Receptor pharmacology in neuroscience practice
Lecture 1: basic terms, experimental approaches and caveats

Neuroscience 201A, October 22nd, 2015

Ionotropic vs. metabotropic neurotransmitter receptors

What is an ionotropic receptor?

Also called a ligand-gated ion channel

Transmembrane proteins which allow ions to pass through in response to binding to a chemical messenger/ligand/neurotransmitter

E.g. GABA-A, glycine (anionic -); nAChR, ZAC, 5HT3 (Cationic +): Cys-loop receptors
AMPA, NMDA, kainate: iGluRs (cationic)
P2XRs: ATP gated channels (cationic)
Kir: PIP2 activated K+ channels

Different from voltage gated channels,
which pass ions depending on membrane potential

What is a metabotropic receptor (GPCR)?

Also called a G protein coupled receptor

Transmembrane proteins which signal through second messengers to
Ion channels in response to binding to a chemical messenger/ligand/neurotransmitter

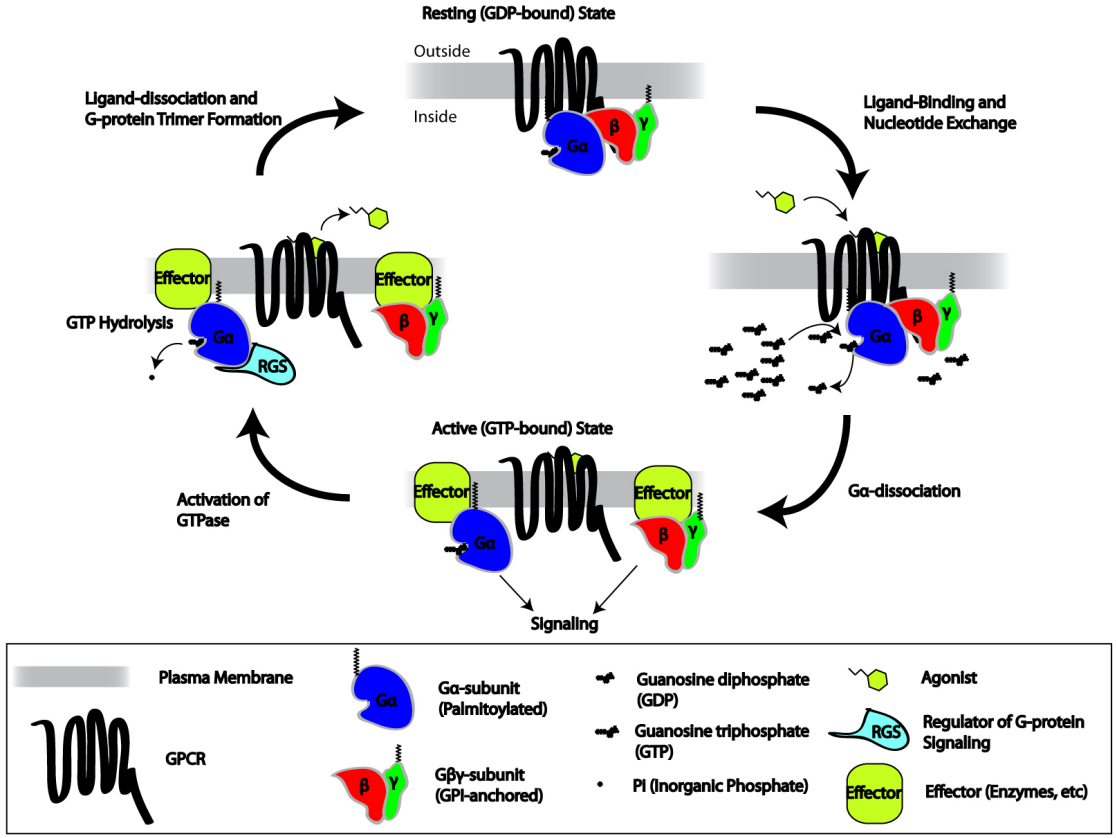
E.g. GABA-B, mGluRs, mAChR, 5HTRs, P2YRs….hmm, these look familiar

As well as norepinephrine, epinephrine, histamine, dopamine, endocannabinoids, adenosine and neuropeptides such as opioids, somatostatin, neurokinins, oxytocin, bradykinin…

Many ligands can act on both ionotropic and metabotropic Receptors…e.g. glutamate, acetylcholine, serotonin, ATP

What is a metabotropic receptor (GPCR)?

It's a G protein coupled receptor….so it couples to G proteins
G proteins are trimeric molecules….with αβγ subunits.



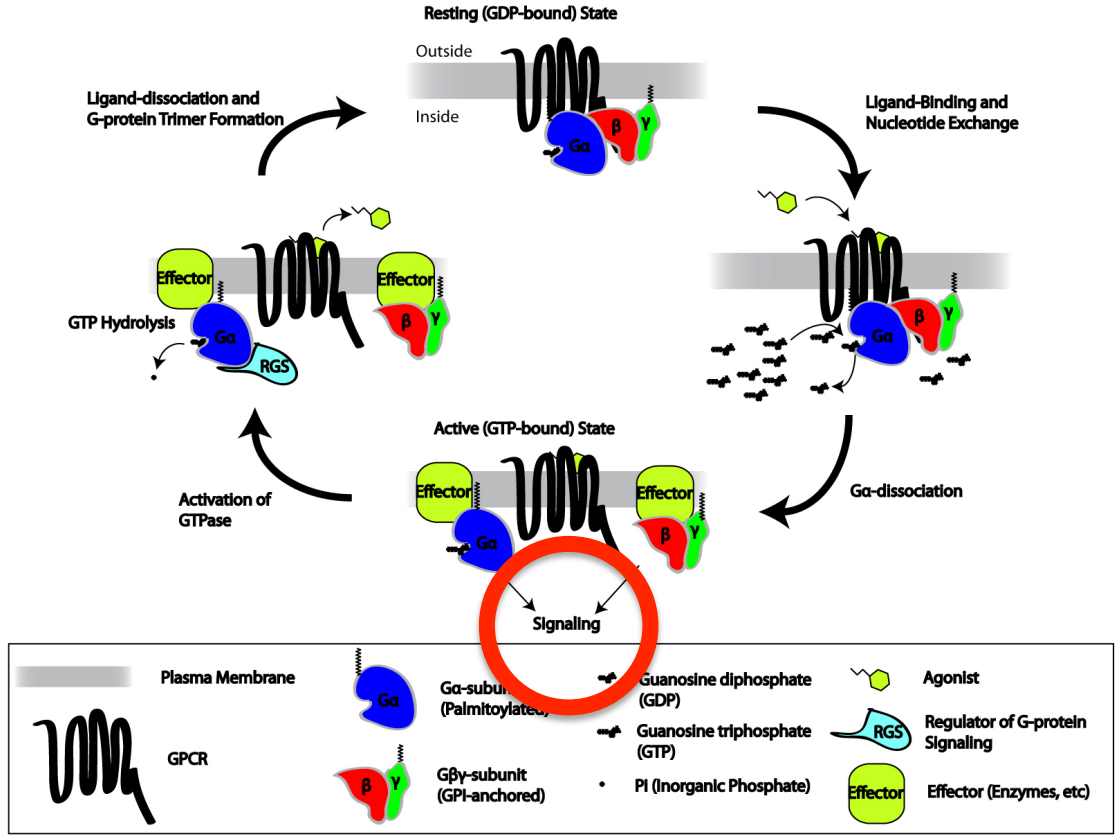
G proteins come in several flavors defined by the α subunit :

