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The human serotonin$_{1A}$ receptor exhibits G-protein-dependent cell surface dynamics

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Abstract Seven transmembrane domain G-protein-coupled receptors constitute the largest family of proteins in mammals. Signal transduction events mediated by such receptors are the primary means by which cells communicate with and respond to their external environment. The major paradigm in this signal transduction process is that stimulation of the receptor leads to the recruitment and activation of heterotrimeric GTP-binding proteins. These initial events, which are fundamental to all types of G-protein-coupled receptor signaling, occur at the plasma membrane via protein–protein interactions. As a result, the dynamics of the activated receptor on cell surfaces represents an important determinant in its encounter with G-proteins, and has significant impact on the overall efficiency of the signal transduction process. We have monitored the cell surface dynamics of the serotonin$_{1A}$ receptor, an important member of the G-protein-coupled receptor superfamily, in relation to its interaction with G-proteins. Fluorescence recovery after photobleaching experiments carried out with the receptor tagged to the enhanced yellow fluorescent protein indicate that G-protein activation alters the diffusion properties of the receptor in a manner suggesting the activation process leads to dissociation of G-proteins from the receptor. This result demonstrates that the cell surface dynamics of the serotonin$_{1A}$ receptor is modulated in a G-protein-dependent manner. Importantly, this result could provide the basis for a sensitive and powerful approach to assess receptor/G-protein interaction in an intact cellular environment.

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Abbreviations

5-HT$_{1A}$ receptor	5-hydroxytryptamine-1A receptor
EYFP	enhanced yellow fluorescent protein
FRAP	fluorescence recovery after photobleaching
GFP	green fluorescent protein
GPCR	G-protein-coupled receptor
PCR	polymerase chain reaction

Introduction

The G-protein-coupled receptor (GPCR)[1] superfamily is one of the largest protein families in mammals and accounts for >1% of the total proteins coded by the human genome [1–3]. Cellular signaling by GPCRs involves their activation upon binding to ligands present in the extracellular environment and the subsequent transduction of signals to the interior of the cell through concerted changes in their transmembrane domain structure [4]. The major paradigm in this signal transduction process is that stimulation of GPCRs leads to the recruitment and activation of heterotrimeric GTP-binding proteins (G-proteins) [5]. These initial events, fundamental to all types of GPCR signaling, occur at the plasma membrane via protein-protein interactions. An important consequence of this is that the dynamics of the activated receptor on the cell surface represents an important determinant in its encounter with G-proteins, and has significant impact on the overall efficiency of the signal transduction process [6]. In this

context, the cell surface organization of GPCRs and G-proteins participating in this signal transduction process assumes relevance. Recent evidence has indicated that a spatiotemporally organized system of interacting molecules rather than a freely diffusible system is responsible for rapid and specific propagation of extracellular stimuli to intracellular signaling molecules [7, 8]. Based on these and other results, it has been proposed that GPCRs are not uniformly present on the plasma membrane but are concentrated in specific membrane microdomains [9]. This heterogenous distribution of GPCRs into domains has given rise to new challenges and complexities in receptor signaling since signaling now has to be understood in context of the three dimensional organization of various signaling components which include receptors and G-proteins.

Serotonin (5-hydroxytryptamine or 5-HT) is an intrinsically fluorescent neurotransmitter [10] and exerts its actions by binding to distinct membrane receptors classified into many groups [11]. Among the 14 subtypes of serotonin receptors, the G-protein-coupled serotonin$_{1A}$ (5-HT$_{1A}$) receptor subtype is the most extensively studied for a number of reasons [12]. Importantly, the 5-HT$_{1A}$ receptor levels and function have been shown to be important indices to diagnose several psychological disorders. These include alterations in the receptor levels in schizophrenia [13] and depression [14], polymorphisms in the upstream repressor region of the 5-HT$_{1A}$ receptor gene in major depression and suicide in humans [15], and altered receptor ligand-binding function in panic disorder [16]. The 5-HT$_{1A}$ receptor has recently been shown to have a role in neural development [17] and protection of stressed neuronal cells undergoing degeneration and apoptosis [18]. In addition, 5-HT$_{1A}$ receptor antagonists represent a major class of molecules with potential therapeutic effects in anxiety- or stress-related disorders [19]. We have earlier shown modulation of ligand binding activity of the 5-HT$_{1A}$ receptor isolated from the bovine hippocampus with agents that perturb G-proteins [20, 21]. In addition, we have solubilized and partially purified the 5-HT$_{1A}$ receptor from both the native bovine hippocampus [22] and heterologously expressed human 5-HT$_{1A}$ receptors from Chinese hamster ovary cells [23], where the receptor was found to possess very similar pharmacological characteristics as in the native system [24].

