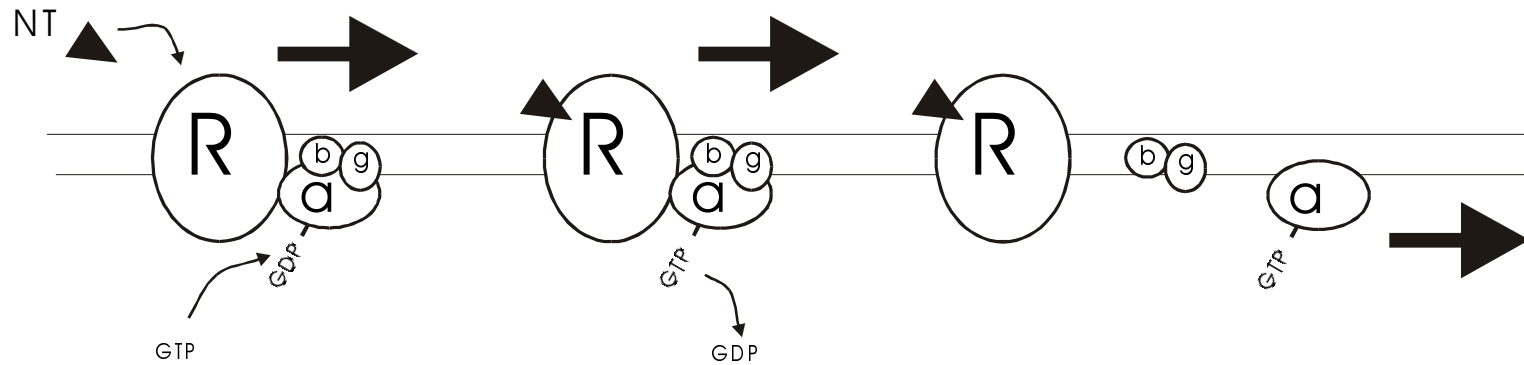


TRANSDUCTION VIA G-PROTEIN COUPLED RECEPTOR: FIRST STEP.



Neurotransmitter (NT) binds to receptor, inducing G-protein to exchange GTP for GDP.

