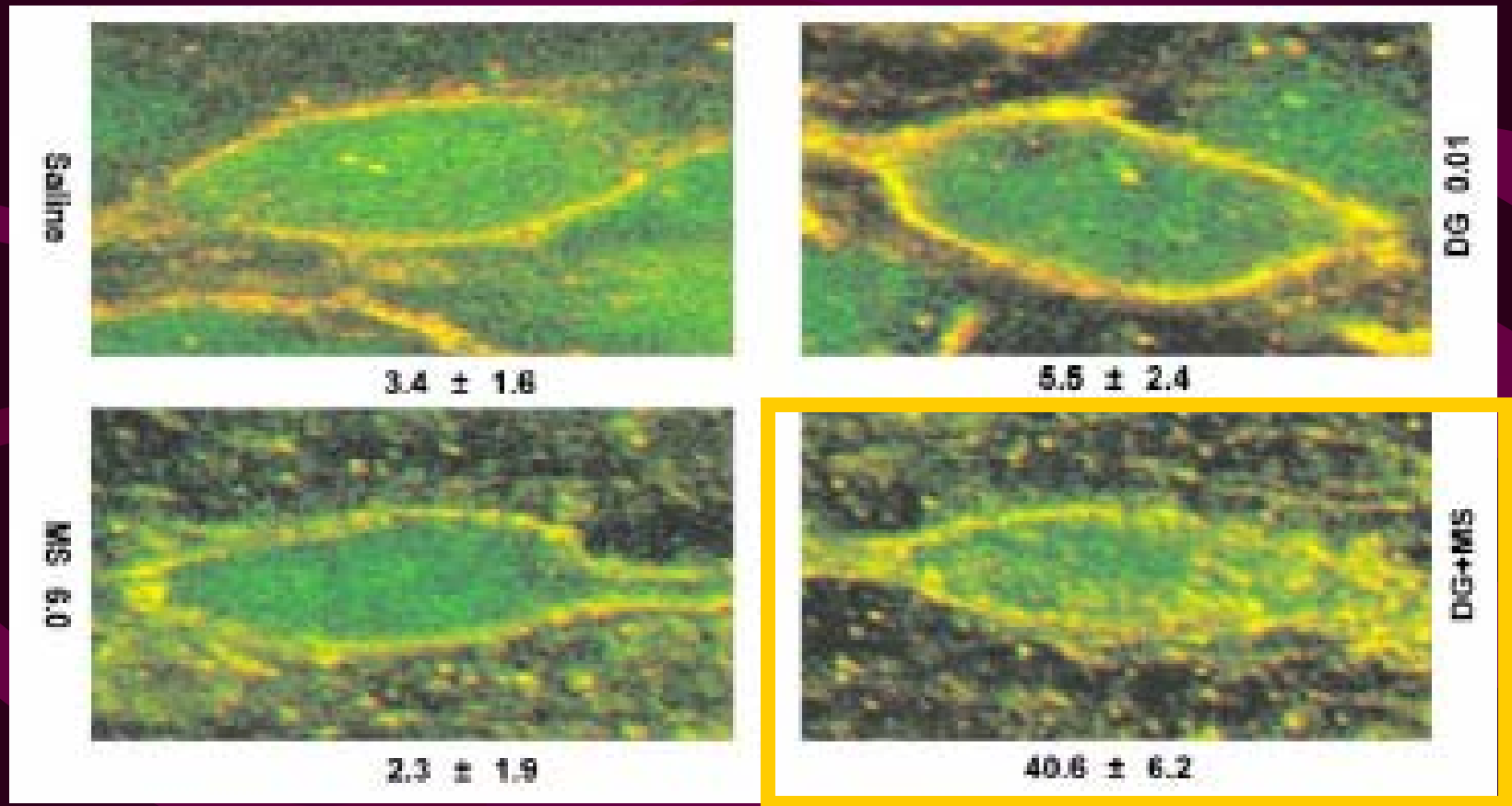
Development of tolerance to morphine



DAMGO inhibits the development of morphine tolerance



endocytosis immediately following behavioral test/
neurons from lamina II of spinal cord dorsal horn



- What might be a better negative control than saline for DAMGO?
- A better control might be another opiate drug, at similarly low doses that does not stimulate MOR endocytosis, I.e. one with high RAVE value.