Monitoring diffusion of cell surface molecules represents a powerful approach to detect their organization on the plasma membrane [25, 26]. In the light of the proposed significance of a spatiotemporally restricted environment in modulating receptor and G-protein interaction (see above), we have utilized the previously characterized serotonin$_{1A}$ receptor tagged to the enhanced yellow fluorescence protein (EYFP) [27] to analyze its cell surface dynamics (diffusion properties) using the technique of fluorescence recovery after photobleaching (FRAP) [28, 29]. Based on the analysis of FRAP data, we provide evidence that cell surface dynamics of this receptor is altered upon receptor independent activation of G-proteins in an intact cellular environment. These results provide novel information on signaling events involving the 5-HT$_{1A}$ receptor in cells and suggest that the association of G-proteins with the 5-HT$_{1A}$ receptor could be an important determinant in its spatiotemporal organization on the cell surface.

Materials and methods

Materials

Penicillin, streptomycin, gentamycin sulfate, mastoparan, and pertussis toxin were obtained from Sigma (St. Louis, MO). D-MEM/F-12 (Dulbecco's Modified Eagle Medium: nutrient mixture F-12 (Ham) (1:1)), lipofectamine, fetal calf serum, and geneticin (G 418) were from Invitrogen Life Technologies (Carlsbad, CA). All other chemicals used were of the highest available purity. Water was purified through a Millipore (Bedford, MA) Milli-Q system and used throughout.

Construction of the vector for EYFP-tagged 5-HT$_{1A}$ receptor and isolation of stable transfectants

The vector coding for the human 5-HT$_{1A}$ receptor tagged at the C-terminal end to EYFP was used to transfect CHO–K1 cells. The construction of the vector, transfection of CHO–K1 cells, and isolation of transfectants stably expressing the fusion protein were carried out as described earlier [27].

Confocal microscopy and fluorescence recovery after photobleaching (FRAP)

Cells were plated at a density of 5×10^4 cells on a 40 mm glass coverslip and were grown in D-MEM/F-12 medium for 72 h. Coverslips were washed with HEPES-Hanks, pH 7.4 buffer and mounted on an FCS2 closed temperature-controlled Bioptechs chamber (Butler, PA). Cells were either treated with AlF_4^-(50 μM $AlCl_3$ and 30 mM NaF) or with 10 μM mastoparan prepared in HEPES-Hanks, pH 7.4 buffer for 5 to 10 min, or were grown in presence of pertussis toxin (200 ng/ml) for 24 h before experiments. Confocal microscopy was carried out using an inverted Zeiss LSM 510 Meta confocal microscope (Jena, Germany), with a 63×, 1.2 NA water-immersion objective using the 514 nm line of an argon laser as the excitation source. Fluorescence emission was collected using the 535–590 nm band pass filter. All images were acquired with the confocal pinhole set

