How much do we know about the coupling of G-proteins to serotonin receptors?

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Abstract
Serotonin receptors are G-protein-coupled receptors (GPCRs) involved in a variety of psychiatric disorders. G-proteins, heterotrimeric complexes that couple to multiple receptors, are activated when their receptor is bound by the appropriate ligand. Activation triggers a cascade of further signalling events that ultimately result in cell function changes. Each of the several known G-protein types can activate multiple pathways. Interestingly, since several G-proteins can couple to the same serotonin receptor type, receptor activation can result in induction of different pathways. To reach a better understanding of the role, interactions and expression of G-proteins a literature search was performed in order to list all the known heterotrimeric combinations and serotonin receptor complexes. Public databases were analysed to collect transcript and protein expression data relating to G-proteins in neural tissues. Only a very small number of heterotrimeric combinations and G-protein-receptor complexes out of the possible thousands suggested by expression data analysis have been examined experimentally. In addition this has mostly been obtained using insect, hamster, rat and, to a lesser extent, human cell lines. Besides highlighting which interactions have not been explored, our findings suggest additional possible interactions that should be examined based on our expression data analysis.

Keywords: G-Proteins, Serotonin receptors, Protein expression, Nomenclature

Introduction
In normal physiology, the neurotransmitter serotonin (5-hydroxytryptamine, 5-HT) and its receptors regulate behaviours such as aggressiveness, anxiety, sex, sleep, mood, learning, cognition and memory. They are involved in numerous disease states, including depression, anxiety, social phobia, schizophrenia, mania, autism, drug addiction, obesity, obsessive-compulsive, panic and eating disorders. Therefore serotonin receptors are the target of a variety of pharmaceutical drugs. With the exception of the 5-HT3 receptor, a ligand-gated ion channel, serotonin receptors are a group of membrane-bound G-protein-coupled receptors, which, by means of G-proteins, activate intracellular pathways to produce an excitatory or inhibitory response [1].

G-proteins are heterotrimers consisting of three subunits: Ga, Gβ and Gγ; they are located on the inner plasma membrane, from which they induce GPCR activation. The Gβ and Gγ subunits form an inseparable complex, the βγ complex [2]. In the absence of receptor stimulation the Ga subunit binds guanosine diphosphate (GDP) and the βγ complex, and remains dissociated from the receptor. Binding of the ligand to the GPCR domain outside the cell induces conformational changes of the intracellular GPCR domain, giving rise to GPCR coupling to the G heterotrimer. Consequently, the Ga protein exchanges GDP for guanosine triphosphate (GTP), causing dissociation of the GTP-bound α-subunit from the βγ complex and their separation from the activated receptor. Ga and βγ therefore activate a cascade of further signalling events that finally result in a change in cell function. The process is terminated with GTP hydrolysis to GDP by Ga [3].

Various Ga families have been described: they can activate different pathways or even exert opposite effects on the same pathway. In general, the 5-HT1 (1A, 1B, 1D, 1E, 1F) receptor family and 5-HT5 receptors couple with Gui/o protein family to inhibit adenylate cyclase (AC) activity, reducing the intracellular cyclic adenosine
monophosphate (cAMP) level whilst the Goα family 5-coupled to HT4, 5-HT6 and 5-HT7 receptors triggers a pathway that leads to AC activation and cAMP production. The 5-HT2 (2A, 2B, 2C) receptor family couple with Goq/11 proteins and stimulate the activity of phospholipase C (PLC) increasing the intracellular inositol trisphosphate (IP3), diacyl-glycerol (DAG) and Ca2+ levels [1]. However Goq can also indirectly alter cAMP production, by decreasing Gas protein abundance [4] or by activating adenylate cyclase 8 (ADCY8) by the PLC/Ca2+/calmodulin pathway [5]. Moreover, the Gai/o family induces a decrease in intracellular cAMP levels through AC inhibition. The Gβγ subunits are closely associated forming a Gβγ complex that can be separated only by denaturation, except in cases when the complex involves β5, whose bond to γ subunits is much weaker. At variance with previous studies, the Gβγ complex does not remain inert after dissociation from the α subunit, but plays a key role both in the inactive and in the active receptor state [6]. The Gβγ complex has the following functions: i) it is required for optimal receptor-G-protein interaction, because it enhances ligand affinity and receptor-G-protein coupling, hence G-protein activation [7]; ii) its subunit composition affects receptor-G-protein coupling specificity [8,9]; iii) it activates specific pathways regardless of the type of Go subunit involved [10].