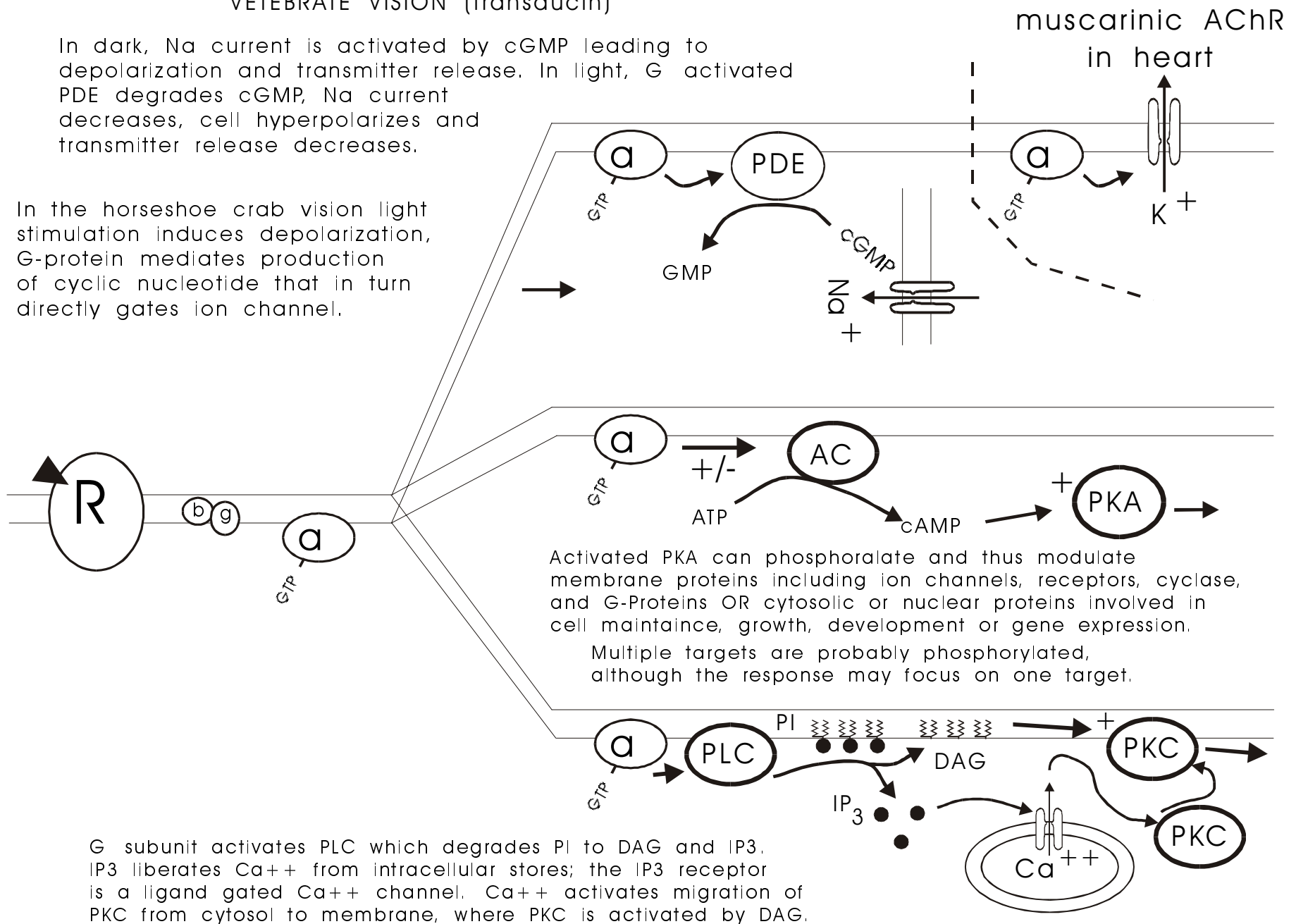
GTP binding induces G-Protein complex to dissociate, activated G $\alpha$  subunit migrates off to activate other proteins.

G $\alpha$  subunit remains active until it spontaneously dephosphorylates GTP to GDP. Following dephosphorylation the G-protein complex reforms, contributing to the inactivation of transduction.

## VETEBRATE VISION (transducin)

In dark, Na current is activated by cGMP leading to depolarization and transmitter release. In light, G activated PDE degrades cGMP, Na current decreases, cell hyperpolarizes and transmitter release decreases.

In the horseshoe crab vision light stimulation induces depolarization, G-protein mediates production of cyclic nucleotide that in turn directly gates ion channel.



G subunit activates PLC which degrades PI to DAG and IP<sub>3</sub>. IP<sub>3</sub> liberates Ca<sup>++</sup> from intracellular stores; the IP<sub>3</sub> receptor is a ligand gated Ca<sup>++</sup> channel. Ca<sup>++</sup> activates migration of PKC from cytosol to membrane, where PKC is activated by DAG.