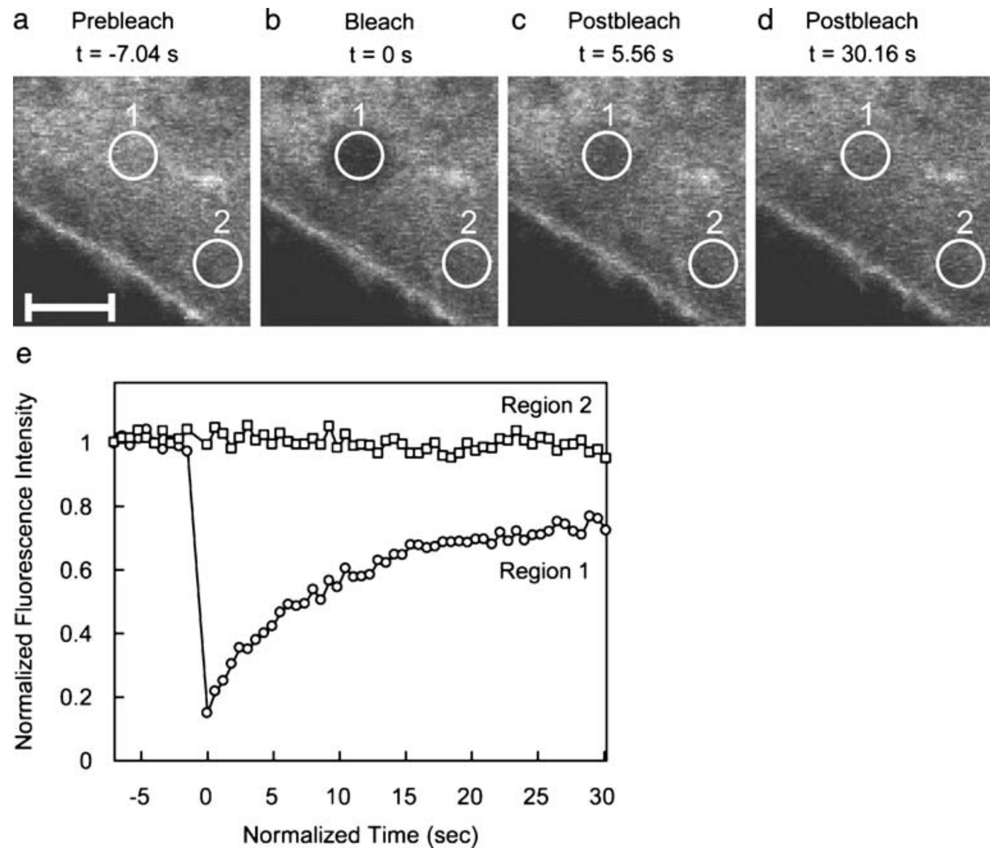

Fig. 1 Fluorescence recovery after photobleaching 5-HT$_{1A}$ receptor tagged to EYFP in CHO–K1 cells. Fluorescence images of cells were acquired at 37°C and represent confocal sections of the base of the cells. Images of the same cell in panels (a), (b), (c), and (d) show fluorescence intensity before and after bleaching for the indicated duration of time. The prebleach images/intensities are shown at time $t < 0$ and the bleach event is shown at time $t = 0$. The normalized fluorescence intensity in Region 1 calculated from these images is plotted in panel (e). The fluorescence intensity in the control Region 2 monitored for the same time period and plotted in panel (e) indicates no significant photobleaching of the field due to repeated imaging. The fluorescence in Region 1 in panel (e) is fitted to Eq. 1 to arrive at the characteristic diffusion time τ_d. Scale bar represents 5 μm. See Materials and methods for other details

between 1 and 2 Airy units and an 8-bit, 512×512-pixel resolution. Fluorescence recovery after photobleaching (FRAP) experiments were carried out after the FCS2 stage reached 37°C which took ~5 min. The uniformly fluorescent surface of well spread CHO–K1 cells stably expressing 5-HT$_{1A}$R-EYFP in contact with the glass coverslip was imaged for bleaching and monitoring fluorescence recovery. In a typical experiment, a square region of interest (ROI) of 14 μm length was scanned. Another circular ROI of 1.4 μm radius was chosen as the bleach ROI within this square ROI. The time interval between successive scans was ~0.63 sec. Analysis with a control ROI drawn a fair distance away from the bleach ROI indicated no significant bleach while fluorescence recovery was monitored. Data representing the mean fluorescence intensity of the bleached ROI were background subtracted using an ROI placed outside the cell boundary and were analyzed to determine the diffusion coefficient (D). FRAP recovery plots were analyzed based on the equation for a uniform disk illumination condition [30]:

$$F(t) = [F(\infty) - F(0)][\exp(-2\tau_d/t)(I_0(2\tau_d/t) + I_1(2\tau_d/t))] + F(0) \quad (1)$$

where $F(t)$ is the mean background corrected and normalized fluorescence intensity at time t in the bleached ROI, $F(\infty)$ is the recovered fluorescence at time $t = \infty$, $F(0)$ is the bleached fluorescence intensity estimated at time $t = 0$ and τd is the characteristic diffusion time. I_0 and I_1 are modified Bessel functions. Diffusion coefficient (D) is determined from the equation:

$$D = \omega^2/4\tau_d \quad (2)$$

where ω is the actual radius of the bleached ROI. Mobile fraction estimates of the fluorescence recovery were obtained from the equation:

$$\text{Mobile fraction} = [F(\infty) - F(0)]/[F(p) - F(0)] \quad (3)$$

where $F(p)$ is the mean background corrected and normalized prebleach fluorescence intensity. Non-linear curve fitting of the recovery data to Eq. (1) was carried out using the Graphpad Prism software version 4.00 (San Diego, CA). Significance levels were estimated by one-way ANOVA using Microcal Origin software version 5.0 (OriginLab Corporation, Northampton, MA).