The role of γ subunits is to transport the Gβγ complex from the endoplasmic reticulum to the plasma membrane. Although all γ proteins share this property, translocation kinetics differs widely among subunits, ranging from 10 sec of the fastest, γ9, to several minutes of the slowest, γ3 [11]. It may be hypothesized that the γ subunits allowing fast translocation are associated with human serotonin receptors with a quicker turnover.

Table 1 G-protein isoforms

| Protein name | Gene name | Validated at protein level | Validated at transcript level |
|--------------|-----------|---------------------------|-----------------------------|
| **G alpha proteins** | | | |
| Ga1 | GNA1 | 2 | - |
| Ga2 | GNA2 | 4 | 6 |
| Ga3 | GNA3 | 1 | - |
| Ga11 | GNA11 | 1 | - |
| Ga12 | GNA12 | 1 | 5 |
| Ga13 | GNA13 | 1 | 2 |
| Ga14 | GNA14 | - | 1 |
| Ga15 (or Ga16) | GNA15 | 1 | 2 |
| Gαolf | GNAL | 3 | 1 |
| Gαo | GNAO | 2 | 1 |
| Gαq | GNAQ | 1 | 2 |
| Gαs | GNAS | 7 | 4 |
| Gαt1 | GNAT1 | 1 | - |
| Gαt2 | GNAT2 | 1 | - |
| Gαt3 | GNAT3 | - | 1 |
| Gαz | GNAZ | - | 2 |
| **G beta proteins** | | | |
| Gβ1 | GNBL | 1 | 2 |
| Gβ1-like | GNBL1 | 1 | 1 |
| Gβ2 | GNBL2 | 1 | 2 |
| Gβ2-like | GNBL2L1 | 1 | 17 |
| Gβ3 | GNBL3 | 1 | 3 |
| Gβ4 | GNBL4 | 1 | 1 |
| Gβ5 | GNBL5 | 3 | 3 |
| **G gamma proteins** | | | |
| Gγ1 | GNGT1 | - | 2 |
| Gγ2 | GNG2 | 1 | 5 |
| Gγ3 | GNG3 | - | 1 |
| Gγ4 | GNG4 | 1 | - |
| Gγ5 | GNG5 | 1 | - |
| Gγ7 | GNG7 | 1 | 1 |
| Gγ8 | GNG8 (alias GNG9) | - | 1 |
| Gγ10 | GNG10 | 1 | - |
| Gγ11 | GNG11 | 1 | - |
| Gγ12 | GNG12 | 1 | - |
| Gγ13 | GNG13 | - | 1 |
| Gγ2 | GNGT2 (alias GNG8 or GNG9) | - | 2 |

For each human G-protein, it is shown the number of the related isoforms subdivided by the validation status, according to UniProt. Note that, GNA15 is the homolog gene encoding the murine Go15 and the human Go16 [13].

### Review

**Many G-protein isoforms, huge number of possible heterotrimers**

We have explored UniProt (www.uniprot.org) and Entrez Gene (www.ncbi.nlm.nih.gov/gene) databases to establish how many protein isoforms are currently known for each G-protein subtype (Table 1). For example, we have found 10 isoforms of the Gai2 subtype. Generally, the isoforms do not derive from new gene loci but from different gene expression regulation of the main transcript. In particular, they are due to alternative splicing, use of alternative transcription start sites (TSS) and alternative start codons. All these detailed data are reported in Additional file 1 along with some annotations. In brief, we found many more protein isoforms than expected: Goα, Gβγ, and Goγ proteins may actually be as many as 53, 38, and 20, respectively, raising the potential heterotrimers to about 40,000 combinations. Most of the isoforms we reported are shorter and lack one or more functional domains compared to
the reference isoforms, so they could have a reduced functional activity. In particular, the shorter Ga subtype isoforms lack GTP domains, the Gβs lack WD domains whereas the Gys have no alterations lying in their functional domains. Surprisingly, up till now, many isoforms have not yet been confirmed at protein level according to UniProt, so we looked for this information in the literature. Unfortunately, the papers merely report the name of the investigated G-protein and do not specify its particular isoform. We also found nomenclature inaccuracies, which are probably due to the former names of G-protein: for example when the authors write “the Gaq protein” it is unclear whether they mean GNAQ (Gαq) or the entire family including also GNA11 (Gaq11), GNA14 (Gaq14), or GNA15 (Gaq15 also Gaq16); similarly, “Gai protein” may indicate GNAI1 (Gai1), GNAI2 (Gai2), or GNAI3 (Gai3). The same applies to “Gβγ” [12]. The nomenclature is not univocal even in some databases. For example, in UniProt GNG8 is also called GNG9, and GNGT2 is also called GNG8 or GNG9; therefore a paper examining GNG9 could refer either to GNG8 or to GNGT2. To overcome this ambiguity, the authors should indicate the protein or gene identifier or protein sequence.

