

Title Page

Functional selectivity and classical concepts of quantitative pharmacology

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Running Title Page

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Functional selectivity – new concept of receptor signaling

Abbreviations

2-AG	2-arachidonylglycerol
5-HT	Serotonin
2C-B-CB	1-aminomethyl-3,6-dimethoxy-4-bromobenzocyclobutane
β 2AR	β_2 adrenergic receptor
AA	arachidonic acid
AFM	atomic force microscopy
DAG	Diacylglycerol
DAMGO	D-Ala ² ,N-Me-Phe ⁴ ,Gly ⁵ -ol]-enkephalin
DHX	dihydroxidine; trans-10,11-dihydroxy-5,6,6a,7,8,12b-hexahydrobenzo[a]phenanthridine
DOB	(-)-1-2, 5-dimethoxy-4-bromophenyl-2-aminopropane
ECM	electron cryomicroscopy
GIRK	G protein-coupled inward rectifying potassium channel.

GPCR	G protein-coupled receptor
GTP γ S	Guanosine 5`-(γ -thio)triphosphate
HGP	Human genome project
HTS	High throughput screen
IP	inositol phosphates
IUPHAR	International Union of Basic and Clinical Pharmacology
LSD	D-lysergic acid diethylamide
MALDI-TOF	Matrix Assisted Laser Desorption Ionization - Time of Flight mass spectroscopy
MAPK	Mitogen-Activated Protein Kinase
MEK	MAP kinase kinase
NC-IUPHAR	Nomenclature committee of IUPHAR
NDI	nephrogenic diabetes insipidus
<i>N</i> -propylDHX	<i>N-n</i> -propyldihydroxidine
PLA	Phospholipase A
PLC	Phospholipase C
SB 242084	6-chloro-5-methyl-N-(6-(2-methylpyridin-3-yloxy)pyridin-3-yl)indoline-1-carboxamide
SILAC	Stable isotope labeling with amino acids in culture
SNP	Single nucleotide polymorphism
TM	Transmembrane helix

Abstract

The concept of intrinsic efficacy has been enshrined in pharmacology for a half-century, yet recent data reveal that many ligands can differentially activate signaling pathways mediated via a single G protein coupled receptor (GPCR) in a manner that challenges the traditional definition of intrinsic efficacy. Some terms for this phenomenon include functional selectivity, agonist-directed trafficking, and biased agonism. At the extreme, functionally selective ligands may be both agonists and antagonists at different functions mediated by the same receptor. Data illustrating this phenomenon are presented from serotonin, opioid, dopamine, vasopressin, and adrenergic receptor systems. A variety of mechanisms may influence this apparently ubiquitous phenomenon. It may be initiated by differences in ligand-induced intermediate conformational states, as shown for the β_2 -adrenergic receptor. Subsequent mechanisms that may play a role include diversity of G proteins, scaffolding and signaling partners, and receptor oligomers. Clearly, expanded research is needed to elucidate the proximal (e.g., how functionally selective ligands cause conformational changes that initiate differential signaling), intermediate (mechanisms that translate conformation changes into differential signaling), and distal mechanisms (differential effects on target tissue or organism). Besides the heuristically interesting nature of functional selectivity, there is a clear impact on drug discovery, as this mechanism raises the possibility of selecting or designing novel ligands that differentially activate only a subset of functions of a single receptor, thereby optimizing therapeutic action. It also may be timely to revise classic concepts in quantitative pharmacology and relevant pharmacological conventions to incorporate these new concepts.

Receptor pharmacology for the new millennium

For the last half century, pharmacological theory has posited that ligands could be characterized by the nature of the functional effects elicited by their interaction with their target receptor. These effects are governed by two important properties: affinity, the property of attraction between a ligand and its receptor, and efficacy, the property that allows ligands, once bound, to produce a response (Kenakin, 1997). This concept has led to the classification of receptor ligands as full agonists, partial agonists, neutral antagonists, or inverse agonists. Perhaps the key idea that governs this classification is the notion of “intrinsic efficacy”, originally proposed by Furchgott (1966) as a measure of the stimulus per receptor molecule produced by a ligand. According to this notion, full agonists possess sufficiently high intrinsic efficacy such that they maximally stimulate all cellular responses linked to a given receptor. Partial agonists possess lower degrees of intrinsic efficacy (leading to submaximal responses), whereas inverse agonists reduce constitutive (ligand-independent, basal) receptor signaling. Neutral antagonists possess no intrinsic efficacy, but occupy the receptor to block the effects of full, partial, or inverse agonists. This idea has also led to the assumption, dearly held in pharmacology, that the ability of the ligand to impart (or reduce) stimulus once that ligand is bound to the receptor is an inherent property of the ligand-receptor complex. Intrinsic efficacy is thus differentiated from the more operational term “intrinsic activity” (Ariens, 1954), that simply refers to the maximal effect (E_{max}) of a ligand relative to a reference agonist in a given experimental system.

In classical pharmacological terms, therefore, intrinsic efficacy has been viewed as a system-independent parameter that is constant for each ligand at a given receptor, irrespective of where that receptor is expressed. Any differences in the expression of agonism by a ligand between cell and tissue types were assumed simply to reflect differences in receptor density and/or the strength of stimulus-response coupling. In other words, the classification of compounds on the basis of intrinsic efficacy only allowed for variations in the *quantity* of the stimulus that was imparted to the cell, but not the *quality*. A full agonist would be expected to activate all of the signaling pathways linked to a receptor to the same degree as the endogenous ligand for that receptor. In contrast, a ligand that antagonizes one signaling pathway via a specific receptor should antagonize

every pathway coupled to that receptor to the same extent. One consequence of this theory was the weight it added to Ehrlich's idea of a therapeutic "magic bullet". It led to a focus on discovery of new receptor ligands with high affinity and having specific functional characteristics at a single target. Although modern drug discovery recently has recognized that there is value in ligands that act by simultaneously targeting multiple receptors (Roth et al., 2004), there is still the widespread view that the characteristics of ligands at target receptors can be described by their "intrinsic efficacy". In fact, the notion that intrinsic efficacy is system-independent forms a major underlying premise in drug discovery today – that the pharmacological characteristics of a drug tested in an experimental model system can be extrapolated to all systems. As discussed below, this premise is frequently incorrect.

This change in perspective has been spurred by data emerging within the past decade in which certain ligands were shown to have quite diverse functional consequences mediated via a single receptor. Because these data were clearly not consistent with classical pharmacological concepts, they were often dismissed as artifacts caused by differences in "strength of signaling," receptor reserve, undetected interactions with unknown receptors, effects of trace contaminants or drug metabolites, and/or similar mechanisms. Yet as more and more data have been amassed with a variety of different receptors, it is becoming clear that the classical concept of "intrinsic efficacy" as a system-independent constant, although once having conceptual utility, is probably not correct. Indeed, almost every researcher reaching this conclusion felt it important enough to coin their own descriptive term, that now include "functional selectivity", "agonist-directed trafficking of receptor stimulus", "biased agonism", "protean agonism", "differential engagement", and "stimulus trafficking" (to name just a few).

It was the concatenation of this research that led to two symposia at the Experimental Biology 2005 meeting whose essence is captured in this Perspective. We propose that ligands induce unique, ligand-specific receptor conformations that frequently can result in differential activation of signal transduction pathways associated with that particular receptor (Clarke and Bond, 1998; Ghanouni et al., 2001; Gonzalez-Maeso et al., 2003; Kenakin, 1995; Mailman et al., 1997; Mailman et al., 1998; Roth and Chuang, 1987). This differential activation may be expressed as differences in intrinsic

activity and/or potency at one signaling pathway versus another, and which are not due to differences in affinity at the mediating receptor. **Throughout this article, this concept is termed “functional selectivity,”** yet it must be made clear this is not a IUPHAR-accepted convention. We chose it for the symposia titles because it is a purely operational term that can accommodate one or any combination of several involved mechanisms (*vide infra*). Indeed, as will be noted below, the participants suggested it may be timely to settle on conventions that will help to focus the relevant literature in this area. Although most of the research that was presented is recent, and uses a variety of modern techniques, we are aware that ideas related to “functional mismatches” had been theorized earlier, sometimes going back decades (Jim et al., 1985; Portoghese, 1965; Roth and Chuang, 1987). We summarize below some of the data from our laboratories that support this concept, but we recognize that others also have made similar observations and come to similar conclusions.

Examples of functional selectivity

Atypical agonists of 5-HT₂ serotonin receptors

There have been a plethora of observations of functional selectivity made by a number of groups investigating serotonin receptor systems. The effector that has been best characterized with respect to 5-HT₂ signaling involves the G α_q stimulation of phospholipase C (PLC), leading to the formation of inositol phosphates (IP) and diacylglycerol, thereby mediating intracellular calcium [Ca²⁺]_i (Hoyer et al., 1994). This receptor family also has been shown to mediate the release of arachidonic acid (AA), presumably through the activation of phospholipase A₂ (PLA₂) (Felder et al., 1990), an enzyme that acts on membrane phospholipids.

Berg et al. (1998b) reported the capability of certain serotonergic ligands to differentially activate these signal transduction pathways associated with the human serotonin 5-HT_{2A} and 5-HT_{2C} receptors. They observed that the relative efficacy of a series of ligands for each of the receptors differed depending upon whether PLC-mediated accumulation of IP or PLA₂-mediated release of arachidonic acid (AA) was measured (Figure 1). Important aspects of the experimental design were that responses to the reference drug were obtained along with each test ligand in every experiment, and

both signal transduction responses were measured simultaneously from the same cells, thereby obviating methodological reasons for response-dependent differences in relative agonist efficacy (Berg et al., 1998a). The significance of these differences in relative efficacy was further supported by the lack of difference in relative agonist efficacy between the PLC-IP response and increases in $[Ca^{2+}]_i$ levels, which agonists would not be expected to regulate differentially.