Key points of He et al 2002

- Small, subanalgesic dose of DAMGO promotes endocytosis and hence desensitization of morphine bound MOR
- This reduces morphine tolerance by enabling endocytosis and consequent receptor recycling
- Therefore, MORs must come in as oligomers, otherwise, DAMGO and Morphine would not be able to bind simultaneously

Questions...

- Why does the DAMGO dose have to be small?
 - So that there is no antinociceptive effect..? Why not?
 - So that there is no interacting with morphine in an unpredicted way?
- What is the mechanism of action of a small DAMGO dose?
- Is DAMGO clinically useful drug?
- Why not just give a high RAVE value opiate by itself?
- Cure for Tolerance?

- Morphine tolerance does not solely involve opioid system, but also all other neurotransmitter systems
- thus lack of tolerance, especially in a non-controlled live animal does not *prove* involvement of DAMGO...
- Further experiments?

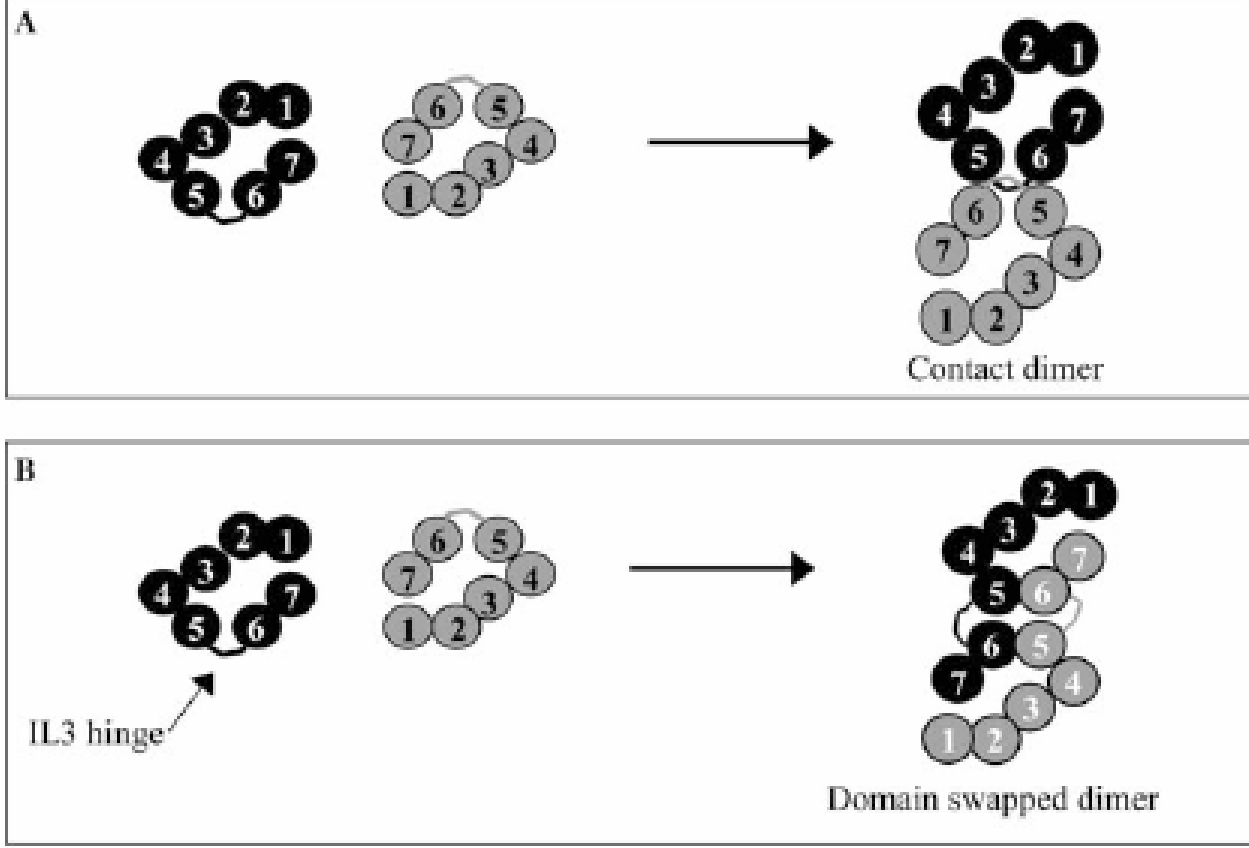
Why Oligomerization? (Kroeger et al 2004)