How heterotrimer composition affects 5-HT receptor behaviour

The mechanisms underpinning formation of one heterotrimer rather than another are poorly understood, but post-translational modifications of G-proteins and of membrane environments are likely to be involved [14]. Nonetheless heterotrimer composition is critical, because it determines what pathway is activated. For example, activation of 5-HT1A receptor inhibits basal phosphoinositide hydrolysis in the dorsal raphe nucleus but not in the hippocampus, most likely due to different heterotrimer compositions in the two tissues [15]. Moreover a single heterotrimer can activate multiple pathways simultaneously, because some Ga proteins have multiple effects. For example, some Gai/o family proteins inhibit AC leading to intracellular cAMP reduction, whereas others can also inhibit Ca2+ or activate K+ channels [16]. To complicate matters further, the same serotonin receptor can couple to different heterotrimers [7,17,18]; the same ligand may therefore simultaneously activate multiple pathways but be unable to regulate a specific one. The mechanism appears to be irrational, since a single switch (receptor) is unlikely to be able to control a large number of lights (pathways).

Ligand-receptor binding affinity affects G-protein-receptor affinity and vice versa, as described in Spodoptera frugiperda S9 cells [7]. Such affinity also depends on heterotrimeric composition; for example, coupling of Gai3 to 5-HT1A or 5-HT1B receptor was more effective than that of Gai2 and Gao in enhancing agonist [3H]-5-HT affinity [14]. Since a variety of psychiatric disorders and/or drug responses are held to be related to altered ligand-receptor affinity, association studies have mainly explored receptor and downstream effector polymorphisms to explain the genetic basis of such different phenotypes [19-26]. However, given that G-proteins can affect ligand affinity, their variations should also be considered in association studies. For these reasons it is important to gain insights into the role, the interactors and the expression of G-proteins in determining cell responses as a consequence of receptor activation.

The observations that in some GPCRs the G-protein complex can modulate receptor activity state and that the transition from active to inactive state depends on the Ga subunit associated with the receptor make the study of G-proteins even more intriguing [14]. In other words, GPCRs can switch from inactive to active even in the absence of binding to an agonist. This mechanism is still poorly understood and may have important pathological as well as physiological implications. In particular significant activation even without serotonin has been described with coupling of Gαz to 5-HT1A receptor, but not to the other 5-HT1 receptors [27].

Finally, a scenario is emerging where different G-protein combinations can bind the same receptor type, conferring a different ligand affinity and activating several pathways. This warrants investigation of the heterotrimeric combinations that may form in humans, their distribution in different tissues, and the differences in ligand binding affinity among the heterotrimers resulting from binding of one receptor type and various G-proteins.