Gα i/o

Gα s/olf

Gα q

Gα 12/13



G proteins come in several flavors defined by the α subunit:

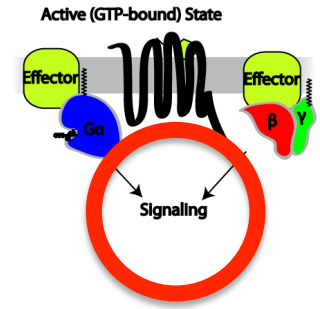
Gα i/o : "inhibitory"; inhibits AC (and PKA), ↑ K+ channels, ↓ Ca2+ channels

Gα s/olf : "stimulatory"; activates AC (and PKA), ↑ cation channels

Gα q : ↑ PLCβ, converts PIP2 to DAG and IP3; activating PKC and releasing Ca2+ from stores

Gα 12/13: activate RhoGEFs, Rho, and ROCK (cytoskeletal rearrangements)

Bγ subunits from Gi/o also ↑ GIRK K+ channels and ↓P/Q- and N -type voltage gated Ca2+ channels



There is also G protein independent signaling from GPCRs…more on that Monday….

Pharmacology Terms You Need to Know

LIGAND — A molecule that binds to the receptor

AGONIST — A ligand that activates the receptor

Full agonist — An agonist that under the specified condition produces the maximal response

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ANTAGONIST — A molecule that blocks the effects of an agonist

Neutral antagonist — Blocks the effects of an agonist (competitive and non competitive) but doesn't alter the "basal" equilibrium of the receptor

Inverse agonist — Blocks the constitutive activity of a receptor

Inverse agonists  agonists

The concentration dependence of the effect is a function of ligand AFFINITY

The magnitude of the effect is dependent on ligand INTRINSIC EFFICACY

Ligand POTENCY is a function of both of these

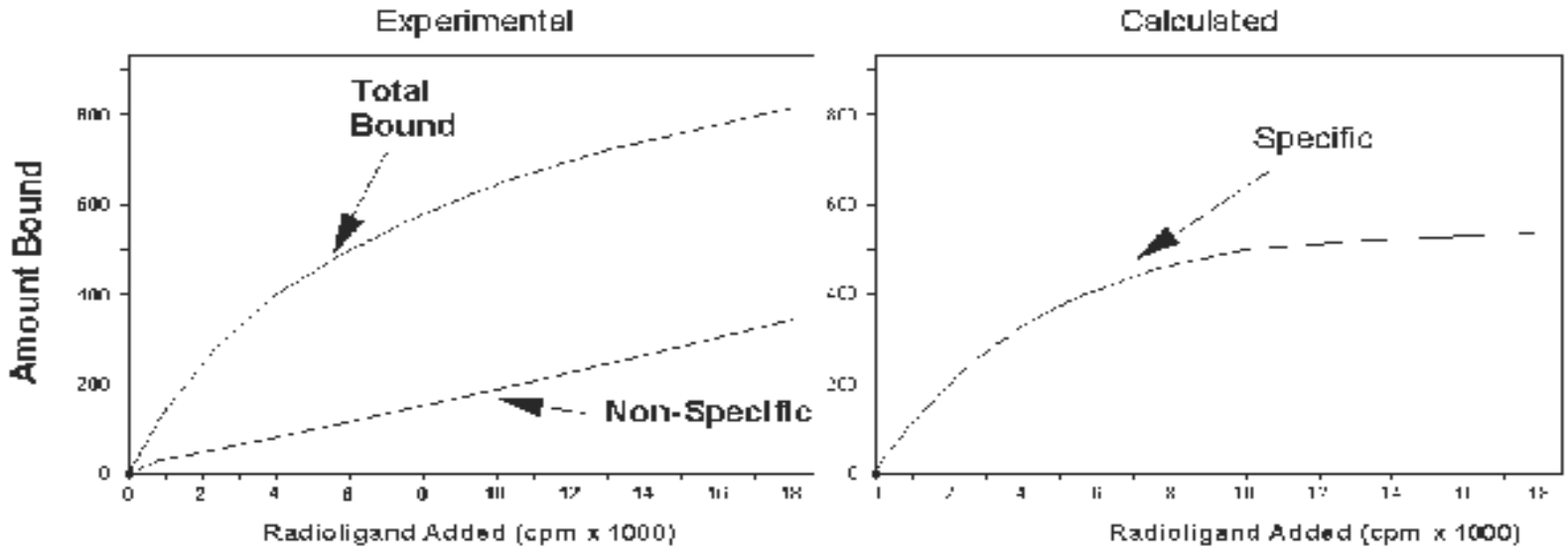
Determining Ligand Affinity

AFFINITY: The ability of a ligand to bind a receptor

Expressed as a dissociation constant, Kd, in molar units
where a lower number is higher affinity

Kd is the concentration of ligand in which half the receptors are occupied.
It is determined experimentally using "saturation binding" with a radioligand (or substitute).

Radioligands bind specifically to receptors and stick to other things too. Specific binding Is determined experimentally by subtraction of non-specific binding.