Further studies of the 5-HT_{2C} receptor demonstrate that the ability of agonists to activate receptor desensitization mechanisms also appears to be ligand-dependent (Figure 1). Marked differences are observed in the relative efficacy of agonists to rapidly desensitize the 5-HT_{2C}-mediated PLC-IP and PLA₂-AA response pathways, but these efficacies of desensitization did not correspond to the relative efficacies of pathway activation (Stout et al., 2002). Examination of the 5-HT_{2C} receptor molecular structure indicates that the second intracellular loop plays a crucial role in transmitting agonist-specific information to the PLC-IP and PLA₂-AA signal response pathways. It was observed that by changing three amino acids within this region (positions 156, 158, and 160 from INI to either VSV or VGV) the capacity of 5-HT_{2C} agonists to differentially signal through these response pathways was compromised (Berg et al., 2001). This example, a result of mRNA editing, highlights the importance of how subtle changes in receptor structure and its effect on conformation (Visiers et al., 2001) can ultimately affect the cellular signaling responses.

The concept of functional selectivity also applies to compounds that are classified as inverse agonists, some of which may have different functional characteristics at other signaling pathways mediated by the targeted receptor. An example of this is SB 242084 (Cussac et al., 2002), a functionally selective ligand that is a strong inverse agonist for 5-HT_{2C} receptor-mediated PLA₂-AA release and G α_i , but an agonist for PLC-IP (De Deurwaerdere et al., 2004). These observations further support the notion of ligand-induced differential signaling, and highlight the importance of referencing response and the conditions of that response when describing the intrinsic activity of a ligand.

Other interesting data have arisen from studies that looked specifically at hallucinogens and their differential signaling through the 5-HT_{2A} receptor. Although the

PLC-IP effector is an easy pathway to evaluate experimentally, there had been no proof that the hallucinogenic effects of compounds such as LSD rely on its activation. A recent report emphasized that there was no correlation between hallucinogenic activity and activation of the PLC effector pathway (Rabin et al., 2002). Kurrasch-Orbaugh *et al.* compared the effects of LSD and DOB (a hallucinogenic phenethylamine derivative) on 5-HT_{2A} receptor-mediated accumulation of IP₃ and found that both had significant potency for this pathway (EC₅₀ = 10 nM and 70 nM, respectively), yet LSD demonstrated little intrinsic activity (22%) relative to that (80%) of DOB (Kurrasch-Orbaugh et al., 2003b).

The discrepancies between PLC activation and *in vivo* pharmacology led to the investigation of other effector pathways associated with 5-HT_{2A} receptor activation. As mentioned previously, 5-HT_{2A} receptor stimulation also leads to the release of AA. A series of 5-HT_{2A} agonists was found to have varying potencies and intrinsic activities for the accumulation of IP₃ or AA (Figure 2), but it appeared that activation of the latter pathway was more likely to be associated with hallucinogenic effects than IP₃ accumulation (Kurrasch-Orbaugh et al., 2003a). Further investigation of hallucinogen-mediated release of AA demonstrated that this functional pathway is more complex than originally thought. *In vitro*, following a 30 minute agonist stimulation, it has been shown that AA signaling probably involves G $\alpha_{i/o}$ and G $\alpha_{12/13}$, as well as G $\beta\gamma$, Rho, MEK1,2, and p38 MAPK (Kurrasch-Orbaugh et al., 2003a), indicating that more than one signaling cascade is responsible for 5-HT_{2A} receptor-mediated AA release. More recent studies have demonstrated that 5-HT_{2A} receptor activation also leads to the formation of the 2-arachidonylglycerol (2-AG), an endocannabinoid whose formation is partially dependent on the PLC-mediated accumulation of IP₃ (Parrish and Nichols, 2006).

It appears, therefore, that activation of the 5-HT_{2A} receptor leads to the production of at least three distinct biochemical signals, IP₃/diacylglycerol, AA, and 2-AG, and that the relative activation of these pathways varies with the ligand under investigation. Moreover, the “transcriptome fingerprints” of various agonists activating only the 5-HT_{2A} receptors were shown to differ (Gonzalez-Maeso et al., 2003). At present, however, there is no clear evidence to suggest what structural characteristics of a ligand promote the activation of one effector over the others. The induction of unique receptor conformations

by each ligand would, in theory, promote distinct affinities and coupling efficiencies to the various G proteins endogenously expressed in cell models, and also possibly to other proteins that interact with the receptors in modulating signal transduction. Virtual docking of enantiomeric phenethylamines into a homology model of the 5-HT_{2A} receptor has been employed in order to investigate the possibility that subtle structural differences among ligands might be responsible for distinct receptor conformations, and therefore the unique pharmacologies associated with a number of the 5-HT_{2A} ligands (Parrish et al., 2005). These studies indicate that there is much more research to be done before the molecular determinants of functional selectivity in any GPCR paradigm are likely to be elucidated (Parrish et al., 2005).

5-HT₂ serotonin receptor “antagonists” and collateral efficacy

Most of the examples of compounds that appear to activate 5-HT receptors differentially are ligands that were originally thought to be typical agonists, yet there are examples in which presumed antagonists actually demonstrate an agonist-like activity. For example, chronic antagonist treatment often leads to compensatory receptor up-regulation (Hess et al., 1988) that may be one of the mechanisms involved in the resultant ‘supersensitivity’ of receptor signaling (Schulz et al., 1979). In spite of this fact, it has long been known that chronic antagonist administration leads to apparent receptor down-regulation and desensitization of signaling with some GPCRs (for recent reviews, see Kenakin, 2005; O'Connor and Roth, 2005). This paradoxical regulation of GPCRs by antagonists was discovered and has been most convincingly demonstrated for 5-HT₂ family serotonin receptors.

Peroutka and Snyder (1980) were the first to demonstrate that chronic treatment with 5-HT_{2A} antagonists led to apparent 5-HT_{2A} receptor down-regulation *in vivo*—a finding that has now been replicated in over 100 published studies (see Gray and Roth, 2001 for review), of which many have shed additional light on this phenomenon. It has been demonstrated that: 1) antagonists induce 5-HT_{2A} receptor internalization and down-regulation *in vitro* when cloned receptors are expressed in heterologous expression systems (Berry et al., 1996; Bhatnagar et al., 2001; Newton and Elliott, 1997; Willins et al., 1999); 2) antagonists induce 5-HT_{2A} receptor internalization *in vivo* in individual

cortical neurons (Willins et al., 1999); 3) antagonist-induced desensitization requires internalization (Hanley and Hensler, 2002); and 4) antagonist-induced receptor down-regulation does not occur due to changes in receptor synthesis (Anji et al., 2000; Roth and Ciaranello, 1991). Antagonist-induced internalization, an example of “collateral efficacy” (Kenakin, 2005), also has been demonstrated with CCK and other peptide receptors (Roettger et al., 1997; Sneddon et al., 2003).

These observations suggest that the antagonist-induced internalization of 5-HT_{2A} and other GPCRs may have therapeutic potential. For example, Elphick *et al.* (2004) recently demonstrated that the JC Virus—the causative agent of progressive multifocal leukoencephalopathy (PML)—uses 5-HT_{2A} receptors to infect cells, and that some 5-HT_{2A} antagonists known to induce receptor internalization can block this viral infection. Further study has shown that antagonists that induce 5-HT_{2A} receptor internalization *in vitro* (e.g. mirtazepine) are able to block viral infection, whereas those that do not (e.g. cyproheptadine) are less protective (O'Connor and Roth, 2005, W. Attwood, personal communication). It stands to reason, therefore, that prophylactic treatment with 5-HT_{2A} antagonists with collateral efficacy is likely to be therapeutic, whereas treatment with antagonists that fail to internalize the 5-HT_{2A} receptor is not (O'Connor and Roth, 2005). Indeed, antagonists with the ability to induce 5-HT_{2A} receptor down-regulation might be therapeutically useful, for example, for individuals at risk for PML as a side-effect of treatment with drugs like natalizumab (Engelhardt and Briskin, 2005; Ransohoff, 2005; Van et al., 2005).

Functional selectivity at μ -opioid, β_2 -adrenergic, and V2 vasopressin receptors

Serotonergic receptors are not the only family of receptors with which the phenomenon of functional selectivity has been observed. There is both pharmacological and biochemical evidence for the agonist-selective regulation of μ -opioid and β_2 -adrenergic receptors (β_2 AR). Some of these observations were made in studies designed to determine the ability of ligands to produce an endocytic effect on the receptor under investigation, thereby affecting receptor regulation. Utilizing epifluorescence microscopy of epitope-labeled μ -opioid receptor, it was possible to visualize differences in μ -opioid receptor agonist induction of receptor endocytosis in transfected fibroblasts

(Keith et al., 1996) and in neurons expressing endogenous receptors *in vivo* (Keith et al., 1998; Sternini et al., 1996). Furthermore, by comparing the “endocytic efficacies” of these compounds with their intrinsic activities for other effectors coupled to μ -opioid receptor (e.g. adenylate cyclase activity and GIRK channel regulation) they were able to illustrate functional differences among agonists that are not predicted by the two-state receptor model. For example, the opioid peptide DAMGO induces significant receptor endocytosis similar to methadone, whereas morphine elicits a much more subtle endocytic response (Keith et al., 1996; Whistler et al., 1999). Morphine is, however, able to activate GIRK channels via μ -opioid receptors in transfected fibroblasts with an intrinsic activity similar to or greater than that of methadone (Whistler et al., 1999). and similar effects have been reported *in situ* (Alvarez et al., 2002). These data suggest that an alternative receptor model is in order, one that takes into consideration at least two different active receptor conformations that can be specifically induced, or selected for, by a particular ligand.