How many couplings between 5-HT receptors and G-proteins are known

In order to find out what is known about heterotrimer associations with serotonin GPCRs, we have performed a literature search. The papers specifically addressing receptor-G-protein complexes were scanty, therefore data were available for quite a small number of complexes out of the possible thousands. Table 2 presents an exhaustive list of all known combinations of the three types of G-proteins and their associations with serotonin receptors in human neural tissues or in similar models. In particular, for each receptor, we have reported the experimentally assessed complexes formed with the G-proteins, the tissues or contexts where the complexes were determined and their references. We have also annotated the couplings assessed as not present along with the particular experimental context. The 5-HT1p and 5-HT3 receptors were excluded, because the former is expressed in the nervous enteric system (not the central nervous system), the latter because it is a serotonin-gated ion channel not coupled to G-proteins, whereas 5-HT5B is
### Table 2 Assessed couplings between G-proteins and serotonin receptors

| Coupling G subunits | Not coupling G subunits | Notes                                                                 | Second messengers | References |
|---------------------|-------------------------|----------------------------------------------------------------------|-------------------|------------|
| 5-HT1A receptor     |                         |                                                                      |                   |            |
| Gai1-?-?            |                         |                                                                      |                   |            |
| Gai2-?-?            | Gai3-?-?                | Human receptor and bovine G-proteins, in vitro reconstitution into E. coli membranes. Affinity order is Gai3 > Gai1 > Gai2 > Gao. |                   | (Bertin, 1992) [20] |
| Gao-?-?             |                         |                                                                      |                   |            |
| Gai1-Gβ1-Gγ1        |                         |                                                                      |                   |            |
| Gai1-Gβ1-Gγ2        |                         |                                                                      |                   |            |
| Gai1-Gβ1-Gγ3        |                         |                                                                      |                   |            |
| Gai1-Gβ1-Gγ5        |                         |                                                                      |                   |            |
| Gai2-Gβ1-Gγ2        | Gai3-Gβ1-Gγ2            | Human receptor, rat Gαs, Gαi1, Gαi2, Gai3, Gao, murine Gαq, human Gαz, bovine Gβγ1 transfected in insect Sf9 cells. Gβ1 less effective than Gβ2, Gγ3, Gγ5, Gγ7. |                   | (Butkerait, 1995) [7] |
| Gao-Gβ1-Gγ2         |                         |                                                                      |                   |            |
| Gai1-?-?            |                         |                                                                      |                   |            |
| Gai2-?-?            | Gai3-?-?                | Human receptor and G-proteins transfected in hamster CHO cells, affinity order is Gai2 > Gai3, Gai1, Gao > Gao |                   | (Garnovskaya, 1997) [32] |
| Gao-?-?             |                         |                                                                      |                   |            |
Table 2 Assessed couplings between G-proteins and serotonin receptors (Continued)