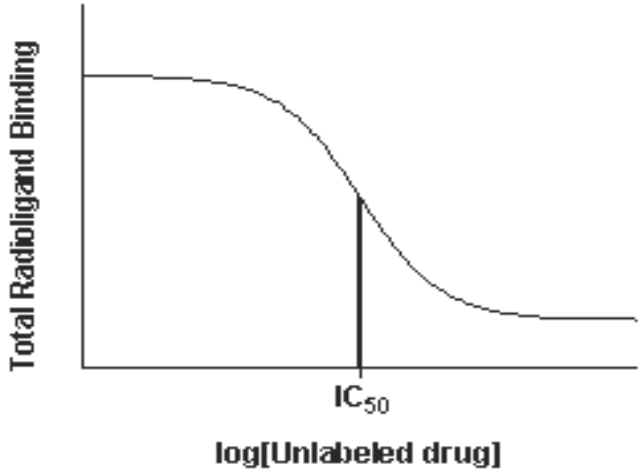


Saturation binding also gives you the total receptor number "Bmax"

Determining Ligand Affinity in practice

Most reported "affinities" are determined using competition binding, not saturation binding. They give a Ki not a Kd.

Competition binding uses a fixed concentration of a radioligand (the "tracer") and competes binding of that tracer with a range of concentrations of cold ligand



Competition Binding gives an IC50 (or EC50 value)
From this value, and the Kd of the tracer, one calculates a Ki value for the new ligand

The Ki is the concentration of competing ligand that would bind half of the receptors in the absence of any other competition.

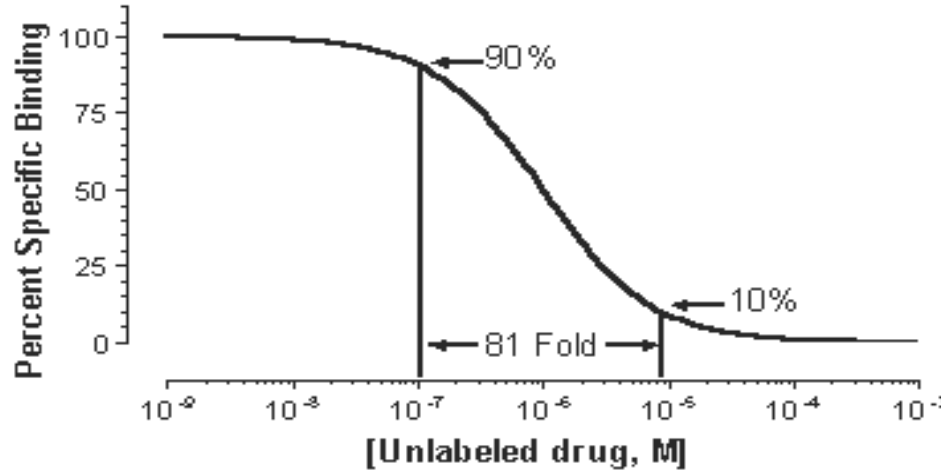
For pure competition at a single binding site Ki is :

$$Ki = \frac{IC50}{1 + \frac{[ligand]}{Kd}}$$

From Cheng and Prusoff (Biochem. Pharmacol. 22: 3099-3108, 1973) there are many equations for varying situations)

What to expect in competition binding

If labeled and unlabeled ligand compete for a single site, the competition binding curve should have a shape defined by the laws of mass action

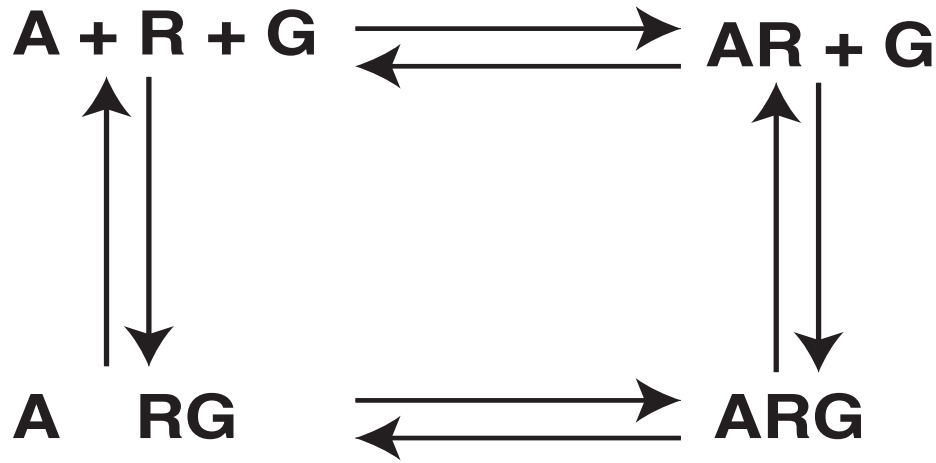


The steepness of the curve is the "Hill slope" which should be ~ -1

For an agonist competing an antagonist this is almost never the case
--affinity is affected by the presence of G protein activation

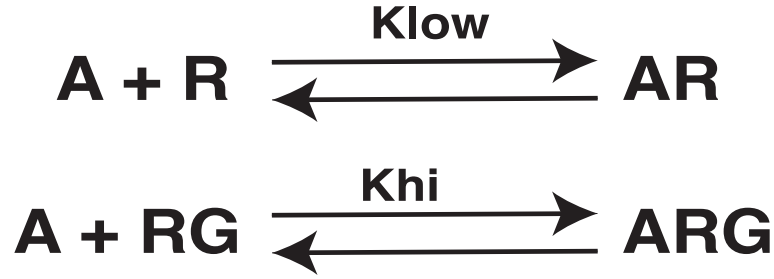
In addition, If your system contains more than one binding site with different affinities the Hill slope will be shallow or biphasic (if affinities are very different)

Agonists have differing affinities depending on G protein coupling



An oversimplified model calculates both the low and high affinity states

(this is normally what is reported if high and low are calculated)



Read more here: RR Neubig, Faseb J. 8:939-946, 1994.