Such observations have prompted further studies designed to elucidate the mechanism behind differential receptor endocytosis. Significant evidence had suggested that ligand-specific differential receptor phosphorylation of the μ -opioid receptor was a potential mechanism by which downstream cellular effectors could distinguish the different functional effects elicited by ligands (He et al., 2002; Whistler and von Zastrow, 1998; Yu et al., 1997; Zhang et al., 1998). Moreover, a similar observation had been made by Benovic et al. (1988) in the β 2AR system *in vitro*. A recent study has examined whether the β 2AR has a single or has multiple agonist-induced phosphorylated states in an intact cell. Trester-Zedlitz et al. (2005) incorporated a protein analytical approach that took advantage of recent improvements in mass spectrometry methods (specifically, MALDI-TOF) and protein enrichment procedures (e.g., SILAC and immobilized metal affinity chromatography), which allow for the biochemical analysis, or “read-out”, of ligand-induced posttranslational modifications to β 2AR isolated from a stable HEK cell line. These studies indicate that there are not only multiple phosphorylation sites between the third intracellular loop and carboxyl-terminal cytoplasmic domain of the β 2AR, but that there appears to be an agonist bias that determines the specific phosphorylation state (Trester-Zedlitz et al., 2005). Although mapping the specific amino acid residues that are

phosphorylated in response to a specific ligand is still a work in progress, the general site locations are consistent with previous studies of ligand-induced desensitization and GRK activity (Hausdorff et al., 1991; Tran et al., 2004). These data lend support to the hypothesis of differential regulation of receptor endocytosis by distinct ligands via agonist-selective posttranslational modifications such as phosphorylation.

More pertinent to the clinic are some preliminary data that suggest there may be a genetic component to functional selectivity as it relates to specific physiological syndromes. A screening approach has identified C5a complement receptor mutations that separate ligand dependence of endocytosis from signaling (Baranski et al., 1999; Whistler et al., 2002). A more recent study reached a similar conclusion for the V2 vasopressin receptor (V2R), and suggested that this distinction is the basis of a newly defined human genetic disease. Several patients, presumptively diagnosed with the syndrome of inappropriate antidiuretic hormone secretion (SIADH), were shown to express one of two gain-of-function point mutations of Arg 137 of the V2R (Feldman et al., 2005). Arg 137 is located in the conserved "DRY/H" motif of the receptor, and the mutations were shown to cause constitutive activation of receptor signaling, defining the "nephrogenic syndrome of inappropriate antidiuresis" or NSIAD (Vargas et al., submitted). This study is particularly interesting because alternate mutation of precisely the same residue to His causes reduced receptor signaling and the opposite disease state of nephrogenic diabetes insipidus (NDI, Rosenthal et al., 1993). Interestingly, the NDI mutant V2R is weakly activated by agonist ligands because it is bound to arrestins and internalized in a constitutive (or ligand-*independent*) manner (Barak et al., 2001). Conversely, the NSIAD mutant V2R exhibits constitutive signaling, yet its internalization and arrestin association are strongly ligand-*dependent* (Vargas et al., submitted). This mutational "reversal" in ligand-dependence for signaling and internalization supports the hypothesis that there may exist multiple ligand-induced conformations of the V2R, differing in relative signaling and internalization activities, and that these may underlie essentially opposite human disease states.

Functional selectivity at D_{2L} and D₁ dopamine receptors

Dopamine receptors represent another subset of GPCRs that have been observed to differentially activate associated signaling cascades in response to certain compounds. In fact, much of the work on functional selectivity through dopamine receptors was an unexpected result of efforts directed at the rational discovery of novel D₁ dopamine ligands (Charifson et al., 1989; Mottola et al., 1996; Qandil et al., 2003). When dihydroxidine (DHX), the first high affinity full agonist for the D₁ dopamine receptor was characterized (Lovenberg et al., 1989), it was found to have only ten-fold selectivity for D₁ versus D₂ receptors (Brewster et al., 1990; Mottola et al., 1992). DHX and its congener *N*-propylDHX were initially characterized as full agonists at the D₂ receptor because they were equally efficacious to dopamine in inhibiting cAMP synthesis and efflux in striatal slices, inhibiting prolactin release *in vivo*, and stimulating GTP γ S binding in rat substantia nigral tissue (Kilts et al., 2002; Mottola et al., 1992). Further characterization of the functional profile of these compounds, however, demonstrated that they are not typical full D₂ receptor agonists. Neither DHX nor *N*-propylDHX are able to inhibit the synthesis and release of dopamine in rat striatum, nor are they able to inhibit the firing of nigral dopaminergic neurons (Figure 3) (Kilts et al., 2002; Mottola et al., 2002). Effects in all of these functional assays would be expected for typical D₂ receptor agonists. Thus, although DHX and *N*-propylDHX bind to the D₂ receptors present in both neural tracks, they cause differential activation of specific signaling pathways, an overall concept that led to the coining of the term “functional selectivity” (Lawler et al., 1994; Lawler et al., 1999; Mailman et al., 1997; Mailman et al., 1998). Interestingly, the functional selectivity of *N*-propylDHX *in vitro* may explain its unexpected behavioral effects *in vivo* (Smith et al., 1997).

Similar trends are observed *in vitro* (rat lactotrophs as well as MN9D & CHO cells stably expressing the D₂ receptor), verifying that the *in situ* observations are not artifacts produced by the activation of other GPCRs, and further confirming that functional selectivity as observed for specific dopaminergic compounds is not an epiphenomenon associated with a particular signaling model (Gay et al., 2004; Kilts et al., 2002; Mottola et al., 2002). This functionally selective profile is not unique to DHX and *N*-propylDHX, as functional selectivity has been shown for other D₂ receptor ligands

[e.g. propylnorapomorphine (NPA) and dinapsoline (DNS)] *in vitro* (Gay et al., 2004 and unpublished)]. Not only have these latter compounds demonstrated varying intrinsic activities among the D₂ receptor-mediated effectors studied, but some of them also exhibit significant variability in their relative potencies for the effectors they do activate (Figure 3).

Ligand-induced differential signaling has been more challenging to demonstrate in D₁ dopamine receptors because there is a relative paucity of functional effectors known to couple to this receptor. A recent report, however, has shown that apomorphine appears to regulate D₁ signaling differentially, as determined by comparing its ability to fully activate D₁ receptor-mediated cAMP accumulation and its inability to induce D₁ receptor internalization (Ryman-Rasmussen et al., 2005).

The therapeutic and clinical relevance of functionally selective compounds has been exemplified by the atypical antipsychotic drug aripiprazole (Abilify). By the time it received FDA approval for the clinical treatment of schizophrenia, aripiprazole had been marketed as a partial agonist at both the 5-HT_{1A} and D₂ receptors as well as a 5-HT_{2A} antagonist. This partial D₂ agonism (Burriss et al., 2002; Cosi et al., 2006) is postulated to lead to “dopamine system stabilization,” i.e. normalization of both dopamine hypo- and hyperactivity in pathologically-affected dopaminergic tracts (Lieberman, 2004; Stahl, 2001a; Stahl, 2001b; Tamminga and Carlsson, 2002).

Further assessment of the literature, however, reveals that the intrinsic activity and potency of aripiprazole for the D₂-mediated inhibition of cAMP accumulation is cell line-dependent, as aripiprazole demonstrates weak partial agonist activity in the CHO-D_{2L} cell line but strong partial agonist activity in HEK-D_{2L} cells (Burriss et al., 2002; Lawler et al., 1999; Shapiro et al., 2003). Moreover, aripiprazole antagonizes both D₂ receptor-mediated GTPγS binding and GIRK channel activity (Shapiro et al., 2003), while acting as a full agonist *in situ* for D₂-mediated inhibition of tyrosine hydroxylase (unpublished observation). Thus aripiprazole appears to elicit D₂-mediated functional effects that encompass a whole range of classic pharmacological traits, and its functional properties probably depend on the signaling machinery associated with the D₂ receptor (and hence, on the type of cell and location in the cell). Indeed, this conclusion is most

consistent with the fact that the drug is reported to antagonize post-synaptic D₂ receptors, but partially activate presynaptic D₂ autoreceptors (Kikuchi et al., 1995). Taken together, these data indicate that if “dopamine stabilization” is a result of aripiprazole treatment, it is primarily via a “correct” mix of direct effects on D₂ receptors rather than “just the right” balance of pre- and post-synaptic actions in competition with dopamine (Urban et al., 2006).

Mechanisms and research directions to understand functional selectivity

Conformational changes at the β 2AR: possible mechanism of functional selectivity

Evidence for multiple receptor states also has been provided by investigations of agonist-induced conformational changes in purified β 2AR protein. The labeling of specific sites on the receptor with fluorescent probes (Gether et al., 1997) allows for the detection of changes in the bound fluorophore, such as fluorescence lifetime, emission maximum and/or intensity, and thereby elucidates variations in receptor conformation (Kobilka and Gether, 2002). This approach has provided evidence that supports the notion that agonists and partial agonists produce distinct active state conformations, and suggests that agonist binding and activation occur through a series of discrete conformational intermediates (Ghanouni et al., 2001; Swaminath et al., 2004).