| Gαi1-?-? | Human receptor and rat Gαs transfected in insect Sf9 cells | - | (Clawges, 1997) [14] |
| Gαi2-?-? | Human receptor and G-proteins transfected in human HeLa cells. affinity order is Gαi1 > Gαi2 >> Gαi3 | - | (Lin, 2002) [35] |
| Gαi3-?-? | Rat receptor and G-proteins transfected in rat GH4C1 cells. cAMP | - | (Liu, 1999) [34] |
| Gαz-Gβ1-Gγ2 | Human receptor and G-proteins transfected in Sf9 cells | - | (Barr, 1997) [33] |
| Gαi1-?-? | No(Gαi1-?-?) | | |
| Gαi2-?-? | No(Gαi3-?-?) | | |
| Gαo-?-? | No(Gαo-?-?) | | |
| Gαz-?-? | In rat hypothalamic paraventricular nucleus | - | (Serres, 2000) [37] |
| Gαi/o-?-? | Human receptor transfected in human HEK293 cells. cAMP | - | (Malmberg, 2000) [38] |
| Gαs-?-? | Human receptor co-expressed with rat G-protein in monkey COS-7 cells. | - | (Dupuis, 2001) [39] |
| Gαi3-?-? | Human receptor transfected in hamster CHO cells. | - | (Newman-Tancred, 2002) [40] |
| Gαi1-?-? | No(Gat-?-?) | Reconstitution in insect Sf9 cell expressing receptor and rat Gα1 and bovine Gat | - | (Slessareva, 2003) [41] |
| Gαq-Gβ1-Gγ2 | Recombinant human receptor, mouse Gaq, rat Gai2, bovine Gβγ co-expressed in insect Sf9. Strong coupling with Gai2, weak with Gaq. | - | (Okada, 2004) [42] |
| Gαi/o-?-? | No(Goq-?-?) | In situ reconstitution in insect Sf9 cells with purified human receptor, squid Gαq, bovine Gai and Gao | - | (Okada, 2004) [42] |
| Gαo-?-? | No(Gai1-?-?) | In rat cortex | - | (Mannoury la Cour, 2006) [43] |
| Gαi3-?-? | No(Gα2-?-?) | | |
| Gαo-?-? | No(Gα3-?-?) | | |
| Gαi3-?-? | In rat anterior raphe area | - | (Mannoury la Cour, 2006) [43] |
| Gαo-?-? | No(Gαz-?-?) | | |
| Gαi1-?-? | No(Gαo-?-?) | In rat hippocampus | - | (Mannoury la Cour, 2006) [43] |
| Gαi3-?-? | No(Gα2-?-?) | | |
| Gα(-?) | No(Gα(-?)) | Coupling Type | Species | Reference |
|--------|-------------|---------------|---------|-----------|
| Ga1-?-? | No(Ga1-?-?) | In rat hypothalamus | - | (Mannoury la Cour, 2006) [43] |
| Ga3-?-? | No(Ga3-?-?) | In rat hippocampus | - | (Martel, 2007) [44] |
| Ga2-?-? | No(Ga2-?-?) | In rat dorsal raphe nucleus | cAMP | (Valdizán, 2010) [45] |
| Ga3-?-? | No(Ga3-?-?) | Human receptor transfected in hamster CHO cells | cAMP | (Rauly-Lestienne, 2011) [46] |
| 5-HT1B receptor | | | | |
| Ga1-?-? | No(Ga1-?-?) | Human receptor and rat Gα transfected in insect Sf9 cells | - | (Clawges, 1997) [14] |
| Ga2-?-? | No(Ga2-?-?) | Reconstitution in insect Sf9 cell expressing receptor and rat Ga1 and bovine Gat | - | (Bae, 1997; Bae, 1999; Slessareva, 2003) [41,47,48] |
| Ga3-?-? | No(Ga3-?-?) | Human receptor and rat G-proteins transfected in human HEK293 cells | cAMP | (Albert, 1999) [36] |
| Ga4-?-? | No(Ga4-?-?) | Chimpanzee receptor transfected in human HEK293 cells | - | (Alberts, 2000) [49] |
| Gai1-Gq1-Gi2 | Human receptor, rat Gα and bovine Gβγ proteins transfected in insect Sf9 cells | - | (Brys, 2000) [50] |
| Ga1-?-? | | Human receptor and G-proteins transfected in human HeLa cells, affinity order is Ga1 > Ga2 > Ga3 | - | (Lin, 2002) [35] |
| Ga2-?-? | | Human receptor and G-proteins transfected in human HeLa cells, affinity order is Ga1 > Ga2 > Ga3 | - | (Lin, 2002) [35] |
| Ga3-?-? | | Human receptor transfected in hamster CHO cells | - | (Newman-Tancredi, 2003) [51] |
| Gαi/Gαo/Gαq/Gα11 | 5-HT1D receptor | 5-HT1E receptor | 5-HT1F receptor | 5-HT2A receptor |
|----------------|-----------------|-----------------|-----------------|-----------------|
| Gαi3-7-7 | Human receptor and rat Gα transfected in insect SF9 cells | - | (Clawges, 1997) [14] | |
| Gαi-7-7 | No(Gαi7-7) | Chimpanzee receptor transfected in human HEK293 cells | - | (Alberts, 2000) [49] | |
| Gαi1-Gβ1-Gγ2 | Human receptor, rat Gαi/o, mouse Gαo and bovine Gβγ proteins transfected in insect SF9 cells | - | (Brys, 2000) [50] | |
| Gαi2-Gβ1-Gγ2 | Gαi1-7-7 | Human receptor and G-proteins transfected in human HeLa cells, affinity order is Gαi1 > Gαi2 > Gαi3 | - | (Lin, 2002) [35] | |
| Gαi3-7-7 | No(Gαi3-7-7) | Human receptor and rat Gα transfected in insect SF9 cells | - | (Clawges, 1997) [14] | |
| Gαi2-7-7 | No(Gαq11-7-7) | Monkey receptor transfected in human HEK293 cells | - | (Alberts, 2000) [49] | |
| Gαi3-7-7 | No(Gαo13-7-7) | In mouse NIH3T3 cells | cPLA2 (not PLC, IP3) | (Kumasch-Orbaugh, 2003) [52] | |
| Gα12/13-7-7 | Rat receptor and G-protein transfected in human HEK293 cells. | Inositol Phosphate | (Bhatnagar, 2004) [53] | |
| Gαq-7-7 | Human receptor and G-protein transfected in human HEK293 cells. | Inositol Phosphate | (Millan, 2012) [54] | |
| Gα11-7-7 | In rat cerebral cortical membranes. | - | (Odagaki, 2014) [55] | |