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The concentration dependence of the effect is dependent on ligand AFFINITY ✔

The magnitude of the effect is dependent on ligand INTRINSIC EFFICACY

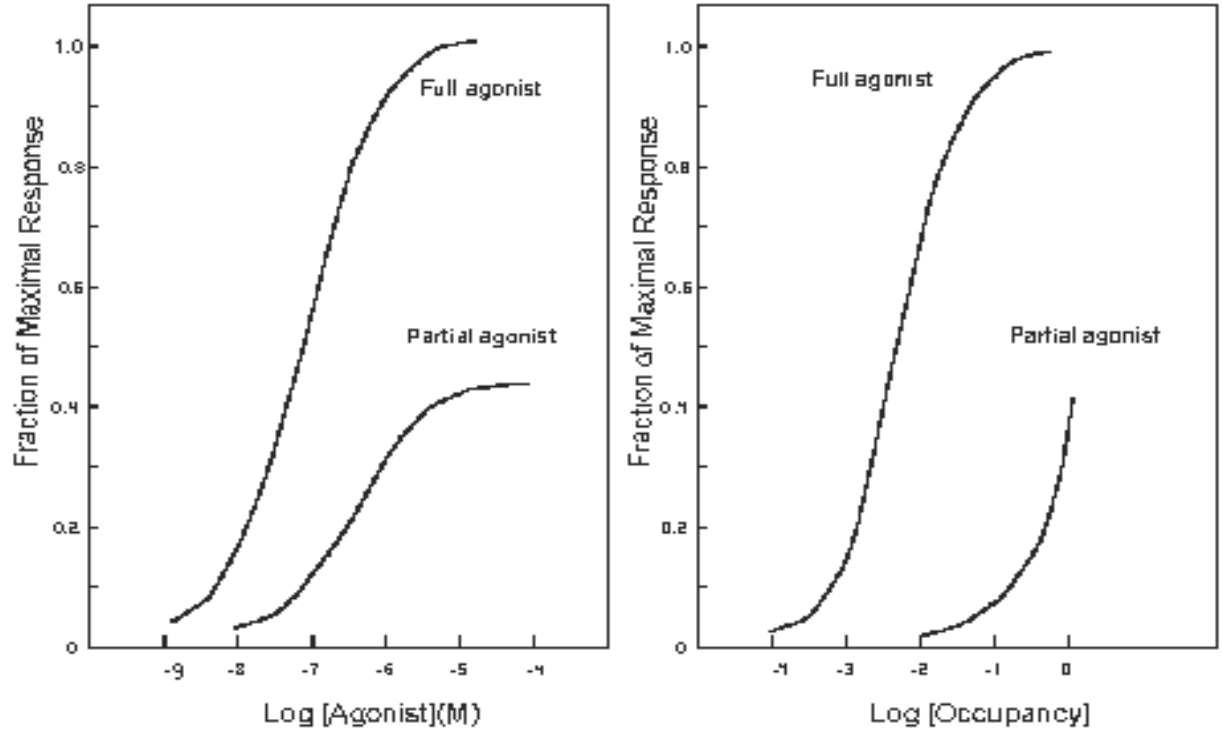
Ligand POTENCY is a function of both of these

Intrinsic Efficacy and Partial Agonism

Intrinsic efficacy is the <u>relative</u> ability of a ligand to produce the maximal effect possible

Caveat: Relative to what? In what system/context?

Relative to endogenous ligand…In a system/context you care about.



Intrinsic efficacy is expressed as "Emax (or % Emax relative to a standard).

Intrinsic Efficacy and Partial Agonism

Intrinsic efficacy is the relative ability of a ligand to produce the maximal effect possible

Caveat: Relative to what? In what system/context?

Relative to endogenous ligand…In a system/context you care about.

What are some things that could affect the intrinsic efficacy of your ligand?

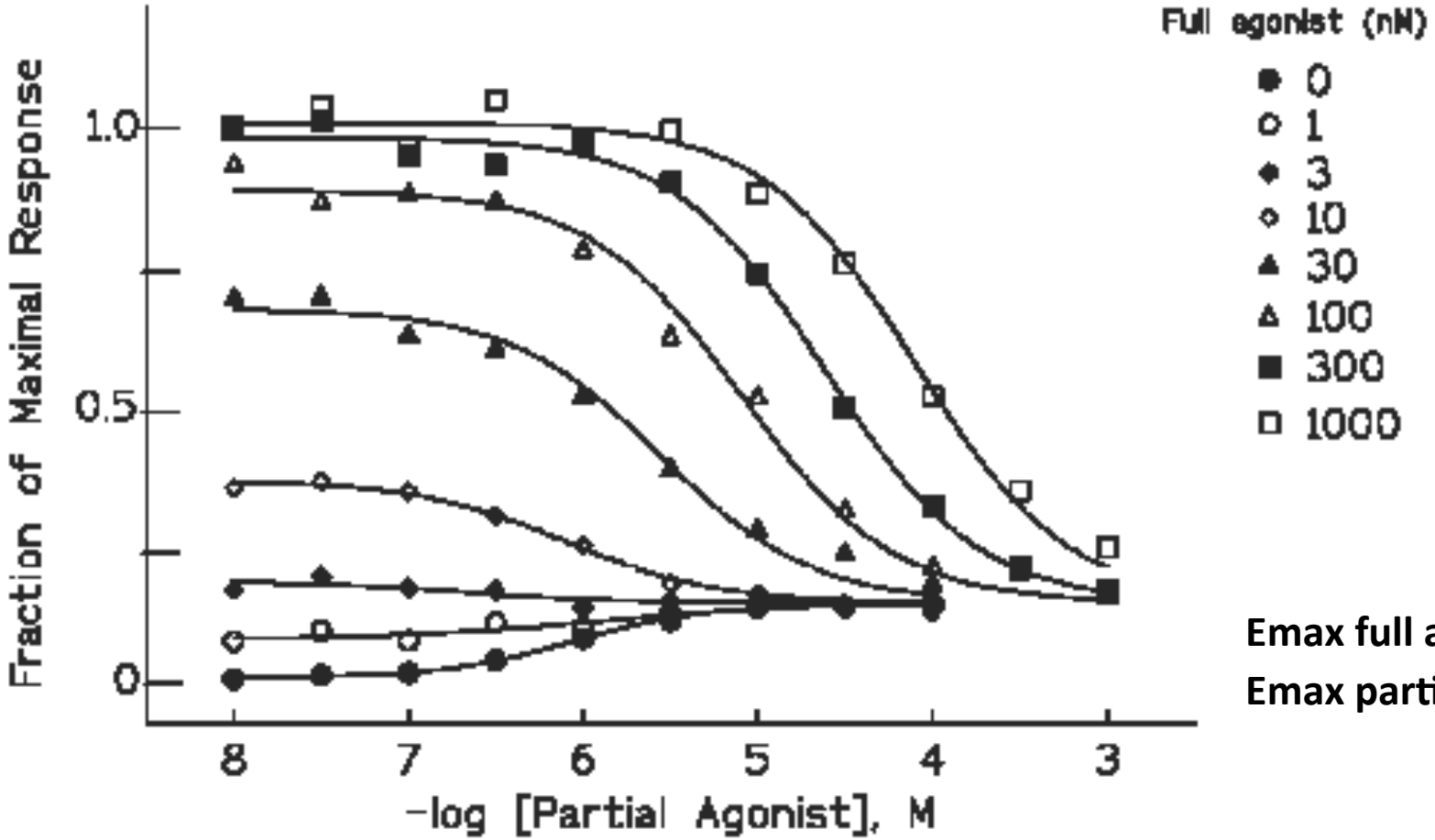
Receptor number
Ligand X needs only 100 occupied receptors to produces maximal response, ligand Y needs 10,000. In systems with only 100 receptors available to talk to your readout Y will look like a partial agonist, but in systems with lots of "spare" receptor talking to your readout, Y will perform like a full agonist (at high enough concentration).