Results

Fluorescence recovery after photobleaching involves generating a concentration gradient of fluorescent molecules by irreversibly photobleaching a fraction of fluorophores in the sample region [28, 29]. The dissipation of this gradient with

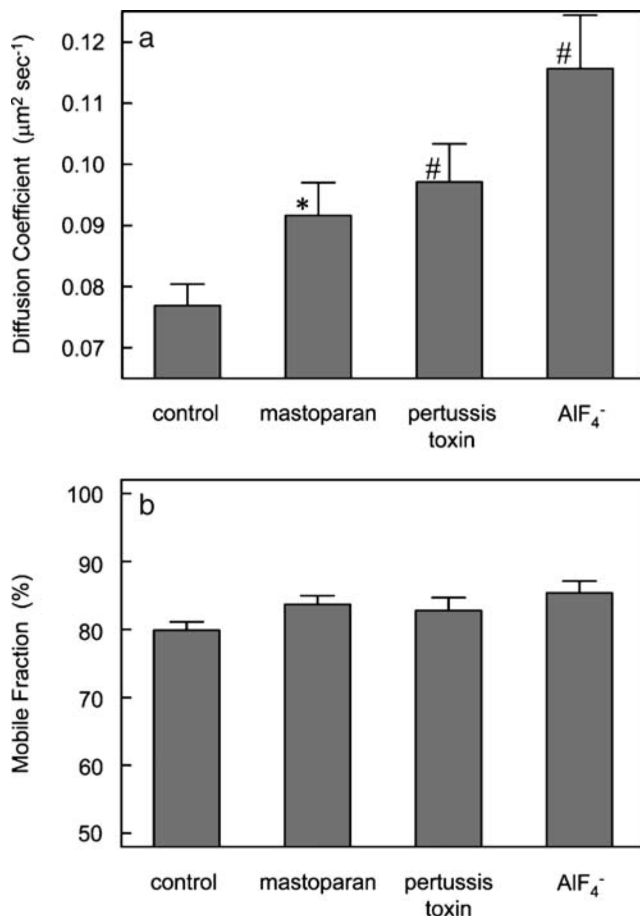

Fig. 2 G-protein-dependent diffusion characteristics of the 5-HT_{1A} receptor tagged to EYFP in CHO-K1 cells. (a) Diffusion coefficients of the 5-HT_{1A} receptor tagged to EYFP are shown under normal conditions (control) and in presence of mastoparan (10 μM), AlF_4^-(50 μM $AlCl_3$+30 mM NaF) and when cells are grown in the presence of pertussis toxin (200 ng/ml). (b) Mobile fractions of the 5-HT_{1A} receptor tagged to EYFP are shown under the same conditions. The data represent the means ± standard error of at least 11 independent experiments for each condition. The means are significantly different from control values at $P < 0.05$ (*), and $P < 0.005$ (#). Data in panel (a) is adapted from Ref. [27]. See Materials and methods for other details

time owing to diffusion of fluorophores into the bleached region from the unbleached regions in the membrane is an indicator of the mobility of the fluorophores in the membrane. A representative panel of images demonstrating the recovery of fluorescence after photobleaching carried out on CHO-K1 cells stably expressing the 5-HT_{1A} receptor tagged to EYFP is shown in Fig. 1. The plots in Fig. 1e indicate the normalized fluorescence intensity in the bleach ROI (Region 1) and control ROI (Region 2) monitored for the entire duration (~47 sec) of the FRAP experiment. The parameters for scanning were optimized to ensure that no significant fluorescence photobleaching occurs due to repeated imaging. This is indicated by the relatively constant mean fluorescence intensity in the control ROI (Region 2; Fig. 1e) during the entire duration of the experiment. Curve fitting analysis of the FRAP recovery plots from control cells indicate a mean diffusion coefficient (*D*) of ~0.077 μm^2 sec^{-1} (Fig. 2a) and a mobile fraction of ~80% (Fig. 2b) for the 5-HT_{1A} receptor tagged to EYFP at 37°C. Importantly, a similar analysis of diffusion rates of pure GFP in 90% glycerol–water mixture at 25°C gave a mean *D* of ~0.65 μm^2 sec^{-1} (T.J. Pucadyil and A. Chattopadhyay, unpublished observations) which is similar to the value of 0.7 μm^2 sec^{-1} calculated for GFP using the Stokes-Einstein equation in a medium of viscosity corresponding to 90% glycerol-water mixture [31] thus validating our method of analysis.