- Oligomerization = GPCRs directly interact to form higher order complexes
 - truncated and mutant receptors dimerize with wild type receptors
 - Heterodimers display novel pharmacological and /or functional properties
- ALTERED LIGAND BINDING → FLEXIBILITY
 - A means of rescuing the function of mutant receptors in heterozygous individuals, through dominant-positive receptor interactions
 - *E.g. receptor fragment that is non-functional when expressed alone, but restores the function of an endogenously expressed mutant receptors, could be delivered throughout the body and only influence those cells in which it is required*
- Currently the only crystal structure available for full length GPCRs are for the light receptor, rhodopsin, which exists as a structural dimer

Mechanism of oligomerization

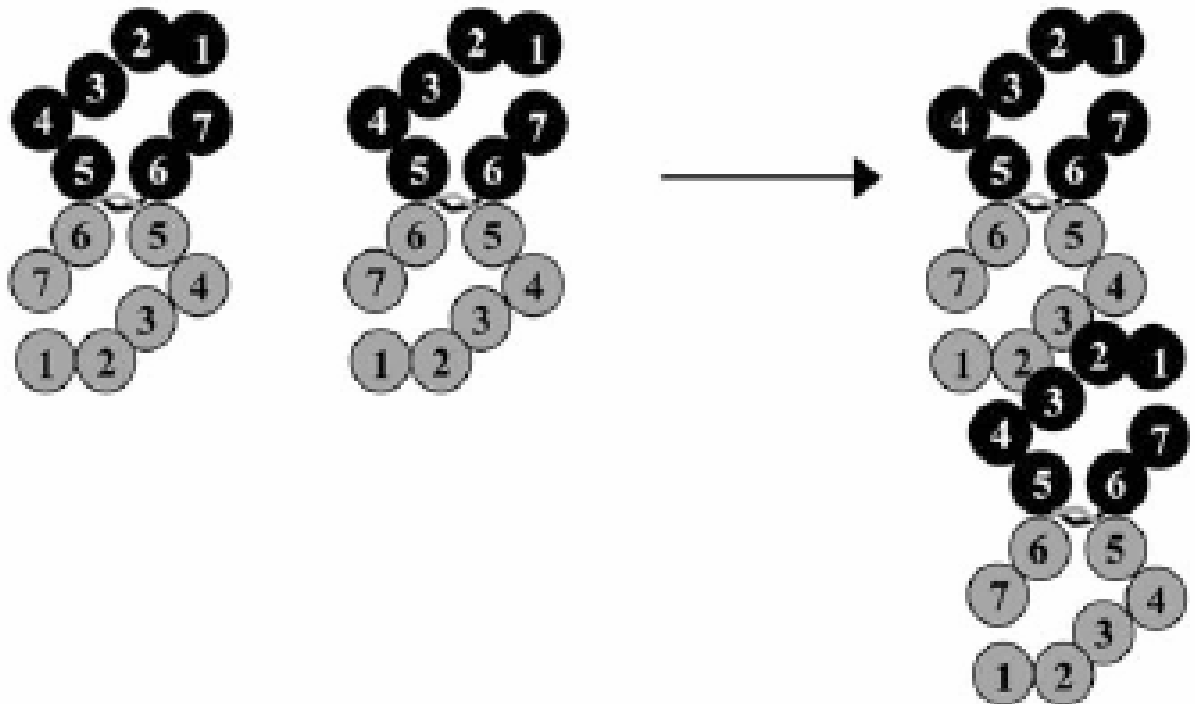
- Dimer interface is unknown at present, likely to be different in different receptors. models exist.
 - Covalent bonds between extracellular domains
 - If have extra large N-terminal domains, oligomerize via disulfide bond formation between N-terminal cystein residues, e.g. mGluR1
 - Interaction of intracellular domains, especially the C-terminal tail
 - E.g. metabotropic GABA_B forms heterodimers via coiled-coil domains within their intracellular carboxy terminal tails
 - Interactions between transmembrane domains.
 - Contact dimerization (lateral packing) would enable the heptahelical integrity of monomer to be maintained, but would require the presence of additional interacting sites on the exterior of the transmembrane bundle. this theoretically enables high-order oligomers to be formed.
 - Domain-swapping would require separation of two independent folding units within the receptor. Produces only dimers.