Membrane potential (or potential at which spike is fired, or inhibited)
In cell X, the resting potential is -40 so you need only 100 receptors occupied by a full agonist to activate enough Ca2+ channels to fire a spike, but I need 10,000 occupied by the partial agonist to do the same job.
What would happen in cell Y where the resting potential is -80?

What other receptors/channels are engaged while ligand is around
Especially relevant for systems that use the same ligand for ionotropic/ metabotropic receptors, or in systems/preparations where you aren't controlling release of other transmitters (glutamate, GABA, glycine), or when comparing effects on pre- vs. pos- synaptic responses to the same ligand.

Intrinsic Efficacy and Partial Agonism

Partial agonists can work as functional antagonists for full agonists (especially if they are high affinity)



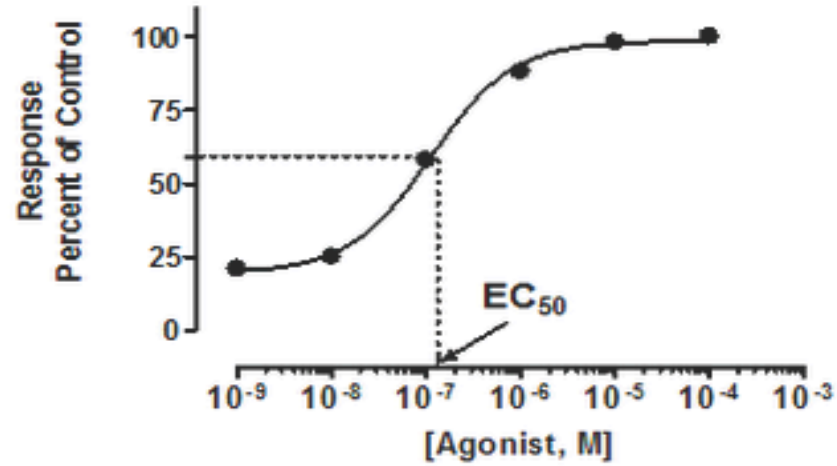
Emax full agonist 100%

Emax partial agonist 20%

Beware: in vivo you rarely know the concentration of your endogenous ligand

Ligand Potency and Selectivity

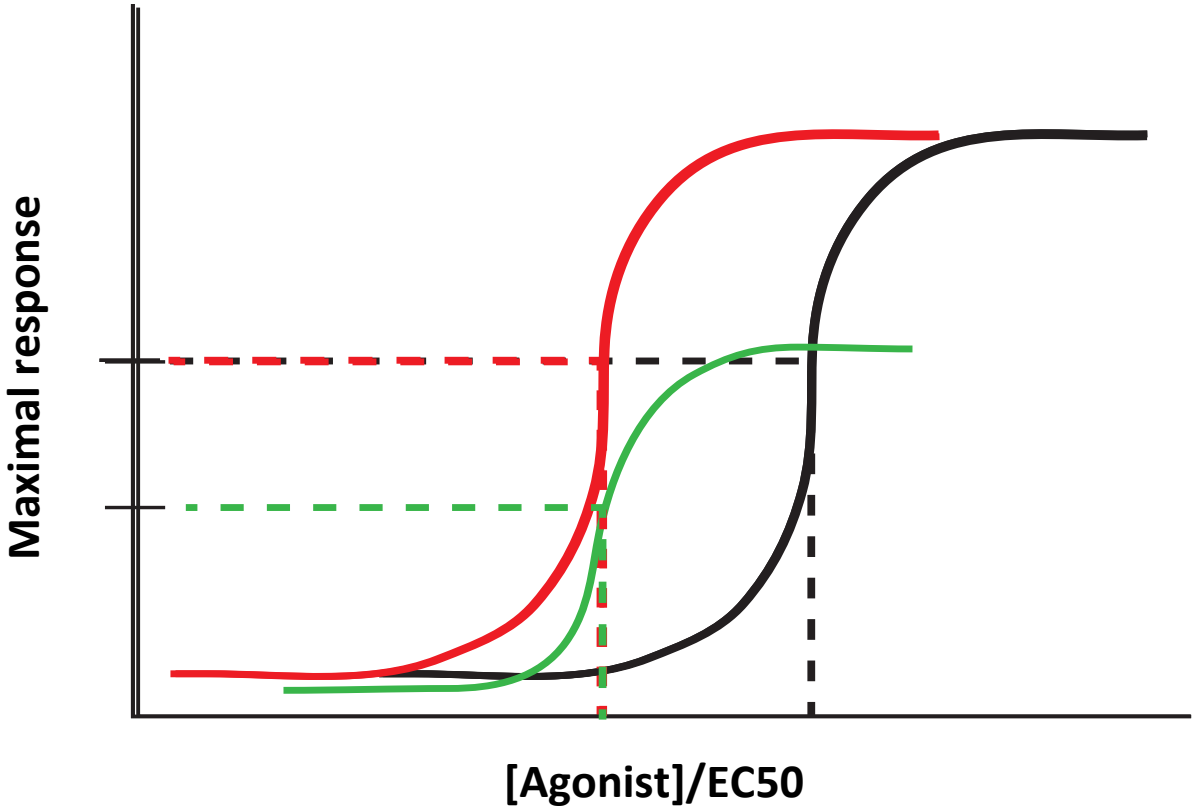
The EC50 (for agonist) and the IC50 (for antagonist) is the concentration of ligand necessary to produce 50% of the maximal effect.



Potency is dependent on BOTH affinity and efficacy of the ligand

In most cases, potency is expressed as 50% maximal response to that ligand not to a reference ligand (for example the endogenous ligand)

Ligand Potency and Selectivity



Higher potency
Same efficacy

Lower efficacy but the
same potency as
RED and higher potency
Than BLACK

In most cases, potency is expressed as 50% maximal response to
that ligand not to a reference ligand (for example the endogenous ligand)

So how is "selectivity" determined?