To determine the dependence of receptor/G-protein interaction on the dynamics of the 5-HT_{1A} receptor, FRAP experiments were carried out on cells stably expressing the 5-HT_{1A} receptor tagged to EYFP in presence of agents that either activate G-proteins or covalently modify them to disrupt their interaction with the receptor. The cationic peptide mastoparan has been shown to catalyze $G_{i/o}$-protein activation in a manner similar to that mediated by GPCRs [32]. Treatment of cells with mastoparan increases the diffusion of the receptor to ~0.092 μm^2 sec^{-1} (Fig. 2a) with a mobile fraction of ~84% (Fig. 2b). It has been proposed that AlF_4^- mimics the γ-phosphate of GTP in the guanine nucleotide binding site of GDP bound Gα subunit [33] and that it can mimic the effects of GTP and GTP-γ-S on G-proteins. Moreover, cell membranes are permeable to AlF_4^-, unlike GTP or GTP-γ-S, and thus are widely used to activate G-proteins in intact cells [34]. Treatment of cells with AlF_4^- (50 μM $AlCl_3$+30 mM NaF) significantly increased the mobility of the receptor (~1.5 fold) to ~0.116 μm^2 sec^{-1} (Fig. 2a) with a mobile fraction of ~85% (Fig. 2b). Taken together, these results suggest that the activation of G-proteins significantly increases the cell surface mobility of the 5-HT_{1A} receptor tagged to EYFP. This increase could be due to the dissociation of G-proteins from the receptor due to activation of G-proteins. If this were true, then prevention of receptor and G-protein interaction *per se* should also lead to an increase in receptor diffusion. To achieve this, cells were treated with pertussis toxin, which ADP-ribosylates and inactivates the α subunit of $G_{i/o}$ class of G-proteins [35]. Importantly, FRAP experiments carried out on cells treated with pertussis toxin indicate an increase in mobility of the receptor to ~0.097 μm^2 sec^{-1} (Fig. 2a) with a mobile fraction of ~83% (Fig. 2b) thus supporting our interpretation on the increase in receptor diffusion in the presence of the activators of G-proteins.

Discussion

We have earlier reported the pharmacological and cellular signaling characteristics of the 5-HT_{1A} receptor tagged to

EYFP at its carboxy terminus and stably expressed in CHO-K1 cells [27]. Thus, binding affinities of the specific agonist 8-OH-DPAT and antagonist *p*-MPPF and extents of receptor/G-protein interaction were found to be similar between the tagged and untagged forms of 5-HT_{1A} receptor. Importantly, the natural agonist serotonin can efficiently catalyze downstream signal transduction events in cells expressing the 5-HT_{1A} receptor tagged to EYFP by reducing the forskolin-stimulated cAMP levels in these cells. These results suggest that the tagged form of the receptor can be reliably used to mimic native 5-HT_{1A} receptors to explore cell surface organization, distribution and dynamics using fluorescence approaches.

The analysis of cell surface dynamics of membrane proteins such as GPCRs is crucial for a comprehensive understanding of their organization and signaling functions. Since G-proteins are, by themselves, membrane anchored proteins, it is evident that cell surface dynamics of both the receptor and G-protein are important determinants of G-protein-coupled signal transduction events. In this report, we have utilized the EYFP fluorescence to monitor the dynamics of the receptor on the cell surface under conditions of G-protein activation/covalent modification using the technique of FRAP. The observed increase in the diffusion of the receptor upon activation of G-proteins with mastoparan and AlF_4^- suggest that the association of G-proteins with the receptor tends to reduce its lateral mobility on the cell surface. Treatment of cells with pertussis toxin that reduces receptor and G-protein interaction also causes an increase in receptor diffusion, thus providing further evidence to support this interpretation. The G-protein heterotrimer is a large protein complex with an average molecular weight of ~88 kDa [36], which would be ~1.2 times the mass of the receptor tagged to EYFP. It is, therefore, possible that their association with the receptor would reduce its diffusion coefficient. Models on G-protein action indicate the activation process stimulates the exchange of a GTP for the existing GDP molecule at the $G\alpha$ subunit [36]. When this occurs, the receptor-bound G-protein heterotrimer complex dissociates until another inactive G-protein binds to the receptor. Independent activation of G-proteins with mastoparan would tend to keep the receptor dissociated from G-proteins. The presence of AlF_4^- would lock the $G\alpha$ subunits in an active state, similar to a GTP-γ-S-bound $G\alpha$ subunit, preventing them from associating with the receptor [33, 34] and thereby increasing the fraction of receptors uncoupled to G-proteins. The treatment of cells with pertussis toxin would inactivate the existing pool of G-proteins and increase the fraction of receptors uncoupled to G-proteins [35]. A recent fluorescence correlation spectroscopy study carried out on rhodamine-labeled galanin bound to galanin receptors supports this possibility [37]. Analysis of the diffusion properties of galanin receptor detected the presence of at least two populations of the receptor. Treatment of cells with pertussis toxin to inactivate endogenous G-proteins led to a complete loss of the slowly diffusing population which was due to receptors coupled to G-proteins [37].