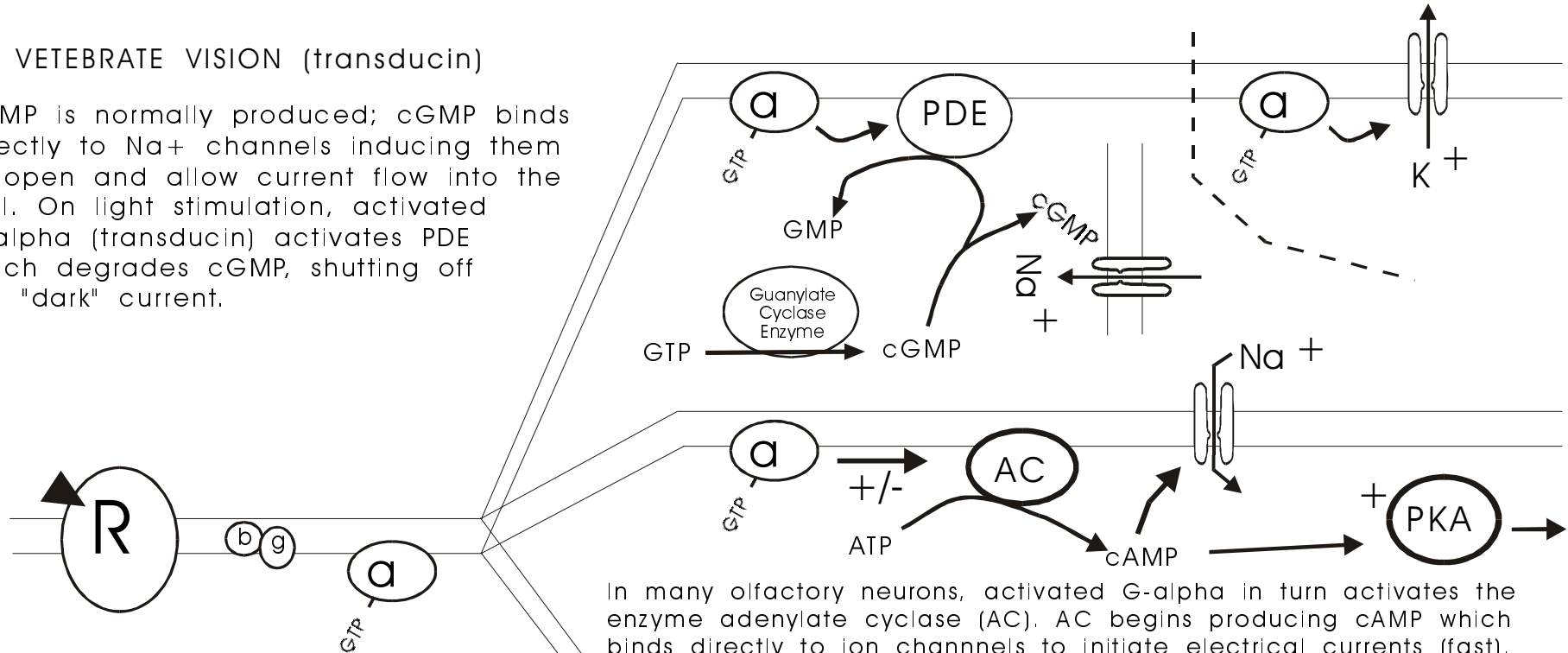
Ca<sup>++</sup> is a potent intracellular regulator, influencing many systems; thus released Ca<sup>++</sup> probably acts on additional systems besides PKC. PKC, as a kinase has similar diverse actions as described for PKA, though ion channels are likely targets.

# SENSORY TRANSDUCTION: VISION AND SMELL

In some invertebrate photodetectors, transduction is mediated by an activated G-alpha binding directly to an ion channel thereby inducing electrical currents.

## VETEBRATE VISION (transducin)

cGMP is normally produced; cGMP binds directly to Na<sup>+</sup> channels inducing them to open and allow current flow into the cell. On light stimulation, activated G-alpha (transducin) activates PDE which degrades cGMP, shutting off the "dark" current.