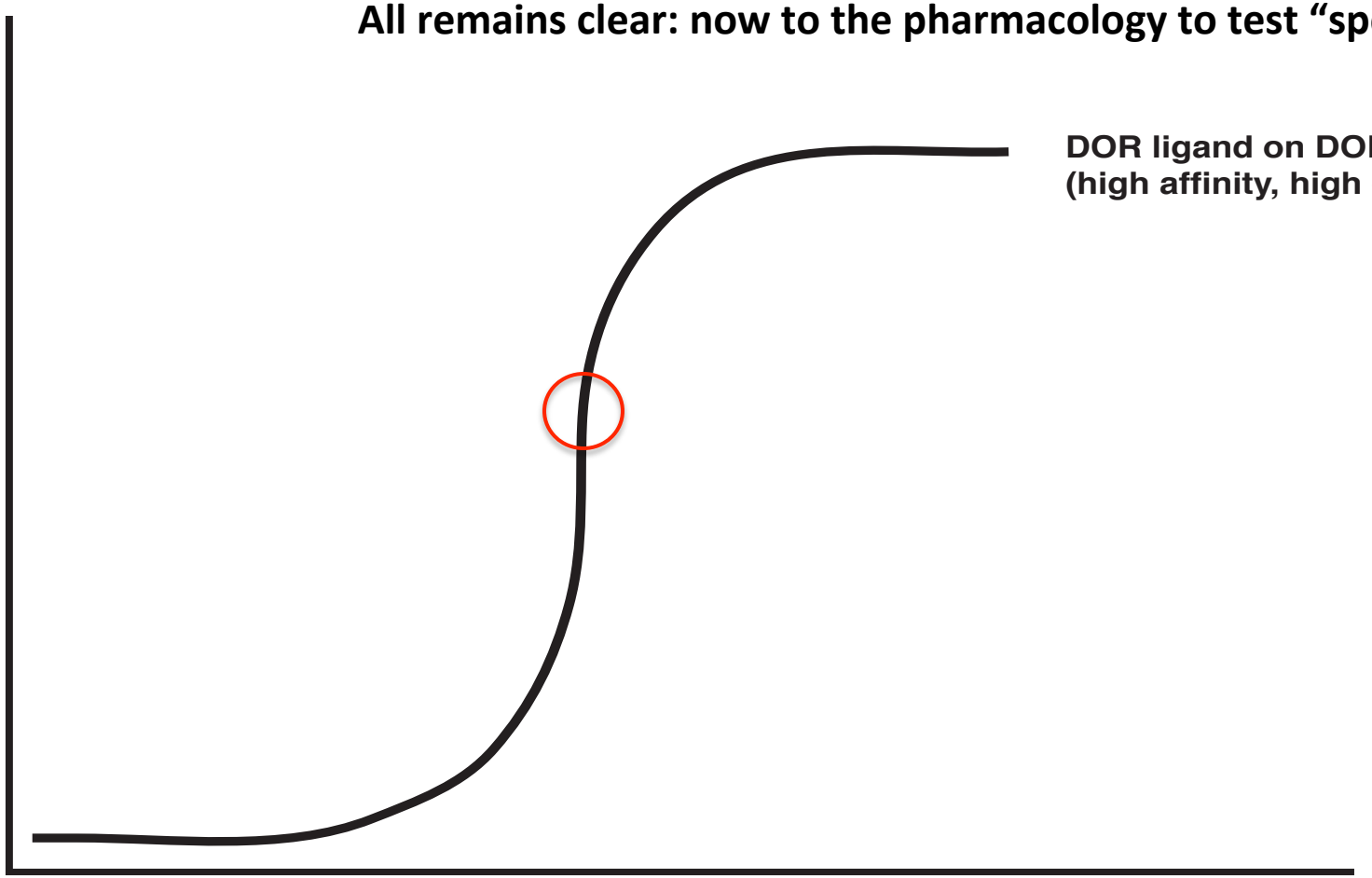
Ligand Potency and Selectivity

SELECTIVITY:

Caveats:

The danger of relying on “selectivity”

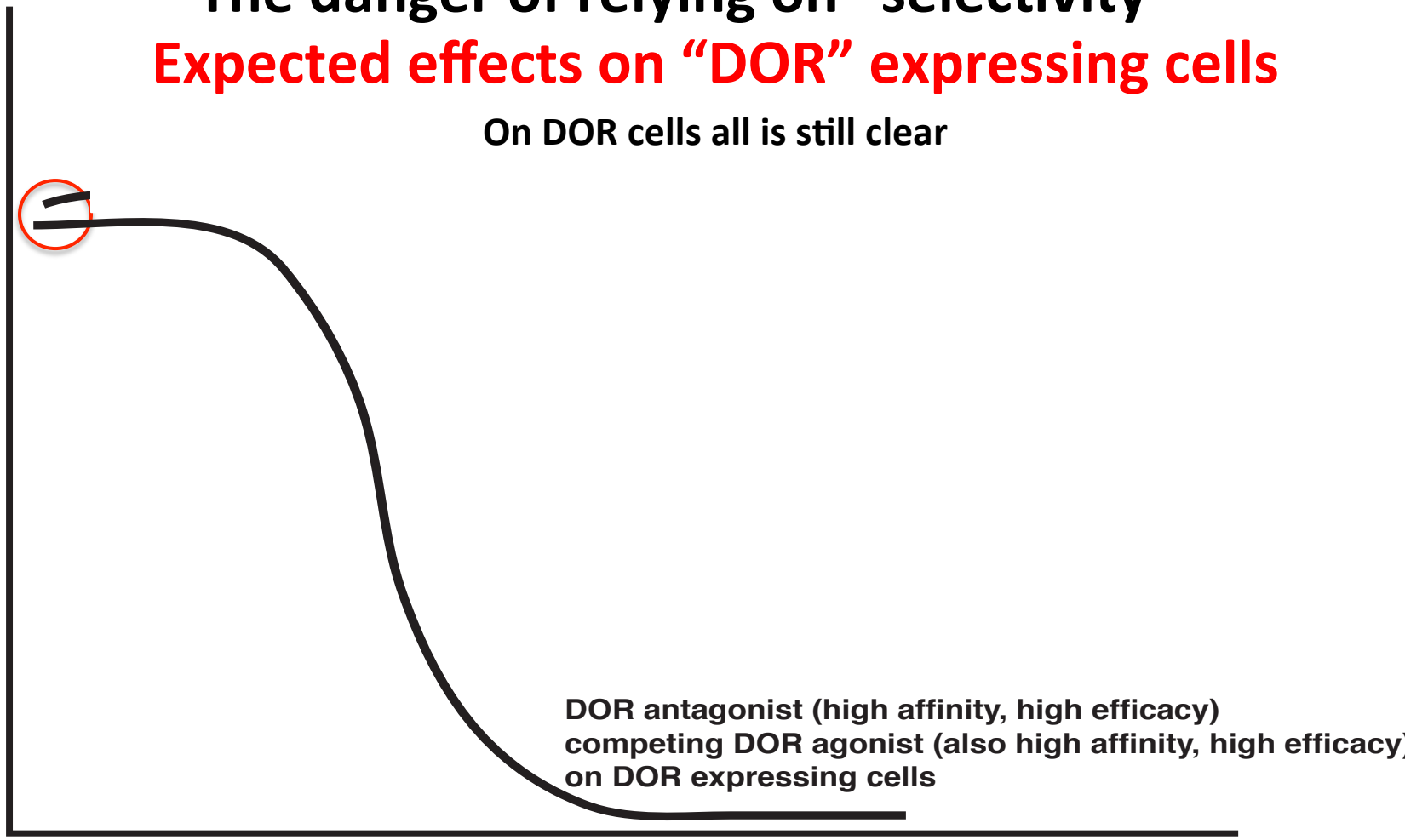
All remains clear: now to the pharmacology to test “specificity”