Diffusion behavior of several integral membrane proteins indicate that the cytoskeleton underlying the cell surface can act as a barrier to free diffusion of these proteins. This is thought to occur due to the steric hindrance imposed by the cytoskeleton on the cytoplasmic domains of these proteins. Treatment of cells with agents that disrupt the cytoskeleton [38], truncation of the cytoplasmic domains of transmembrane proteins [39], or a lack of interaction of membrane proteins with cytoplasmic effector molecules [40] tends to increase their mobility on the cell surface. Likewise, the presence of the bulky heterotrimeric G-protein complex associated with the receptor (since G-proteins, when bound to membrane receptors, could be considered as equivalent to cytoplasmic domains of membrane proteins) could further reduce (over the differences arising due to molecular mass of G-proteins) receptor diffusion, which would be partially relieved when the G-protein dissociates from the receptor. Another possibility could be that the increase in receptor diffusion could reflect changes in the oligomeric state of the receptor, as has been shown for the δ-opioid receptor [41], and the cholecystokinin receptor [42] or their partitioning into or out of domains proposed to exist on the cell surface [7, 9]. Incidentally, there is growing evidence on the compartmentalized localization of G-proteins in cholesterol-rich membrane domains [43] that have been reported to diffuse as separate entities on the membrane [44]. Whether the differences in the diffusion properties of the receptor while being associated with or without G-proteins results from the movement of receptors into or out of such domains represents an interesting possibility.

In summary, we conclude that the cell surface dynamics of the 5-HT_{1A} receptor is modulated in a G-protein dependent manner. Our results on the G-protein dependent cell surface dynamics of the 5-HT_{1A} receptor provide novel insight and present a sensitive and powerful approach to assess receptor/G-protein interaction in an intact cellular environment. This approach could prove to be useful in analyzing molecular mechanism of signal transduction of 5-HT_{1A} receptors in particular and G-protein-coupled receptors in general.

