The Journal of Physiology

CROSSTALK

Rebuttal from Nevin A. Lambert and Jonathan A. Javitch

Nevin A. Lambert[1]
and Jonathan A. Javitch[2,3]
[1]*Department of Pharmacology and Toxicology, Medical College of Georgia, Georgia Regents University, Augusta, GA 30912-2300, USA*
[2]*Departments of Psychiatry and Pharmacology, College of Physicians and Surgeons, Columbia University, New York, NY 10032, USA*
[3]*Division of Molecular Therapeutics, New York State Psychiatric Institute, New York, NY 10032, USA*
Email: nelambert@gru.edu

In their review Bouvier and Hebért (2014) demonstrate how 'dimer-coloured' glasses can filter perception, impair recall, and perhaps blind us to alternative hypotheses and contradictory findings. For example, Whorton *et al.* (2008) demonstrated that rhodopsin *monomers* reconstituted from detergent into nanodiscs activate transducin as rapidly as native rod membranes, not that reconstituted oligomers activate more quickly than monomers, which was what our colleagues somehow took away. Indeed, forced dimerization of rhodopsin if anything slows transducin activation (Bayburt *et al.* 2007). Likewise, rows of rhodopsin dimers were cited as structural evidence for oligomerization (Fotiadis *et al.* 2003), but older observations suggesting that such rows may be related to sample preparation were overlooked (Roof & Heuser, 1982). Large receptor arrays are also very difficult to reconcile with rapid lateral and rotational diffusion of rhodopsin in intact outer segments (Poo & Cone, 1974). Redka *et al.* (2013) observed some degree of binding cooperativity for most agonists even in detergent, where the receptors they studied were thought to be monomers. Nevertheless, since cooperativity was greater in phospholipid than in detergent it was said to be 'lost' when receptors were monomerized. This is a generous interpretation, particularly since detergent also decreased the affinities of both high- and low-affinity sites by 10- to 20-fold. Fonseca & Lambert (2009) was cited as evidence for transient *dimers*, although this study failed to detect evidence of dimers of any sort. In our view these examples illustrate the degree to which oligomerization has become the default explanation for many observations, and a concept that is more widely accepted than a careful consideration of all available evidence warrants. There is little doubt that class A GPCRs can and do interact in the membrane, but despite initial inferences that these interactions are stable and constitutive, new more discriminating methods suggest that these interactions are transient (Hern *et al.* 2010; Kasai *et al.* 2011). Using highly-engineered approaches we and others (Mesnier & Banères, 2004; Vilardaga *et al.* 2008; Han *et al.* 2009; Urizar *et al.* 2011) have shown that receptors in proximity can affect each other, but whether this is relevant in native systems is extraordinarily difficult to establish and simply not yet clear. The potential of heterodimers for improved pharmacotherapies is exciting and has drawn many of us to the field. We certainly agree with our colleagues that 'much work remains to irrefutably demonstrate [the] functional importance [of dimers] *in vivo*', but we must continue to approach this work with unfettered vision, without preconception, and with healthy skepticism.

Call for comments

Readers are invited to give their views on this and the accompanying CrossTalk articles in this issue by submitting a brief comment. Comments may be posted up to 6 weeks after publication of the article, at which point the discussion will close and authors will be invited to submit a 'final word'. To submit a comment, go to http://jp.physoc.org/letters/submit/jphysiol;592/12/2449

References

Bayburt TH, Leitz AJ, Xie G, Oprian DD & Sligar SG (2007). Transducin activation by nanoscale lipid bilayers containing one and two rhodopsins. *J Biol Chem* **282**, 14875–14881.

Bouvier M & Hébert TE (2014). CrossTalk proposal: Weighing the evidence for Class A GPCR dimers, the evidence favours dimers. *J Physiol* **592**, 2439–2441.

Fonseca JM & Lambert NA (2009). Instability of a class A G protein-coupled receptor oligomer interface. *Mol Pharmacol* **75**, 1296–1299.

Fotiadis D, Liang Y, Filipek S, Saperstein DA, Engel A & Palczewski K (2003). Atomic-force microscopy: Rhodopsin dimers in native disc membranes. *Nature* **421**, 127–128.

Han Y, Moreira IS, Urizar E, Weinstein H & Javitch JA (2009). Allosteric communication between protomers of dopamine class A GPCR dimers modulates activation. *Nat Chem Biol* **5**, 688–695.

Hern JA, Baig AH, Mashanov GI, Birdsall B, Corrie JE, Lazareno S, Molloy JE & Birdsall NJ (2010). Formation and dissociation of M_1 muscarinic receptor dimers seen by total internal reflection fluorescence imaging of single molecules. *Proc Natl Acad Sci U S A* **107**, 2693–2698.

Kasai RS, Suzuki KG, Prossnitz ER, Koyama-Honda I, Nakada C, Fujiwara TK & Kusumi A (2011). Full characterization of GPCR monomer-dimer dynamic equilibrium by single molecule imaging. *J Cell Biol* **192**, 463–480.

Mesnier D & Banères JL (2004). Cooperative conformational changes in a G-protein-coupled receptor dimer, the leukotriene B_4 receptor BLT1. *J Biol Chem* **279**, 49664–49670.

Poo M & Cone RA (1974). Lateral diffusion of rhodopsin in the photoreceptor membrane. *Nature* **247**, 438–441.

Redka DS, Heerklotz H & Wells JW (2013). Efficacy as an intrinsic property of the M_2 muscarinic receptor in its tetrameric state. *Biochemistry* **52**, 7405–7427.

Roof DJ & Heuser JE (1982). Surfaces of rod photoreceptor disk membranes: integral membrane components. *J Cell Biol* **95**, 487–500.

Urizar E, Yano H, Kolster R, Gales C, Lambert N & Javitch JA (2011). CODA-RET reveals functional selectivity as a result of GPCR heteromerization. *Nat Chem Biol* **7**, 624–630.

Vilardaga J-P, Nikolaev VO, Lorenz K, Ferrandon S, Zhuang Z & Lohse MJ (2008). Conformational cross-talk between α_{2A}-adrenergic and μ–opioid receptors controls cell signaling. *Nat Chem Biol* **4**, 126–131.

Whorton MR, Jastrzebska B, Park PS, Fotiadis D, Engel A, Palczewski K & Sunahara RK (2008). Efficient coupling of transducin to monomeric rhodopsin in a phospholipid bilayer. *J Biol Chem* **283**, 4387–4394.

Additional information

Competing interests

None declared.

Funding

The authors' research is supported by grants from the National Institute of General Medical Sciences (GM078319), National Institute of Drug Abuse (DA022413), and National Institute of Mental Health (MH54137).

DOI: 10.1113/jphysiol.2014.274241