The danger of relying on “selectivity”

Expected effects on “DOR” expressing cells

On DOR cells all is still clear



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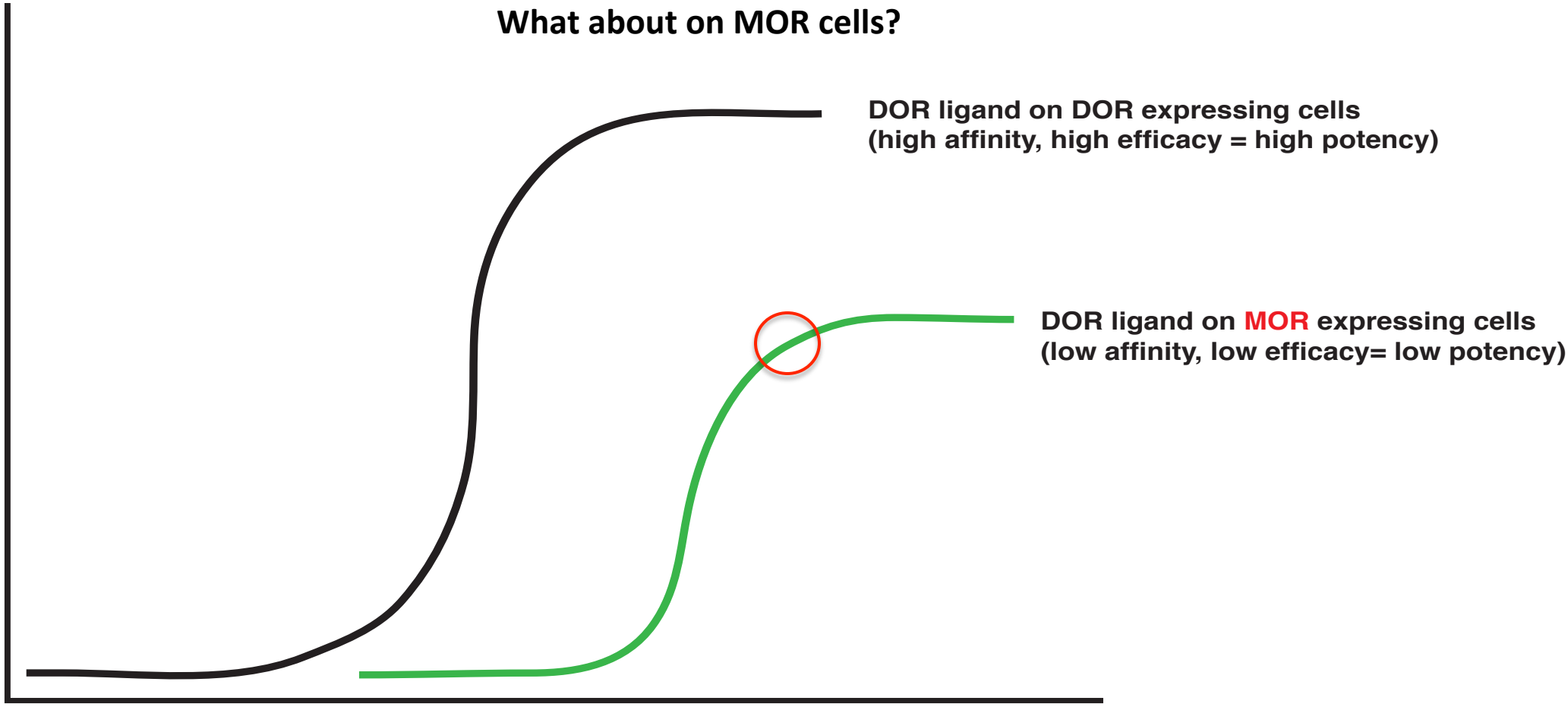