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References

1. Pierce, K.L., Premont, R.T., Lefkowitz, R.J.: Seven-transmembrane receptors. Nat. Rev. Mol. Cell Biol. **3**, 639–650 (2002)
2. Perez, D.M.: The evolutionarily triumphant G-protein-coupled receptor. Mol. Pharmacol. **63**, 1202–1205 (2003)
3. Fredriksson, R., Lagerstrom, M.C., Lundin, G.-C., Schioth, H.B.: The G-protein-coupled receptors in the human genome form five main families. Phylogenetic analysis, paralogon groups, and fingerprints. Mol. Pharmacol. **63**, 1256–1272 (2003)
4. Gether, U.: Uncovering molecular mechanisms involved in activation of G protein-coupled receptors. Endocr. Rev. **21**, 90–113 (2000)
5. Hamm, H.E.: How activated receptors couple to G proteins. Proc. Natl. Acad. Sci. USA **98**, 4819–4821 (2001)
6. Peters, R.: Lateral mobility of proteins and lipids in the red cell membrane and the activation of adenylate cyclase by beta-adrenergic receptors. FEBS Lett. **234**, 1–7 (1988)
7. Neubig, R.R.: Membrane organization in G-protein mechanisms. FASEB J. **8**, 939–946 (1994)
8. Hur, E.-M., Kim, K.-T.: G protein-coupled receptor signalling and cross-talk: Achieving rapidity and specificity. Cell. Signal. **14**, 397–405 (2002)
9. Ostrom, R.S., Insel, P.A.: The evolving role of lipid rafts and caveolae in G protein-coupled receptor signaling: Implications for molecular pharmacology. Br. J. Pharmacol. **143**, 235–245 (2004)
10. Chattopadhyay, A., Rukmini, R., Mukherjee, S.: Photophysics of a neurotransmitter: Ionization and spectroscopic properties of serotonin. Biophys. J. **71**, 1952–1960 (1996)
11. Hoyer, D., Hannon, J.P., Martin, G.R.: Molecular, pharmacological and functional diversity of 5-HT receptors. Pharmacol. Biochem. Behav. **71**, 533–554 (2002)
12. Pucadyil, T.J., Kalipatnapu, S., Chattopadhyay, A.: The serotonin$_{1A}$ receptor: A representative member of the serotonin receptor family. Cell. Mol. Neurobiol. **25**, 553–580 (2005)
13. Sumiyoshi, T., Stockmeier, C.A., Overholser, J.C., Dilley, G., Meltzer, H.Y.: Serotonin$_{1A}$ receptors are increased in postmortem prefrontal cortex in schizophrenia. Brain Res. **708**, 209–214 (1996)
14. Fajardo, O., Galeno, J., Urbina, M., Carreira, I., Lima, L.: Serotonin, serotonin 5-HT_{1A} receptors and dopamine in blood peripheral lymphocytes of major depression patients. Int. Immunopharmacol. **3**, 1345–1352 (2003)
15. Lemonde, S., Turecki, G., Bakish, D., Du, L., Hrdina, P.D., Bown, C.D., Sequeira, A., Kushwaha, N., Morris, S.J., Basak, A., Ou, X.M., Albert, P.R.: Impaired repression at a 5-hydroxytryptamine 1A receptor gene polymorphism associated with major depression and suicide. J. Neurosci. **23**, 8788–8799 (2003)
16. Neumeister, A., Bain, E., Nugent, A.C., Carson, R.E., Bonne, O., Luckenbaugh, D.A., Eckelman, W., Herscovitch, P., Charney, D. S., Drevets. W.C.: Reduced serotonin type 1A receptor binding in panic disorder. J. Neurosci. **24**, 589–591 (2004)
17. Gross, C., Zhuang, X., Stark, K., Ramboz, S., Oosting, R., Kirby, L., Santarelli, L., Beck, S., Hen, R.: Serotonin$_{1A}$ receptor acts during development to establish normal anxiety-like behaviour in the adult. Nature **416**, 396–400 (2002)
18. Adayev, T., Ray, I., Sondhi, R., Sobocki, T., Banerjee, P.: The G protein-coupled 5-HT_{1A} receptor causes suppression of caspase-3 through MAPK and protein kinase Cα. Biochim. Biophys. Acta **1640**, 85–96 (2003)
19. Griebel, G.: 5-HT_{1A} receptor blockers as potential drug candidates for the treatment of anxiety disorders. Drug News Perspect. **12**, 484–490 (1999)
20. Harikumar, K.G., Chattopadhyay, A.: Differential discrimination of G-protein coupling of serotonin$_{1A}$ receptors from bovine hippocampus by an agonist and an antagonist. FEBS Lett. **457**, 389–392 (1999)
21. Javadekar-Subhedar, V., Chattopadhyay, A.: Temperature-dependent interaction of the bovine hippocampal serotonin$_{1A}$ receptor with G-proteins. Mol. Membr. Biol. **21**, 119–123 (2004)
22. Chattopadhyay, A., Harikumar, K.G., Kalipatnapu, S.: Solubilization of high affinity G-protein-coupled serotonin$_{1A}$ receptors from bovine hippocampus using pre-micellar CHAPS at low concentration. Mol. Membr. Biol. **19**, 211–220 (2002)
23. Chattopadhyay, A., Jafurulla, M.D., Kalipatnapu, S.: Solubilization of serotonin$_{1A}$ receptors heterologously expressed in Chinese hamster ovary cells. Cell. Mol. Neurobiol. **24**, 293–300 (2004)
24. Kalipatnapu, S., Pucadyil, T.J., Harikumar, K.G., Chattopadhyay, A.: Ligand binding characteristics of the human serotonin$_{1A}$ receptor heterologously expressed in CHO cells. Biosci. Rep. **24**, 101–115 (2004)
25. Lagerholm, B.C., Weinreb, G.E., Jacobson, K., Thompson, N.L.: Detecting microdomains in intact cell membranes. Annu. Rev. Phys. Chem. **56**, 309–336 (2005)
26. Kusumi, A., Ike, H., Nakada, C., Murase, K., Fujiwara, T.: Single-molecule tracking of membrane molecules: Plasma membrane compartmentalization and dynamic assembly of raft-philic signaling molecules. Semin. Immunol. **17**, 3–21 (2005)
27. Pucadyil, T.J., Kalipatnapu, S., Harikumar, K.G., Rangaraj, N., Karnik, S.S., Chattopadhyay, A.: G-protein-dependent cell surface dynamics of the human serotonin$_{1A}$ receptor tagged to yellow fluorescent protein. Biochemistry **43**, 15852–15862 (2004)
28. Klonis, N., Rug, M., Harper, I., Wickham, M., Cowman, A., Tilley, L.: Fluorescence photobleaching analysis for the study of cellular dynamics. Eur. Biophys. J. **31**, 36–51 (2002)
29. Lippincott-Schwartz, J., Snapp, E., Kenworthy, A.: Studying protein dynamics in living cells. Nat. Rev. Mol. Cell Biol. **2**, 444–456 (2001)
30. Soumpasis, D.M.: Theoretical analysis of fluorescence photobleaching recovery experiments. Biophys. J. **41**, 95–97 (1983)
31. Kubitscheck, U., Kuckmann, O., Kues, T., Peters, R.: Imaging and tracking of single GFP molecules in solution. Biophys. J. **78**, 2170–2179 (2000)
32. Higashijima, T., Burnier, J., Ross, E.M.: Regulation of Gi and Go by mastoparan, related amphiphilic peptides, and hydrophobic amines. Mechanism and structural determinants of activity. J. Biol. Chem. **265**, 14176–14186 (1990)
33. Higashijima, T., Graziano, M.P., Suga, H., Kainosho, M., Gilman, A.G.: ^{19}F and ^{31}P NMR spectroscopy of G protein alpha subunits. Mechanism of activation by Al^{3+} and F^{-}. J. Biol. Chem. **266**, 3396–3401 (1991)
34. Inoue, Y., Fishman, P.H., Rebois, R.V.: Differential activation of the stimulatory and inhibitory guanine nucleotide-binding proteins by fluoroaluminate in cells and in membranes. J. Biol. Chem. **265**, 10645–10651 (1990)
35. Sternweiss, P.C., Robishaw, J.D.: Isolation of two proteins with high affinity for guanine nucleotides from membranes of bovine brain. J. Biol. Chem. **259**, 13806–13813 (1984)
36. Casey, P.J., Gilman, A.G.: G protein involvement in receptor-effector coupling. J. Biol. Chem. **263**, 2577–2580 (1988)
37. Pramanik, A., Olsson, M., Langel, U., Bartfai, T., Rigler, R.: Fluorescence correlation spectroscopy detects galanin receptor