To understand the multistep process of GPCR activation better, catecholamine agonist fragments (such as catechol and dopamine) have been utilized to study the structural and functional properties of these conformational intermediate states (Swaminath et al., 2005). Considering that receptor activation is thought to result from the ability of an agonist to disrupt certain intramolecular interactions responsible for maintaining the basal state of the receptor, it was hypothesized that specific ligand moieties could be employed to realize these interactions.

Swaminath et al. (2005) have reported that catechol, a weak partial agonist for GTP γ S binding, was able to activate the rotamer toggle switch of transmembrane helix 6 (TM6), but unable to produce a break in the ionic lock between helices TM3 and TM6. Dopamine, which is able both to activate the TM6 rotamer toggle switch and break the

TM3-TM6 ionic lock, demonstrated strong partial activation of GTP γ S binding but failed to induce β_2 adrenergic receptor internalization. The extended dopamine congeners norepinephrine and epinephrine produced additional conformational changes that promoted receptor internalization, suggesting that a ligand must induce a number of conformational changes for typical agonist activity to be satisfied. The non-catechol partial agonist salbutamol, however, is able to break the ionic lock but fails to activate the rotamer toggle switch. Molecular modeling indicates that the salbutamol aromatic ring does not interact with the same receptor residues as the catechol moiety of catecholamines, suggesting that salbutamol induces an active conformation distinct from those induced by catecholamines (Swaminath et al., 2005).

These results suggest that the disruption of all of the interactions that maintain the basal conformational state is not required for a ligand to produce some level of receptor activation. It is likely that structurally distinct ligands are able to break different combinations of the basal state stabilizing interactions either directly by binding to amino acids that are involved in these intramolecular interactions, or indirectly by stabilizing new intramolecular interactions. These ligand-specific conformational changes may be responsible for differential activation of the signaling cascades of a receptor (Swaminath et al., 2005). Furthermore, structurally distinct ligands are able to break different combinations of the basal state stabilizing interactions in order to produce unique conformational states, which ultimately may be responsible for differential activation of the signaling cascades of a receptor (Swaminath et al., 2005).

Computational and theoretical approaches to understanding functional selectivity

In addressing the apparent disruption of traditional thought in pharmacology generated by the functional selectivity phenomenon, there has been a focus on the reasons that make the observations leading to the definition of “functional selectivity” appear uncommon to classical pharmacology. It has been suggested that in order to incorporate such apparently discordant observations into the pharmacological characterization of drug action, it may be necessary to re-evaluate the current axioms underlying the pharmacological definitions. This re-evaluation is provoked by experimental results from newly acquired abilities to: (1) identify and test naturally-occurring molecular variations

of both receptors and ligands (e.g., peptide hormones); (2) engineer novel molecular structures (e.g., constitutively active constructs) that exhibit new types of pharmacological properties; (3) create uncommon adjacencies of receptors on the cell surface, allowing for new types of heterodimerizations; and (4) test a much larger variety (structurally and chemically) of artificially created ligands for the receptors.

As a result of these developments, the phenomena that lead to the definition of “functional selectivity” must be examined to establish whether: A) the novel (or puzzling) phenotypes simply reveal underlying mechanisms that had not been challenged in classical pharmacology; and B) these mechanisms depend on the same underlying structure-function rules that explain classical pharmacology, but are richer in phenotypes.

In the context of the latter point, it has been suggested that the approaches must address structure-based mechanisms for modes of receptor activation, including a mechanistic understanding of the molecular processes involved in the discrimination between agonists and antagonists, full vs. partial agonists, neutral antagonists vs. inverse agonists, and the molecular details of constitutive activity to various degrees (Ebersole et al., 2003). Moreover, the protein-protein interaction responsible for homo- and hetero-oligomerization (Filizola and Weinstein, 2005), as well as for the integration of the receptors in the signaling pathways in the cell (Weinstein, 2006), are likely to play important roles in determining the properties underlying the observed departures from what is considered to be “classical pharmacological activity.”

Point B can be illustrated by examining results from combined experimental and computational approaches applied to investigating mechanisms essential to the hallucinogenic drug action of several classes of 5-HT₂ receptor compounds. The goal of this endeavor is to uncover the subtle consequences of ligand-receptor interactions as they are mechanistically related to the subcellular elements ultimately responsible for hallucinogenic action (reviewed in Weinstein, 2006). It provides an excellent example of apparent pharmacological conundrums related to functional selectivity of the 5-HT_{2A} receptor demonstrated by the elegant definition of the “transcriptome fingerprint” (Gonzalez-Maeso et al., 2003).

In seeking the structural context of the ligand-determined receptor mechanisms, this interdisciplinary research has illuminated a number of conserved structural motifs in the configurations of rhodopsin-like GPCRs that are implicated in receptor signaling by acting as functional microdomains (Weinstein, 2006). Examples include (a) the NPxxY motif of TM7 (Prioleau et al., 2002), and (b) the cluster of aromatic residues in TM6 that straddles a universally conserved proline and is thought to act as a “toggle switch,” thereby conveying the consequences of the ligand-binding event to the rearrangements associated with receptor activation (as reviewed in Visiers et al., 2002).

Given this defined set of sites involved in ligand-dependent transitions in the conformation of the GPCRs, these studies have shown that structurally similar ligands can produce different, ligand-dependent modes of receptor activation by aligning themselves in different positions within the receptor-binding pocket. For example, it appears that bulky substitutions of the cationic moiety in the hallucinogenic compounds allow these ligands to adopt unique positions within the 5-HT_{2A} receptor binding pocket relative to their non-hallucinogenic congeners (Ebersole et al., 2003). These observations highlight the idea that ligand-dependent conformations are able to produce functionally selective responses at the cellular level that ultimately can be translated to a unique physiological effect. This mechanistic translation depends on the interactions of the receptor molecule with its environment, such as in oligomer formation (see below).

The oligomerization mechanism is only one form of integration of the GPCR into the signaling networks of the cell, as different receptor conformations produced by ligand binding could select for interaction different proteins (e.g., PDZ domains, kinases) from the downstream signaling pathway (Figure 4). Consequently, it is important to integrate the examination of the putative mechanisms leading to the functional selectivity paradigm with the subcellular, cellular, and physiological levels of GPCR signaling. A powerful tool for this integrative approach is quantitative modeling of signaling pathways, as mathematical representations that are amenable to computational simulations (Campagne et al., 2004). To produce useful models of these signaling systems, the components must be represented with the highest level of physical realism possible at our current understanding of cell physiology.

The potential role of GPCR oligomers in functional selectivity

The specific mechanisms of ligand-specific GPCR pathway activation may also be revealed as more is understood concerning the functional consequences of receptor dimerization/oligomerization. Many GPCRs have been observed to exist as homo- and hetero-dimers or oligomers, and in some cases this heterodimerization has been reported to lead to novel functional consequences. The potential structural interactions responsible for receptor oligomerization and crosstalk are, however, largely unknown.

To understand better the functional interactions between GPCRs, it is necessary to recognize where potential receptor-receptor interfaces are located. It has been proposed that functional cross-talk between GPCRs is likely the result of conformational changes at these dimer interfaces. To investigate this hypothesis, Guo et al. (2005) have utilized two rhodopsin-based structural models to study the potential dimer interface of the dopamine D₂ receptor. A critical aspect of this investigation involved mapping the homodimer interface in the D₂ receptor over the entire length of the fourth transmembrane segment (TM4) by cross-linking of substituted cysteines. The susceptibility of the cysteine mutants to cross-linking is altered differentially by the presence of agonists and inverse agonists. The TM4 dimer interface in the inverse agonist-bound conformation is consistent with the dimer of the inactive form of rhodopsin modeled with constraints from atomic force microscopy (Liang et al., 2003). Cross-linking of a different set of cysteines in TM4 was slowed by inverse agonists and accelerated in the presence of agonists. Cross-linking of the latter set locks the receptor in an active state, as shown in both GTP γ S binding and cAMP accumulation assays. Thus, a conformational change at the TM4 dimer interface is part of the receptor activation mechanism (Guo et al., 2005). This is shown schematically in Figure 5.

These results demonstrate that conformational changes in, and ultimately the rearrangement of, TM4 is critical to the activation of the D₂ receptor dimer, and that this aspect of the dimer interface is coupled to the structure of the binding site (Guo et al., 2005). It has been proposed that a single heterotrimeric G protein may interact with two GPCR protomers (Baneres and Parelo, 2003; Filipek et al., 2004), and conformational changes at the interface may thereby mediate G protein coupling and activation. Based on X-ray crystallography studies of the soluble N-terminal binding sites of metabotropic

glutamate receptors, agonist binding is thought to alter the relative orientation of the dimer formed by the two extracellular binding domains (Kniazeff et al., 2004; Kunishima et al., 2000; Tsuchiya et al., 2002). Moreover, in metabotropic glutamate receptors, agonist binding has been shown to lead to a change in the distance between the cytoplasmic loops of the protomers (Tateyama et al., 2004). Such a movement must be associated with an altered relationship between the transmembrane domains of the two protomers. Although it has yet to be examined, it is proposed that known dopamine D₂ functionally-selective compounds (e.g. dihydrexidine, dinapsoline, aripiprazole) might produce conformational changes that generate unique dimer interfaces that ultimately result in selective dimer-effector coupling.