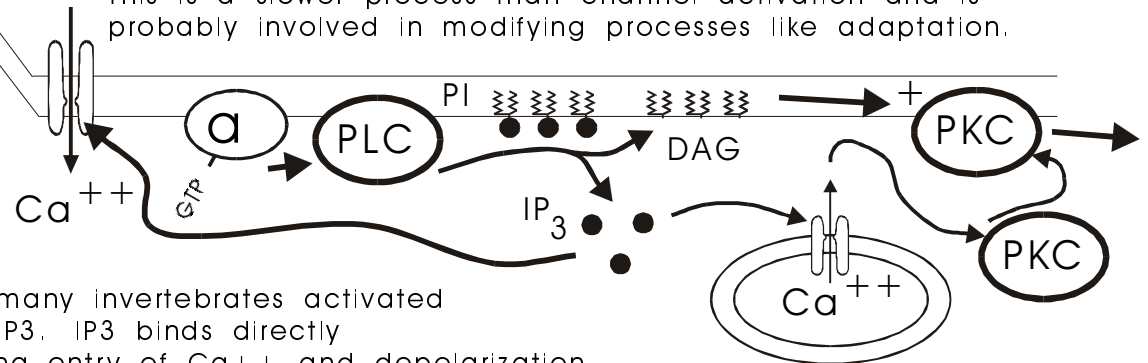


In many olfactory neurons, activated G-alpha in turn activates the enzyme adenylate cyclase (AC), AC begins producing cAMP which binds directly to ion channels to initiate electrical currents (fast).

cAMP also activates PKA and its resulting phosphorylations. This is a slower process than channel activation and is probably involved in modifying processes like adaptation.

## DIVERSITY ALLOWS SPECIFICITY.

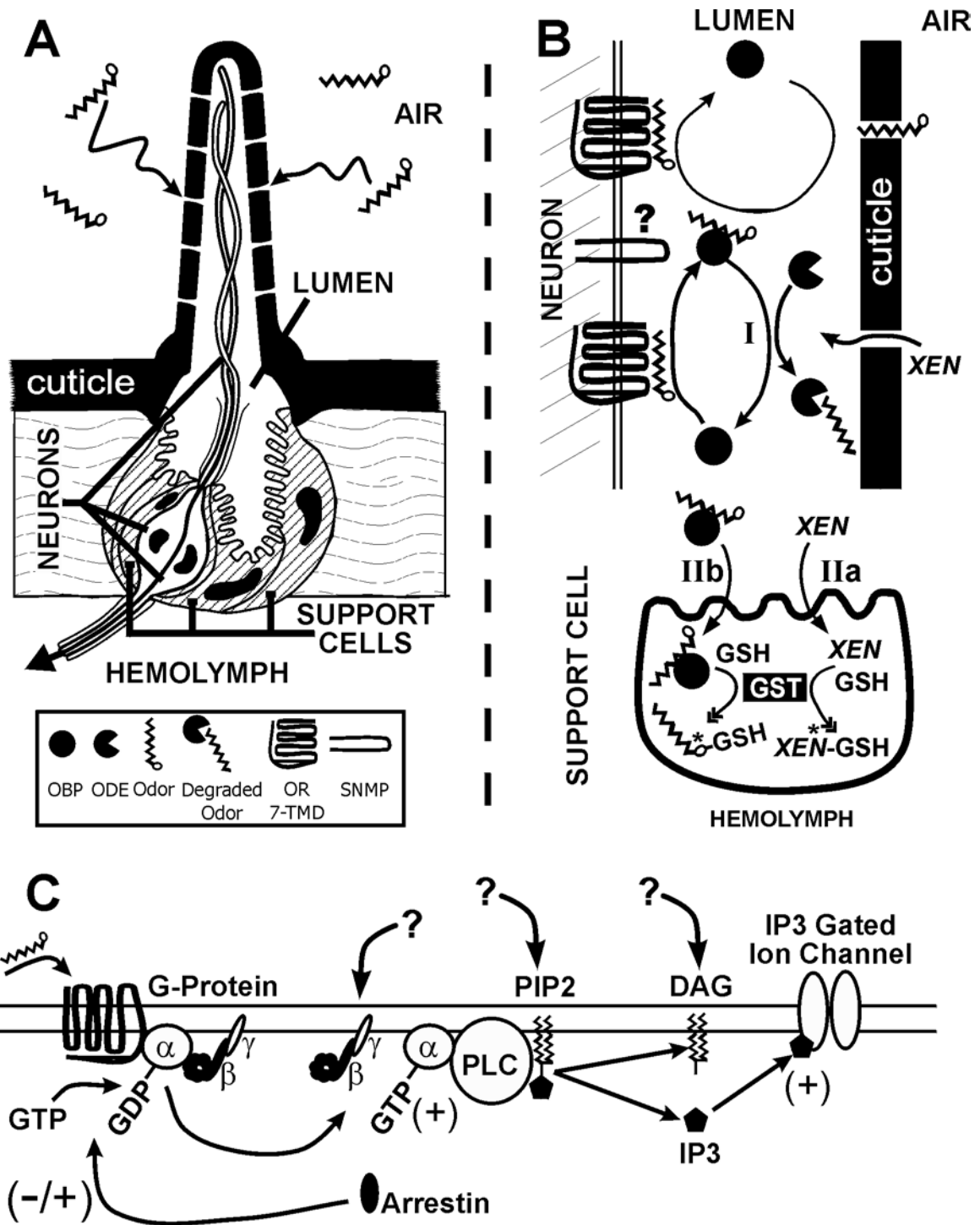
There are multiple type of G-alpha subunit. Each type interacts with only a specific class of receptor protein AND with a specific class of target. The G-alpha that activates PDE is different than those functioning in olfaction, etc.