diversity on insulinoma cells. Biochemistry **40**, 10839–10845 (2001)
38. Fujiwara, T., Ritchie, K., Murakoshi, H., Jacobson, K., Kusumi, A.: Phospholipids undergo hop diffusion in compartmentalized cell membrane. J. Cell Biol. **157**, 1071–1081 (2002)
39. Edidin, M., Zuniga, M.C., Sheetz, M.P.: Truncation mutants define and locate cytoplasmic barriers to lateral mobility of membrane glycoproteins. Proc. Natl. Acad. Sci. USA **91**, 3378–3382 (1994)
40. Zhang, F., Yang, B., Odin, J.A., Shen, Z., Lin, C.T., Unkeless, J.C., Jacobson, K.: Lateral mobility of FcγRIIa is reduced by protein kinase C activation. FEBS Lett. **376**, 77–80 (1995)
41. Cvejic, S., Devi, L.A.: Dimerization of the delta opioid receptor: Implication for a role in receptor internalization. J. Biol. Chem. **272**, 26959–26964 (1997)
42. Cheng, Z.-J., Miller, L.J.: Agonist-dependent dissociation of oligomeric complexes of G protein-coupled cholecystokinin receptors demonstrated in living cells using bioluminescence resonance energy transfer. J. Biol. Chem. **276**, 48040–48047 (2001)
43. Huang, C., Hepler, J.R., Chen, L.T., Gilman, A.G., Anderson, R.G., Mumby, S.M.: Organization of G proteins and adenylyl cyclase at the plasma membrane. Mol. Biol. Cell **8**, 2365–2378 (1997)
44. Pralle, A., Keller, P., Florin, E.L., Simons, K., Horber, J.K.: Sphingolipid-cholesterol rafts diffuse as small entities in the plasma membrane of mammalian cells. J. Cell Biol. **148**, 997–1008 (2000)