Impact on research and training

The impact of functional selectivity on quantitative pharmacology

The initiation of the era of “analytical pharmacology,” i.e. the development and application of quantitative modeling to describe drug-receptor interactions and their consequences, can largely be attributed to the work of A.J. Clark. Along with Clark, the field has continued to develop and progress thanks in large part to the achievements of Ariens, Gaddum, Schild, Stephenson, Furchgott, Black, Kenakin, and others. Indeed pharmacology, by its very nature, is a quantitative discipline, and as such biological and mathematical models are indispensable as tools for designing and interpreting experiments, and providing objective insights into potential mechanisms of action.

An understanding of the simple model-building process provides a foundation for subjecting experimental data to mechanistic scrutiny. There are three possibilities that must be considered if the data are not in accord with the predictions of the model: (1) the incorporation of more complex mechanisms into the model is necessary; (2) the assumptions underlying the model fail to describe accurately the process used to generate the data; or (3) the process/quality of data generation needs to be scrutinized.

According to this process, functional selectivity initially might be described by the simplest mechanism (i.e., a single state receptor model), as variations in stimulus-response coupling efficiency are sufficient to model many experimentally-observed examples of tissue/cell-dependent differences in the expression of agonism for a given

ligand. If one assumes for the sake of argument that possibilities (2) and (3) do not apply to the experimental approach and resulting data, then the simplistic premise of a single-state receptor model becomes clearly inadequate as a mechanism that can explain phenomena such as reversals in agonist rank potencies, reversals in rank efficacies, and reversals of agonism to inverse agonism.

One direction for reconciling these experimental observations is to build more complex drug-receptor models that not only accommodate our current understanding of protein biophysics, but also incorporate multiple receptor states (Christopoulos and Kenakin, 2002). The heuristic nature of these models may yield further insight into biological behavior, although the very nature of their complexity may sometimes make it difficult to test the model adequately. A potential pitfall of multi-state models is the temptation to avoid critical scrutiny of the underlying assumptions. Thus, the very model that was built to accommodate novel data might fail to describe accurately the conditions under which the data were generated. As an example, the most commonly used multi-state models assume equilibrium between all reactants, an assumption that often may not be met. As shown in the cartoon in Figure 6, steady-state or kinetic models may provide more realistic mechanisms for understanding biological behavior under such circumstances (Christopoulos et al., 1999; Lew et al., 2000; Woolf and Linderman, 2000). Indeed, it may be that it is timely to bring non-linear dynamic approaches into the receptor pharmacology arena.

Functional selectivity: do we need to update pharmacological terminology?

In light of the pharmacological questions raised by the concept of functional selectivity, there is a need to appropriately classify GPCRs and the novel mechanisms that have been put forth to describe adequately the anomalies observed in functional pharmacology. In fact, the Nomenclature Committee of the International Union of Basic and Clinical Pharmacology (NC-IUPHAR) is developing a plan to initiate such a project. The mission of NC-IUPHAR is to: (1) issue guidelines for receptor and ion channel classification; (2) classify the major receptor and ion channel systems; and (3) facilitate the interface between discovery of new sequences from the human genome project (HGP) and designation of the derived proteins as functional receptors and ion channels.

Indeed, the publication of the HGP marks a unique moment in scientific history in that all the potential sequences for receptors and ion channels that have any analogy to previously known classes are now available. It is unlikely that many new ion channels or receptors have yet to be discovered, so it is the mechanistic understanding of these hypothesized ion channel and receptor sequences (as well as their biologically-important polymorphisms) that is presently an important pursuit of the medical and pharmacological community. The initial difficulties lie in the fact that the primary receptor sequences must be linked to general pharmacology, as well as to the affinity and potency values of classic agonists and antagonists. It is clear, however, that for standard receptor sequences there can be extremely diverse affinities and potencies reported in the literature (for a hands-on example of this the reader is referred to <http://pdsp.cwru.edu/pdsp.asp>, an interactive database of ligand-receptor affinities resulting from the NIMH-sponsored Psychoactive Drug Screening Program in Bryan Roth's laboratory, and to the IUPHAR site www.iuphar-db.org).

If functional selectivity is indeed a crucial mechanism that must be considered in the drug development process, then initial screens using reconstituted systems may not show the appropriate pharmacology. To select the best compounds (or, indeed, to understand selected lead compounds), assays dependent on several effector systems are necessary to screen for differential activation. This will provide a more accurate reflection of the characteristics of given compound, but even then, appropriate *in vivo* physiological models will still be required. This clearly creates a massive, but necessary task for pharmacologists as was recently proposed in detail (Spedding et al., 2005). If this is the case, then drug discovery may be back to drug-specific pharmacology (where each drug may have a distinct profile), rather than specific-drug pharmacology (where agents specific for a receptor have a predicted action). This is particularly the case for nuclear receptors where the local availability of co-activators and co-repressors markedly modifies drug responses (NC-IUPHAR reports in preparation).

Functional selectivity and its impact on drug discovery.

There are two general questions concerning functional selectivity as it relates to industry: (1) can functional selectivity be observed during the GPCR drug discovery

process, especially within *in vitro* systems? and (2), if observed, can functional selectivity *in vitro* be applied toward measures of *in vivo* efficacy and/or safety liabilities?

Of course the answer to the first question is “yes.” Functional selectivity is often observed with GPCRs expressed in recombinant systems, which can have an immediate impact on lead identification. High throughput screening (HTS) campaigns, however, often employ a single functional endpoint with which to characterize the activities of test compounds, an approach that can potentially lead to overlooking or triaging important compounds that signal through alternative pathways via the same receptor. For example, it has been demonstrated with both 5-HT_{2C} and D₂ ligands that relatively small structural modifications can elicit functional selectivity (more than 100-fold) without affecting receptor affinity (Gay et al., 2004; Miller et al., 2000). It is important, therefore, to collect data on ligand affinity as well as on ligand activity through multiple functional endpoints. Within the realm of industry, however, the addition of multiple functional effectors to a HTS operation is a substantial additional cost, and it is partially for this reason that industry has been slow to move in this direction.

Even when functional selectivity can be demonstrated *in vitro*, it is not completely clear how it may affect *in vivo* efficacy and/or improved safety profiles. The 5-HT_{2C} compounds that illustrated functionally selective profiles *in vitro* (i.e., high 5-HT_{2C} receptor affinity, high GTP γ S potency, but a 100-fold lower potency for the 5-HT_{2C}-mediated production of IP₃) were found to produce a similar level of efficacy in both acute and chronic feeding models as those compounds considered typical potent 5-HT_{2C} receptor agonists (Largent et al., 2002). Further *in vivo* examination of cFos activation in regions of the brain known to be involved in appetite regulation showed no difference among the compounds in the number or localization of activated neurons. Conversely, propylidihydroxidine (Figure 3), a compound with functionally selective *in vitro* properties (Kilts et al., 2002), causes quite unexpected behavioral effects (Smith et al., 1997). These examples highlight the immaturity of this field, and the need for additional research.

Ultimately, functional selectivity is a concept that must be considered when dealing with drug discovery. Both further discovery, and a better understanding of the

multiple signaling pathways coupled to various GPCRs will allow for the design of more integrated HTS assays and thus lead to more informed interpretations of ligand-receptor structure-activity relationships. Appreciating this concept also may help to explain unexpected *in vivo* findings of functionally weak compounds that illustrate *in vivo* efficacies equivalent to typical agonists.

Future directions

A major point of discussion is how these observations of functionally selective ligands might impact on traditional pharmacological principles, from the bench and clinic to the classroom. For example, there is the line of thinking that the underlying mechanisms for this phenomenon should be more carefully illuminated before a significant push is made to establish functional selectivity as an accepted pharmacological mechanism. Proponents for this more conservative approach feel that current knowledge of local receptor milieus, the involvement of accessory and structural proteins in signaling, receptor dimerization, kinetics, and the like, is rather limited, and that this uncertainty opens the door for a variety of mechanisms that might provide alternate explanation(s) without requiring major revisions in current pharmacological theory.

Although it is clear there are many gaps in our knowledge of receptor signaling, it seems to us that functional selectivity is a real phenomenon with a plethora of supporting examples. Indeed, we are not aware of any data from appropriately controlled studies that *invalidate* the functional selectivity hypothesis. Furthermore, some receptor mutations have been shown to cause standard ligands to become functionally selective, or to alter the signaling pattern of existing functionally selective ligands. As such, the concept must be given wider consideration by pharmacologists because of its potential relevance to both mechanistic interpretation of data, as well as its impact on ligand characterization and even drug discovery. We might consider, as an example, the dynamic nature of ligand-receptor modeling that has been continually updated as new data and new methodologies have been incorporated. Of course, the basic pharmacological tenet of “intrinsic efficacy” is a particular difficulty because of the inability to reconcile it with so many examples of functional selectivity.

If one accepts that functional selectivity is a widely applicable phenomenon (regardless of whether or not it requires scrapping of traditional quantitative pharmacology), it seems clear that it will affect the way that pharmacology is taught. If the concept proves to be universal, it impacts directly on training of future scientists who will affect future research and drug discovery. How this more complicated mechanism will be taught to others (e.g., medical or pharmacy students) is a challenge that will certainly need to be sorted out.

Another major point of discussion relates to agreement on a common name for functional selectivity to avoid a pharmacological Tower of Babel. As noted in the Introduction, this phenomenon carries many monikers, some of which have different connotations as to what is being described. What is evident from the data is that the examples of GPCR ligands considered to be “functionally selective” run the gamut in terms of their functional profiles. Thus, there are examples of ligands that have a broad functional profile for a specific receptor (antagonist or inverse agonist through full agonist behaviors), whereas others demonstrate less drastic differences in their receptor-specific functional activity (e.g., full and partial agonist activity). Some current terms imply either specific mechanisms or a narrow range of functional possibilities (e.g., “protean agonism,” “agonist-directed trafficking of stimulus,” and “biased agonism”). These terms fail to accommodate situations in which there is functional “inactivity” (i.e., antagonism), and the use of the term “trafficking” is often confused with established terminology that describe receptor relocalization. Another phrase, “relative activity versus endocytosis (RAVE),” although a catchy acronym, is mechanistically restrictive.