In other olfactory cells AND in visual cells of many invertebrates activated G-alpha activates PLC and the production of IP3. IP3 binds directly to ion channels in the outer membrane allowing entry of Ca<sup>++</sup> and depolarization.

Ca<sup>++</sup> entry represents the initial electrical current (fast) but Ca<sup>++</sup> also induces many secondary responses in the cell which presumably act to modify sensory neuron response (e.g. adaptation) (slow).

**Schematic of an olfactory sensillum and a generalized biochemical pathway of insect odor reception.**



From: Vogt RG (2004) Molecular basis of pheromone detection in insects. In *Comprehensive Insect Physiology, Biochemistry, Pharmacology and Molecular Biology*. (eds. LI Gilbert, K Iatrou, S Gill). Elsevier, London. In Press.

## Insect Odor Detection Figure Description

**A. Anatomy.** An olfactory sensillum includes 2-3 neurons surrounded by 3 support cells; olfactory dendrites/cilia project up the fluid filled lumen of a cuticular hair. The sensillum lumen is isolated from hemolymph by a cellular barrier. Figure is modified from Steinbrecht (1969). Olfactory sensilla come in a range of shapes, including plate-like and long and short hair-like structures, and olfactory neurons may have branched or unbranched dendrites; sensilla classes are in part defined by the shape of the cuticular hair and the branched nature of the neurons (see Steinbrecht 1997, 1999). There is little data on the functional differences between different sensilla classes of a given individual, although in Lepidoptera, attractant sex pheromones are detected by the long trichoid sensilla, illustrated here.

**B. Perireceptor Events.** Hydrophobic odor molecules enter the aqueous sensillum lumen via pores penetrating the cuticular hair wall. Hydrophilic OBPs are proposed to bind and transport odors to receptor proteins located in the neuronal membranes. ODEs (pathway I) in the sensillum lumen are proposed to degrade these odor molecules. Cytoplasm of support cells contains xenobiotic inactivating enzymes (pathway IIa), such as glutathione-S-transferase (GST), which may also serve to inactivate odor molecules (pathway IIb). Interactions between OBPs and ORs and the function of SNMP are unclear. Figure is modified from Rogers et al. (1999).