| Gαq-7-7 | Human G-proteins transfected in rat cortex A1A1v cells | Inositol Phosphate | (Shi, 2007) [56] | |
| Gα11-7-7 | Human G-proteins transfected in rat cortex A1A1v cells | Inositol Phosphate | (Shi, 2007) [57] | |
| Gαq/11-7-7 | Human receptor transfected in hamster CHO cells. | Ca²⁺ | (Cussac, 2008) [58] | |
| | In rat cortex. | - | (Mannoury la Cour, 2009) [59] | |
| Table 2 Assessed couplings between G-proteins and serotonin receptors (Continued) |
|---------------------------------------------------------------|
| **5-HT2B receptor**                                           |
| Gαq/11-Gβ1-Δγ2 | Mouse receptors stably expressed in mouse fibroblast cells | Ras | (Launay, 1996) [60] |
| Gα13-7-7 | No(Gαs-7-7) | In mouse LM6 and 1C11 and in M. natalensis carcinoid tumor primary cultured cells | IP3, NOS, cGMP | (Manivet, 2000) [61] |
| Gαq/11-7-7 | No(Gαq/11-7-7) | Human receptor transfected in hamster CHO cells | Ca²⁺ | (Cussac, 2008) [58] |
| Gαq-7-7 | Human receptor and G-protein transfected in human HEK293 cells | Inositol Phosphate | (Millan, 2012) [54] |
| **5-HT2C receptor**                                           |
| Gαi-7-7 | Mouse receptor and rat G-proteins expressed in Xenopus oocytes | - | (Chen, 1994) [62] |
| Gαo-7-7 | No(Gαs-7-7) | Mouse receptor and G-proteins expressed in Xenopus oocytes | Inositol Phosphate | (Quick, 1994) [63] |
| Gα11-7-7 | No(Gαt-7-7) | Rat receptor and squid Gαo, bovine Gαt, Gαi/o proteins transfected in insect Sf9 cells | - | (Hartman, 1996) [64] |
| Gαi/o-7-7 | No(Gαi/o-7-7) | Human receptor in human HEK293 cells | IP3, cAMP | (Alberts, 1999) [65] |
| Gα11-7-7 | No(Gαi/o/7-7) | In rat choroid plexus epithelial cells | Inositol Phosphate | (Chang, 2000) [66] |
| Gα11-7-7* | No(Gα11-7-7)* | Human receptor and mouse G-proteins (except human Gα16) transfected in mouse NH3T3 cells | Inositol Phosphate | (Price, 2001) [67] |
| Gα12-7-7* | No(Gα12-7-7)* | * with not edited receptor form | | |
| Gα13-7-7* | No(Gα13-7-7)* | # with edited receptor form | | |
| Gα14-7-7* | No(Gα14-7-7)* | | | |
| Gα15-7-7* | No(Gα15-7-7)* | | | |
| Gα16-7-7* | No(Gα16-7-7)* | | | |
| Gα3-7-7 | Human receptor transfected in hamster CHO cells | - | (Cussac, 2002) [68] |
| Gα11-7-7 | No(Gαs-7-7) | In rat choroid plexus epithelial cells and rat receptor transfected in mouse NH3T3 cells | Inositol Phosphate (via PLD, not PLC) | (McGrew, 2002) [69] |
| Gα13-7-7 | Human receptor and squid G-protein reconstitution in insect Sf9 cells | Ca²⁺ | (Okada, 2004) [70] |
| Gαq-7-7 | Human receptor and G-protein transfected in human HEK293 cells | Inositol Phosphate | (Millan, 2012) [54] |
| Coupling | Species | Cell Type | Coupling Type | References |
|----------|---------|-----------|---------------|------------|
| Gaq-Gβ1-Gy2 | Recombinant human receptor, mouse Gaq, rat Gαi2, bovine Gβγ co-expressed in insect Sf9. Strong coupling with Gαi2, weak with Gaq. | - | (Okada, 2004) [42] |
| Gai/o-?-? | No(Gai/o-?-?) | In situ reconstitution in insect Sf9 cells with purified human receptor, squid Gaq, bovine Gai and Gas | - | (Okada, 2004) [42] |
| Gaq11-?-? | Human receptor transfected in hamster CHO cells | Ca²⁺ | (Cussac, 2008) [58] |
| 5-HT4 receptor | Gαq-Gβ1-Gy2 | No(Gai2-Gβ1-Gy2) | Murine receptor and G-proteins transfected in insect Sf9 cells | cAMP | (Ponimaskin, 2002) [71] |
| Gai/o-?-? | No(Gai2-Gβ1-Gy2) | Human receptor transfected in human HEK293 cells; SHT4a receptor coupled only to Gas, while SHT4b isoform coupled to Gai/o | cAMP, Ca²⁺ | (Pindon, 2002) [72] |
| Ga13-Gβ1-Gy2 | No(Gai2-Gβ1-Gy2) | Murine receptor and G-proteins transfected in insect Sf9 cells | Inositol Phosphate, RhoA | (Ponimaskin, 2002) [73] |
| 5-HT5A receptor | Gas-?-? | Human receptor transfected in monkey COS-7 cells | cAMP, Inositol Phosphate | (Pellissier, 2011) [74] |
| Gai1-Gβ1-Gy2 | No(Gai1-Gβ1-Gy2) | Human receptor, rat Gai/o, human Gαz and Gα16, bovine Gas, mouse Gaq, Ga11, Ga12 and Ga13, bovine Gβγ reconstituted in insect Sf9 cells | - | (Francken, 2000) [75] |
| Gai2-Gβ1-Gy2 | No(Gai1-Gβ1-Gy2) | - | (Francken, 2001) [76] |
| Gai3-Gβ1-Gy2 | No(Gai2-Gβ1-Gy2) | Human receptor, rat Gai/o, human Gαz, bovine Gas and Gβγ reconstituted in insect Sf9 cells | - | (Francken, 2001) [76] |
| 5-HT6 receptor | Gaai/o-?-? | Human receptor transfected in rat C6 glioma cells | IP3, Ca²⁺, cAMP, cADPR | (Noda, 2003) [77] |
| Gas-?-? | In human HEK293 cells stably expressing human receptor. | cAMP | (Baker, 1998) [78] |
| Human receptor and G-protein, assessed in vitro. | cAMP | (Kang, 2005) [79] |
Table 2 Assessed couplings between G-proteins and serotonin receptors (Continued)