Although the term “functional selectivity” is descriptive and mechanistically neutral (both desirable characteristics), its use to describe the concept of ligand-dependent differential regulation of receptor-coupled effector pathways is weakened by the fact that a PubMed search for “functional selectivity” results in 133 hits. Of these, only a few actually refer to the concept of ligand-dependent differential regulation of signaling via a single receptor (the concept inherent to this Perspective), whereas the majority utilizes the phrase in a manner that describes ligand activity as a function of receptor-subtype. Thus, although “functional selectivity” may convey an appropriately neutral functional tone, its “misuse” in the literature may potentially create confusion for

future publication on the issue. A term that has been suggested as a viable alternative is “ligand-induced differential signaling” (LIDS). It also is mechanism-neutral, would not be confused with other aspects of cellular signaling and regulation, and evokes a catchy acronym. The importance of an appropriate mechanistic classification of functional selectivity has been echoed by members of the Nomenclature Committee of IUPHAR. In fact, the NC-IUPHAR urges those who are interested to actively participate in the ongoing dialogue on the issue of terminology by submitting their opinions directly (<http://www.iuphar.org/nciuphar.html>). It is important to note that although this Perspective has taken a GPCR-centric view of the receptor world, we are keenly aware that similar phenomena (e.g., SERM - selective estrogen receptor modulation) have been recognized with other receptor superfamilies (e.g., see Gronemeyer et al., 2004 for recent review). Thus, the impact of this issue spans the breadth of pharmacology

In closing, there is a need for even greater understanding of local receptor environments and the factors that might play a role in the types of anomalous observations reviewed here. These range from accessory proteins (both catalytic and organizational), receptor dimerization (both homo- and heterodimers), alternative receptor splicing, mRNA editing, receptor polymorphisms (SNPs), trafficking of receptors, etc. There is also a need to understand how subtle changes in ligand structure can sometimes have profound effects on functional properties. Not only will such data provide a firm mechanistic base for functional selectivity (by whatever name it is called), but this knowledge also will help us to understand many apparent pharmacological anomalies, and potentially lead to novel drug discovery. Some years ago, it was written that functional selectivity “...could yield important therapeutic advances, although it introduces a new level of complexity that will require significantly greater understanding of receptor dynamics and the interaction with transduction mechanisms” (Mailman et al., 1997; Mailman et al., 1998). The discussions resulting from the paradoxical data found in the literature suggests that the time is ripe to explore these issues further.

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Footnotes

Conflicts of interest

Dr Michael Spedding is both chairman for NC-IUPHAR and deputy director of research for Servier. Dr. Keith Miller is an employee of Bristol-Myers Squibb, Inc. Drs. Mailman and Nichols, the University of North Carolina and Purdue University have a financial interest in BioValve Technologies, the licensee of compounds mentioned in this Perspective. The opinions presented herein are those of the authors, and not those of any other party.

Legends for Figures

Figure 1. Functional selectivity at the 5-HT_{2C} receptor. Bars represent the ratio of the maximal effect of the test ligands to that of the reference ligand, 5-HT. Note for example, that bufotenin is a full agonist at AA release, and a partial agonist at IP accumulation, whereas TFMPP is a full agonist at IP accumulation but has only partial intrinsic activity at AA release. Other examples violating the classically-held tenet of intrinsic efficacy (i.e. response-independence) are also seen in the data (Data from Stout et al., 2002).

Figure 2: Functional selectivity at 5-HT_{2A} receptors. The panel on the left illustrates relative potency of two agonist ligands in stimulating PLC, measured by the accumulation of inositol phosphates in a stable rat 5-HT_{2A} NIH-3T3 cell line. The EC₅₀ values for DOB and (R)-2C-B-CB are 23 nM and 43 nM, respectively, only two-fold different, with similar intrinsic activities. By contrast, the panel on the right shows the potency of the same two ligands in stimulating arachidonic acid release from these cells, with EC₅₀ values of 58 nM and 1.6 μM, respectively, their potencies differing in this signaling pathway by nearly 30-fold. Although DOB is clearly not functionally selective, (R)-2C-B-CB, a structurally related ligand, is 36-fold selective in stimulating the PLC signaling pathway over AA production.

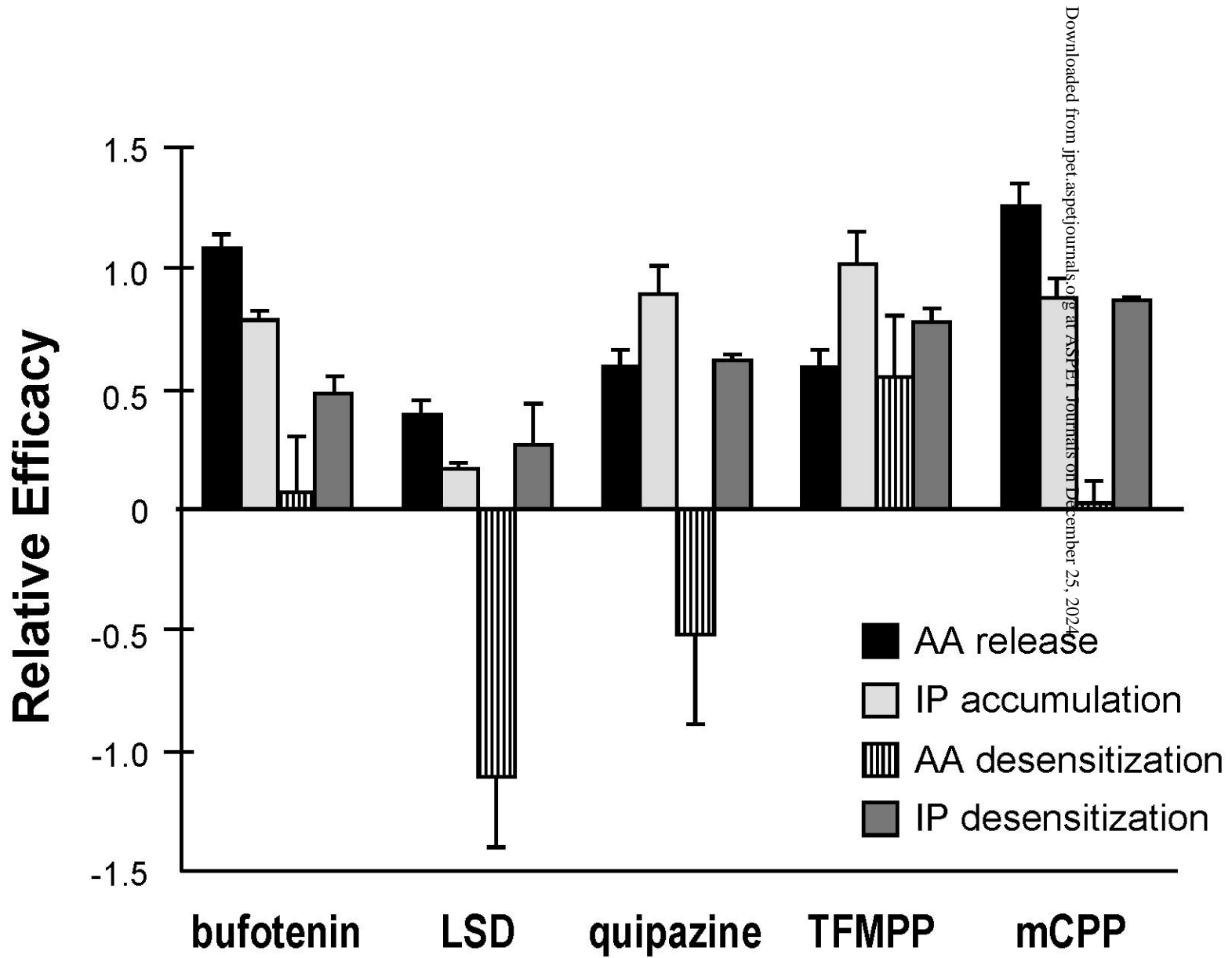
Figure 3. Functional selectivity can be expressed as differences in intrinsic activity or potency although the two are interrelated. **A.** Quinpirole and propylidihydroxidine (PrDHX) are full agonists at the D_{2L} receptor in MN9D cells, whose effects are completely blocked by the D₂ antagonist (butaclamol), **B.** Conversely, only quinpirole inhibits receptor-mediated DA release, and both PrDHX and butaclamol block quinpirole effects. **C.** As shown above in Figure 2, the relative potency of ligands can be markedly different even when the intrinsic activity is the same. Data modified from Kilts et al. (2002) and Gay et al. (2004)