**C. Transduction Events.** Odor-OR interaction stimulates an IP<sub>3</sub> second messenger cascade in which the alpha-subunit of a G-protein activates Phospholipase C (PLC) to cleave the membrane lipid phosphatidylinositol 4,5-bisphosphate (PIP<sub>2</sub>) to diacyl glycerol (DAG) and inositol trisphosphate (IP<sub>3</sub>). IP<sub>3</sub> is thought to bind directly to and activate transmembrane cation channels (IP<sub>3</sub> receptors) (reviewed by Krieger and Breer, 2003). Processes such as those mediated by phosphorylation (e.g. Schleicher et al., 1994) or arrestins (e.g. Merrill et al., 2002) may provide modulatory feedback on receptor-G-protein interactions. "?" pointing to Gβγ, PIP<sub>2</sub> and DAG are to imply yet uncharacterized but possible modulatory roles for these signals.

### Also, from text of review... Gβγ, DAG and Arrestins...

Two loose ends depicted in panel C (above) are the βγ subunits of the G-protein (Gβγ) complex and DAG. "Gβγ is now known to directly regulate as many different protein targets as the Gα subunit" (Clapham and Neer, 1997). In vertebrates, Gβγ has been shown to activate G-protein coupled inwardly rectifying K<sup>+</sup> channels (GIRKs) (e.g. Salvador et al., 2003) (GIRKs are K<sup>+</sup> channels that regulate the membrane resting potential). Gβγ has been shown to activate RGS proteins (regulators of G-protein signaling) which in turn activate GAP protein (GTPase activating protein) which regulates the GTPase activity in the Gα subunit (turning off Gα activity) (e.g. Witherow and Slepak, 2003). Gβγ has been shown to regulate G-protein coupled receptor kinases (GRKs) which phosphorylate and down regulate G-protein coupled receptors (e.g. Inglese et al., 1992; Pitcher et al., 1998; Eichmann et al., 2003). Gβγ has been shown to regulate the activities of certain PLC enzymes and the subsequent production of IP<sub>3</sub> (e.g. Akgoz et al., 2002). In vertebrate chemoreception, Gβγ has been shown to regulate PLC activity in mammalian vomeronasal neurons (Runnenburger et al., 2002) and in bitter taste neurons (Rossler et al., 2000a). Active Gβγ signaling in insect olfaction has yet to be reported.

Arrestins are proteins which bind to the phosphorylated state of a GPCR, and can mediate the uncoupling of GPCRs from G-proteins (desensitization) (Inglese et al., 1993; Pippig et al., 1993; Freedman et al., 1996) as well as the internalization of GPCRs (with presumed subsequent dephosphorylation in microsomes with subsequent recycling of the receptor, i.e. resensitization) (Ferguson *et al.*, 1996). In an IP<sub>3</sub> mediated pathway, GPCR phosphorylation may be mediated by GRKs, which may be mediated by Gβγ (see above). Arrestins have been identified in insect antennae and shown to be critical in the olfactory transduction process in *Drosophila* and the mosquito *Anopheles gambiae* (Merrill et al., 2002). In this study, an arrestin was cloned from *A. gambiae*, and was shown to express in both olfactory and visual sensory neurons. Of three *Drosophila* arrestins, *DmArr1* and *DmArr2* were shown to express in both visual and olfactory tissue while *DmKrz* was non-visual but also expressed in olfactory tissue. A double mutant (*arr1*<sup>2</sup>;*arr2*<sup>3</sup>) showed reduced EAG (electroantennogram) response to butanol and ethyl acetate, suggesting that arrestins play a key role in olfactory transduction in these animals (Merrill et al., 2002).