Dimerization



Oligomerization

C



Oligomerization

Regulation of oligomerization

- Little is known about how dimerization is regulated.
- dimer formation may be influenced by receptor expression levels and/or relative affinities of particular receptor combinations
 - Ligands may modulate receptor oligomers by
 - Promoting association or dissociation of dimers
 - Binding to preformed oligomers to alter the conformation of oligomer
 - Evidence that dimers are formed in endoplasmic reticulum (e.g. GABA)
 - GPCRs may traffic to cell surface as dimers, where upon ligand binding the dimer may
 - Dissociate; Increase in number; Become oligomeric; Change its conformation
 - maybe dimerization requires and/or is modulated by additional proteins
 - Candidates include GPCR chaperones such as the receptor activity modifying proteins

Functional Significance of Oligomerization in vivo

- Majority of studies on GPCR dimerization performed in recombinant systems.
- Evidence for in vivo speculated
- Significance DAMGO: there is a site that DAMGO can bind to!

Contradictory findings

- **Bailey et al 2003**
- Looked at mature mammalian neurons in Locus Cereleus in brain slices of rats and guinea pigs killed by cervical dislocation,
 - LC has homogenous population of noradrenergic neurons that express MORs but not other opioid receptors
- MORs were coupled to GPC inwardly rectifying potassium channels(GIRK). Potassium current reflects receptor activation
 - measured desensitization by measuring change in potassium current via whole-cell patch clamp recordings.
- Coapplication of DAMGO and Morphine did not increase morphine-induced desensitization
- Coapplication caused a lesser degree of desensitization than DAMGO alone
- They saw no effect in HEK293 cells
- In fact morphine inhibits DAMGO induced desensitization
- **Blanchet et al 2003 (same methods)**
- DAMGO causes desensitization and triggers rapid internalization of MORs (within 15 min). Morphine gives rise to smaller, but sustained currents.
- -coapplication of DAMGO and Morphine revealed competition between the two agonists and normal desensitization .

Reconciliation?

- potassium channels were said to be activated in K-opioid receptors...
- Different cell preparation...

More contradictions...

- **Zastrow et al 2003**
- If disrupt /knockout gene coding for beta-arrestin-2...
- ...agonist-induced desensitization of MOR - endocytosis - cannot happen
- Mice show reduced analgesic tolerance (cited in He et al)
- Mutant mice show increased sensitivity to acute anti-nociceptive effects of morphine as well as decreased development of anti-nociceptive tolerance, but not physical dependence following prolonged administration of morphine
- Therefore receptor desensitization may contribute directly to morphine tolerance



Thank you!

Phospholipase D 2 - Koch et al 2004

- *PLD2 activity is elevated in response to DAMGA (inducing endocytosis) but not to morphine treatment (not inducing endocytosis)*
- ***Inhibition** of PLD2 by 1-butanol or overexpressoin of a negative PLD2 mutant led to a **robust decrease of DAMGO induced rat mu-opioid receptor endocytosis**, indicating that PLD2 activation is required for the agonist-mediated mu-opioid receptor endocytosis*
- ***Activation of PLD2 is essential for resensitization** resulting in a decrease of agonist-induced mu-opioid receptor desensitization, indicating that PLD2 is a modulator of mu-opioid receptor reactivation after agonist treatment.*
- *agonist induced clathrin-mediated endocytosis of MOR requires receptor phosphorylation and subsequence beta-arresting binding. But mechanisms not completely understood. Important step in initiation of clathrin-mediated endocytosis is the formation of beta-arrestin/beta two-adaptin/clathrin complex at the plasma membrane. PLD2 seems to initiate the clathrin vesicle formation*