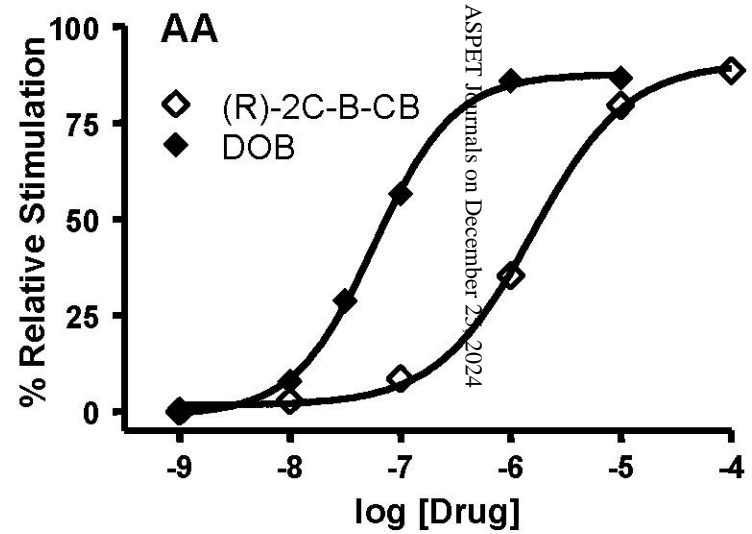
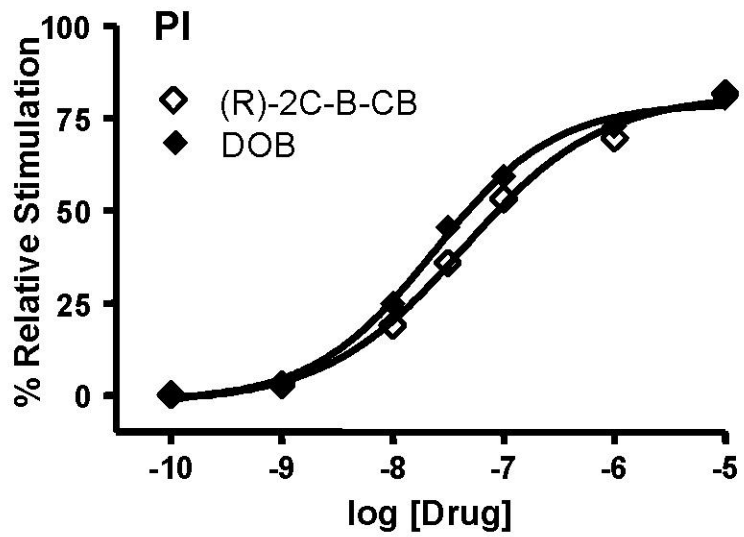
Figure 4. The protein-protein interaction interface between a GPCR and its signaling environment is regulated by intramolecular interactions involving the NPxxY motif: The interaction between Y7.53 and the F7.60, which is in Hx8, controls the position of the helix and the C-terminal. This can regulate the interaction interface between the GPCRs and other proteins in the signaling cascade (e.g., PDZ domains), and may be affected differentially by ligands, providing one mechanism for functional selectivity.

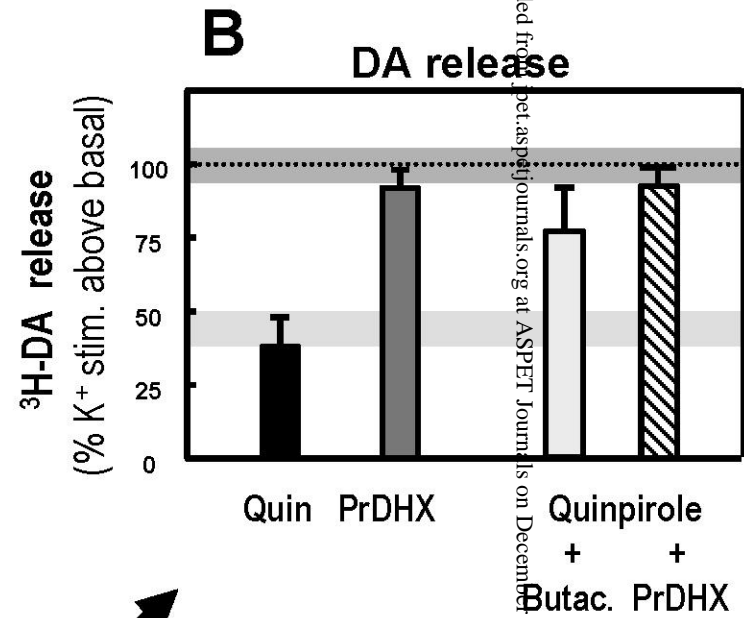
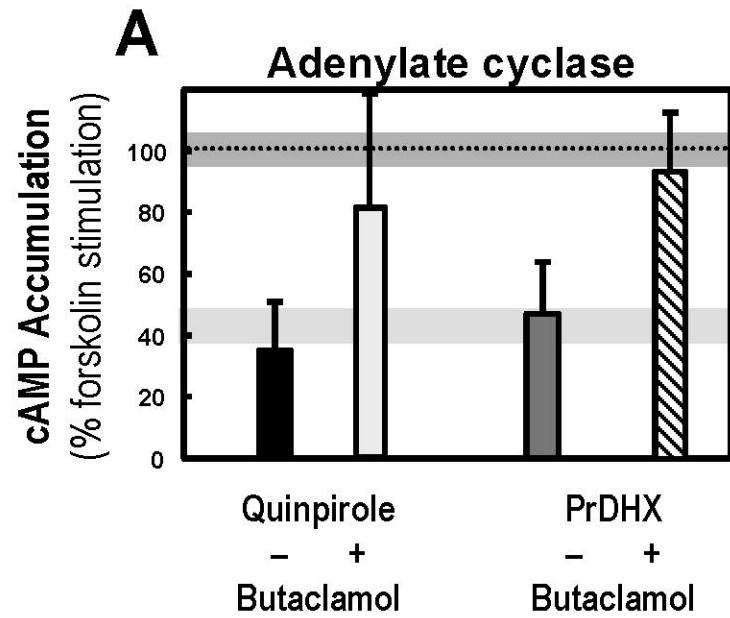
Figure 5, Illustration of potential dimer arrangements and molecular model representations of the proposed dimer interfaces and effects of disulfide trapping of the respective interfaces on receptor activation. (see Guo et al., 2005 for additional detail). (Left) Extracellular and side views of a symmetric TM4-TM5 interface (yellow) as proposed by Liang et al. (2003). (Right) Extracellular and side views of a symmetric TM4 interface (red) deduced from the squid rhodopsin 2D electron density map (Davies et al., 2001). These two models are proposed to correspond to the inactive and active states, respectively. The backbone of the transmembrane segments is rendered as cylinders. (Middle) The change in [cAMP] in the presence of forskolin (100 μ M) after treatment with CuP (100 μ M) in cells stably expressing the appropriate cysteine mutants is shown as the mean \pm SEM (n = 3-6). The x axis is plotted so that activation of the receptor, which causes inhibition of adenylate cyclase through G_i, is shown to the right of the y intercept. In C168^{4,58}S, the maximal change of cAMP induced by dopamine was -74%. Thus, disulfide trapping of residues at the red interface activates the receptor, whereas cross-linking of residues at the yellow interface decreases spontaneous activation.

Figure 6. The three different types of models applied to biological systems are amenable to a pendulum analogy. Equilibrium models, which encompass the ternary complex model (TCM), extended TCM (ETCM) and cubic TCM (CTCM) are akin to a pendulum that is static in nature. Steady-state models, such as the ternary complex activation model (TCAM), are more realistic in that they encompass the contribution

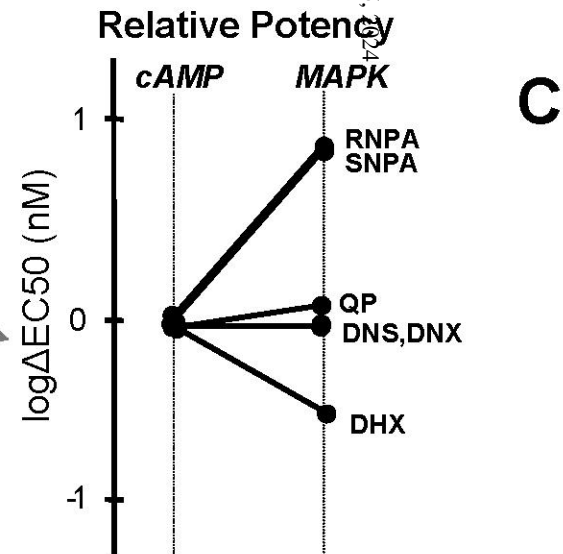
of energy (e.g., wind for the pendulum) to maintain a behavior that appears static. The most biologically relevant models, however, are dynamic models, because they reflect the fact that the system is constantly changing in time. However, the more biologically relevant, the more complex the model becomes. Adapted from Woolf and Linderman (2000).