## References – Insect Odor Detection, G $\beta\gamma$ , DAG and Arrestins

- Akgoz M, Azpiazu I, Kalyanaraman V, Gautam N (2002) Role of the G protein gamma subunit in beta gamma complex modulation of phospholipase C $\beta$  function. *J. Biol. Chem.* 277, 19573-19578.
- Clapham DE, Neer EJ (1997) G protein beta gamma subunits. *Annu. Rev. Pharmacol. Toxicol.* 37, 167-203.
- Eichmann T, Lorenz K, Hoffmann M, Brockmann J, Krasel C, Lohse MJ, Quitterer U (2003) The amino-terminal domain of G-protein-coupled receptor kinase 2 is a regulatory G $\beta\gamma$  binding site. *J. Biol. Chem.* 278, 8052-8057.
- Ferguson SS, Downey WE 3rd, Colapietro AM, Barak LS, Menard L, Caron MG (1996). Role of beta-arrestin in mediating agonist-promoted G protein-coupled receptor internalization. *Science* 271, 363-6.
- Inglese J, Koch WJ, Caron MG, Lefkowitz RJ (1992) Isoprenylation in regulation of signal transduction by G-protein-coupled receptor kinases. *Nature* 359, 147-150.
- Inglese, J., Freedman, N. J., Koch, W. J., and Lefkowitz, R. J. (1993). Structure and mechanism of the G protein-coupled receptor kinases. *J. Biol. Chem.* 268, 23735-8.
- Krieger J, Breer H (2003) Transduction mechanisms of olfactory sensory neurons. In *Insect Pheromone Biochemistry and Molecular Biology*. (eds. GJ Blomquist and RG Vogt), pp. 593-607. Elsevier Academic Press, London.
- Merrill CE, Riesgo-Escovar J, Pitts RJ, Kafatos FC, Carlson JR, Zwiebel LJ (2002) Visual arrestins in olfactory pathways of *Drosophila* and the malaria vector mosquito *Anopheles gambiae*. *Proc. Natl. Acad. Sci. USA* 99, 1633-1638.
- Pippig S, Andexinger S, Daniel K, Puzicha M, Caron MG, Lefkowitz RJ, Lohse MJ (1993). Overexpression of beta-arrestin and beta-adrenergic receptor kinase augment desensitization of beta 2-adrenergic receptors. *J. Biol. Chem.* 268, 3201-3208.
- Pitcher JA, Freedman NJ, Lefkowitz RL (1998) G-protein coupled receptor kinases. *Ann. Rev. Biochem.* 67, 653-692.
- Rogers ME, Jani MK, Vogt RG (1999) An olfactory specific glutathione S-transferase in the Sphinx moth *Manduca sexta*. *J. Exp. Biol.* 202, 1625-1637.
- Rosler P, Boekhoff I, Tareilus E, Beck S, Breer H, Freitag J (2000a) G protein betagamma complexes in circumvallate taste cells involved in bitter transduction. *Chem Senses.* 25, 413-21.
- Runnenburger K, Breer H, Boekhoff I (2002) Selective G protein beta gamma-subunit compositions mediate phospholipase C activation in the vomeronasal organ. *Eur. J. Cell Biol.* 81, 539-547.
- Salvador C, Mora SI, Ordaz B, Antaramian A, Vaca L, Escobar LI (2003) Basal activity of GIRK5 isoforms. *Life Sci.* 72, 1509-1518.
- Schleicher S, Boekhoff I, Konietzko U, Breer H (1994) Pheromone-induced phosphorylation of antennal proteins from insects. *J. Comp. Physiol. B* 164, 76-80.
- Steinbrecht RA (1969) Comparative morphology of olfactory receptor. In *Olfaction and Taste III* (ed. C Pfaffmann) pp. 3-21, Rockefeller Univ. Press, New York.
- Steinbrecht RA (1997) Pore structures in insect olfactory sensilla: a review of data and concepts. *Int. J. Insect. Morphol. Embryol.* 26, 229-245.
- Steinbrecht RA (1999) Olfactory Receptors. in *Atlas of Arthropod Sensory Receptors, Dynamic Morphology in Relation to Function* (eds. E Eguchi, Y Tominaga) Springer-Verlag, Tokyo.
- Witherow DS, Slepak VZ (2003) A novel kind of G protein heterodimer: the G beta5-RGS complex. *Receptors Channels.* 9, 205-212.