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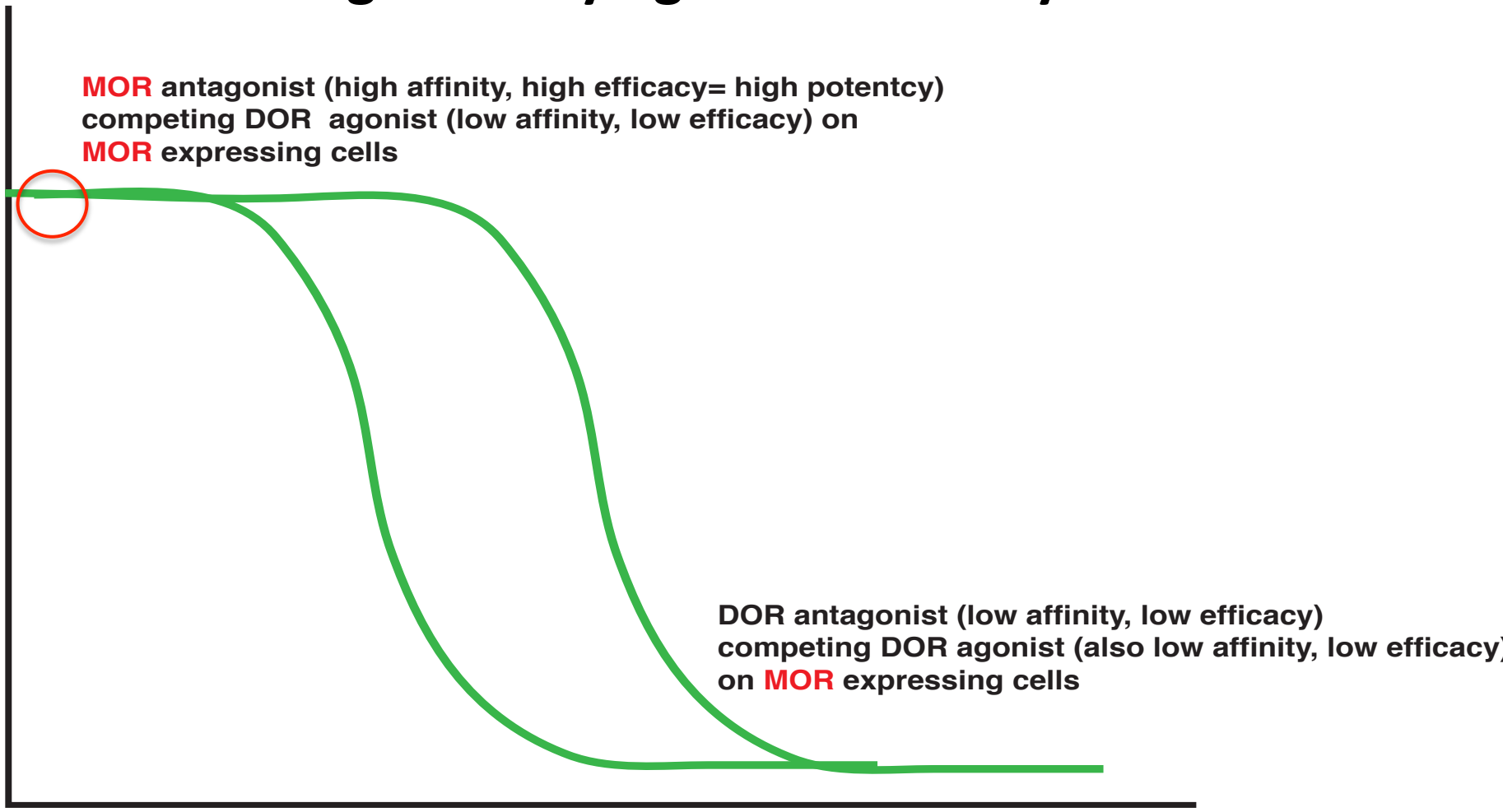

When you are reading the literature…
pay attention! Because now you know the benefits and limitations of a rich pharmacopeia and how to interpret others’ data

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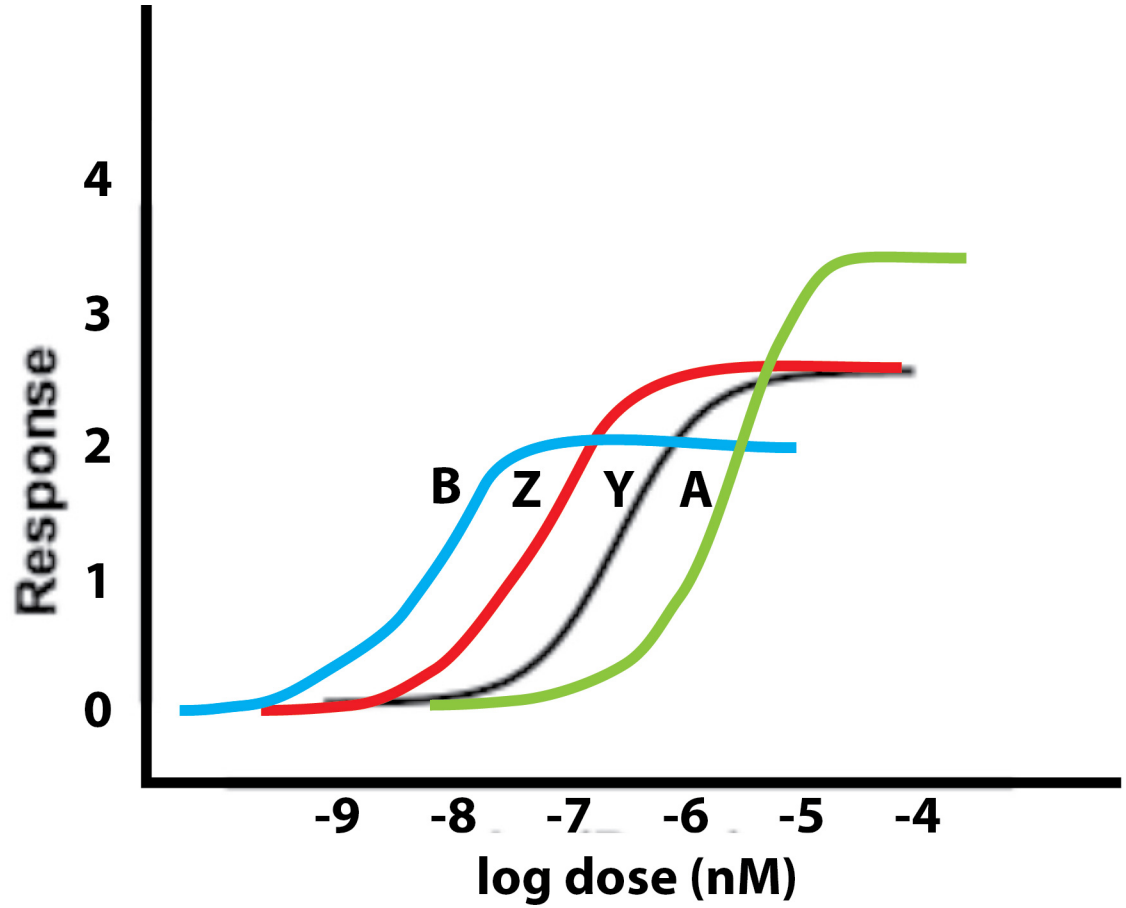
Response
4
3
2
1
0
B
Z
Y
A
-9
-8
-7
-6
-5
-4
log dose (nM)

If you thought that was complicated…..

There is significant, mounting evidence for "Functional Selectivity", "Biased agonism" "differential engagement" or "RAVE" (relative activity vs. endocytosis) at GPCRs…

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.