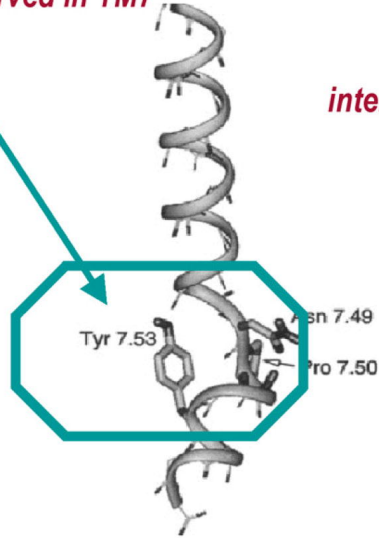


Functional selectivity can be manifest as differences in *intrinsic activity* or *potency*

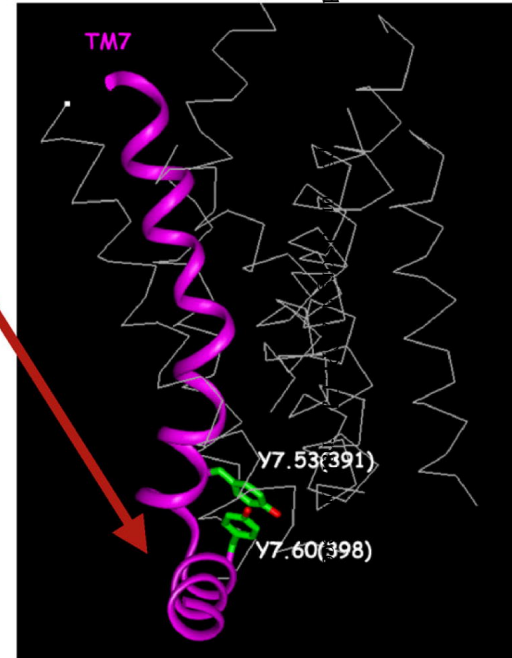


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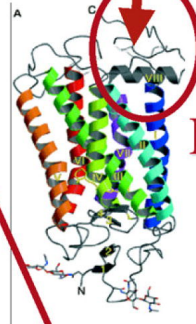
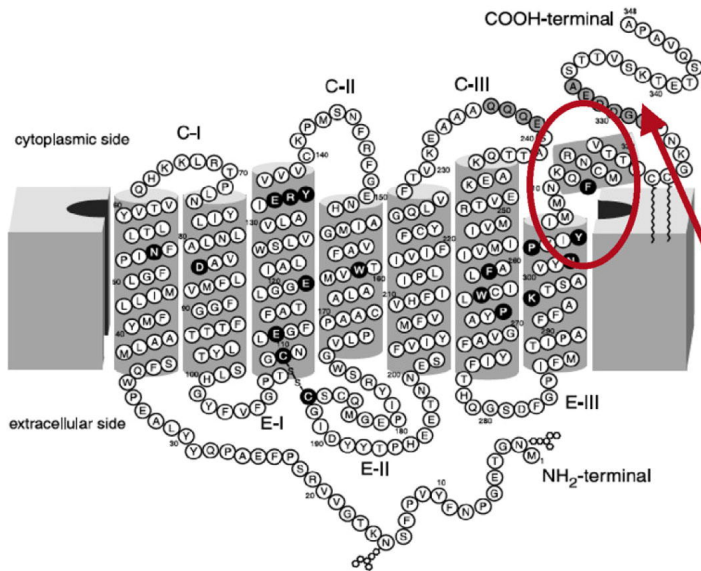
The NPxxY motif conserved in TM7 of Class A GPCRs



interacts with Helix 8

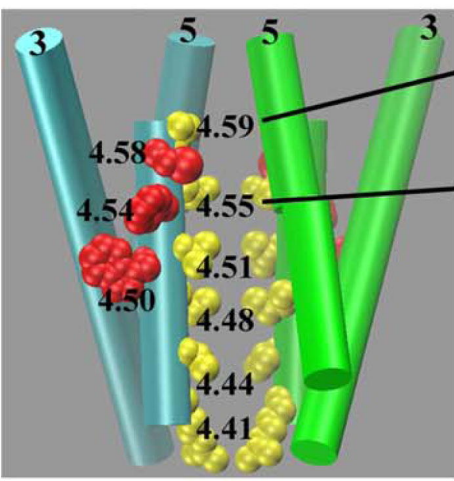
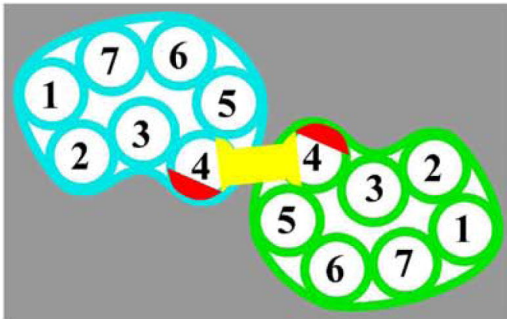


(shown for TM 7 of a 5-HT receptor model)

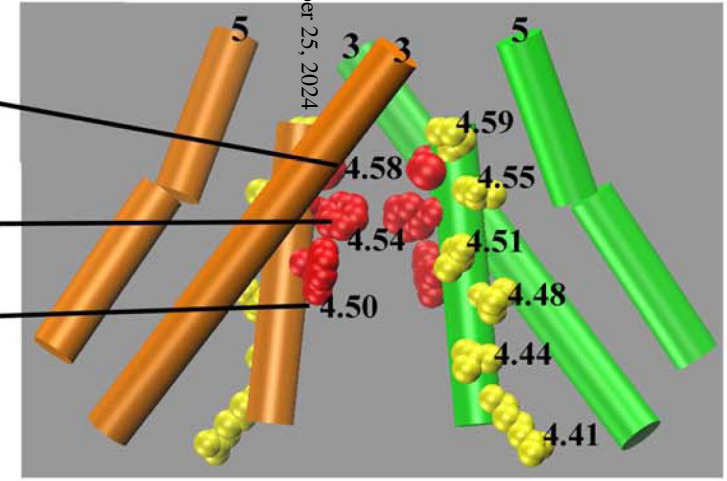
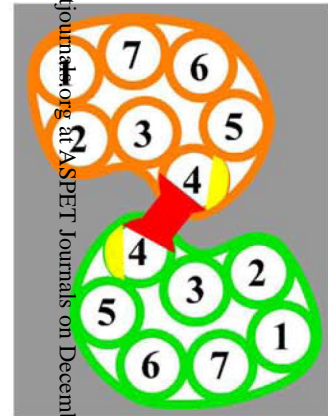
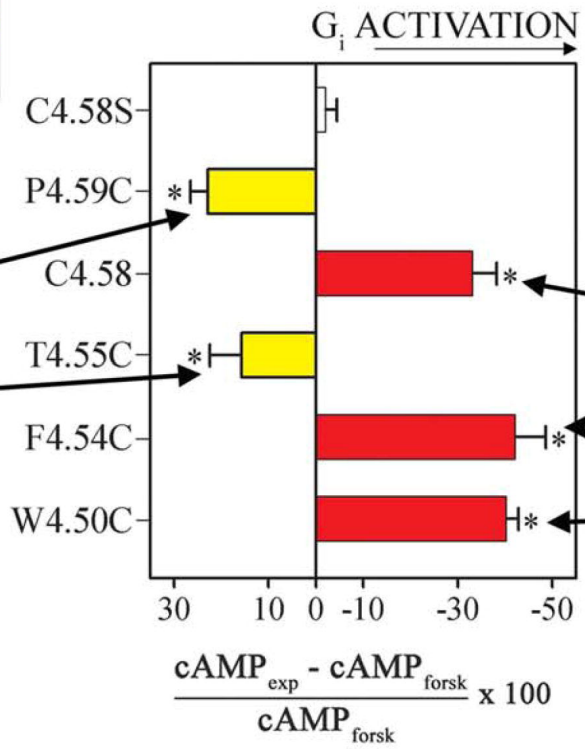


Helix 8

and is involved in positioning the C-terminus for interaction (shown for rhodopsin)

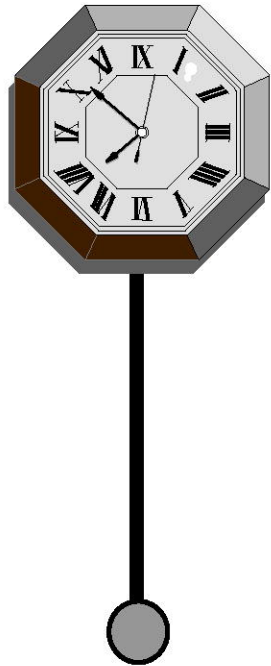


AFM model



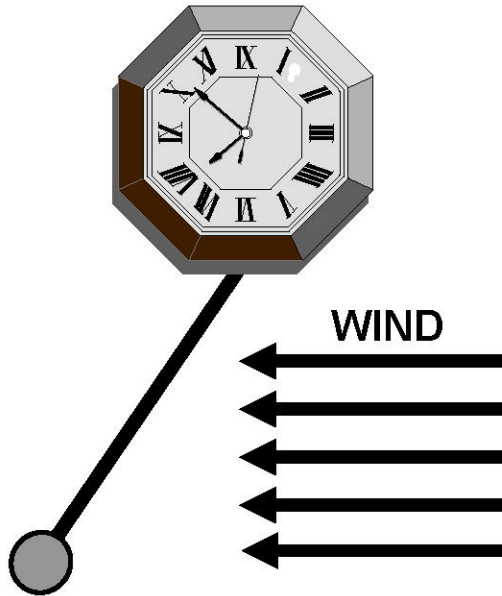
ECM model

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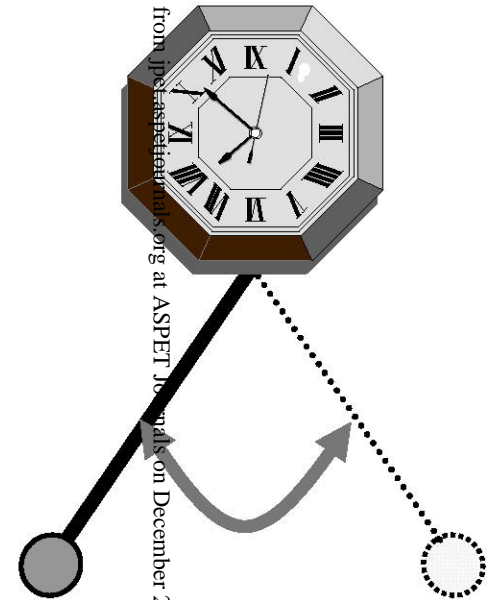
Equilibrium

TCM
ETCM
CTCM
etc.



Steady-State

TCAM



Dynamic

BIOLOGICAL RELEVANCE

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