| Gαi-?-?         | Gαi-?-?          | 5-HT7 receptor                                                                 | Second messenger                  | Reference |
|------------------|------------------|--------------------------------------------------------------------------------|-----------------------------------|-----------|
| Gαi-?-?          | No(Gαi-?-?)      | In human HEK293 cells stably expressing human receptor                         | cAMP, Ca2+/calmodulin            | (Baker, 1998) [78] |
| Gαi-?-?          | No(Gαq/11-?-?)   | Human receptor transfected in human HEK293 cells                               | cAMP                              | (Adham, 1998) [80] |
| Gαi-?-?          | No(Gαq/11-?-?)   | Human receptor transfected in human HEK293 cells, affinity order is Gαi > Gαq/11| cAMP                              | (Alberts, 2001) [81] |
| Gαi-?-?          | No(Gαi3-?-?)     | Murine receptor and G-proteins transfected in insect Sf9 cells                 | RhoA, Cdc42 (NOT Rac1)            | (Kvacchnina, 2005) [82] |
| Gαi-?-?          | No(Gαi3-?-?)     | Murine receptor and G-proteins transfected in insect Sf9 cells                 | cAMP                              | (Kvacchnina, 2009) [83] |

**Note:** "?" means that the particular Gβ or Gγ protein has not be identified in the reference paper. "-" means that the second messenger has not be assessed in the reference paper. Note: unfortunately some papers reported Gαi/o, Gαi, or Gαq/11 without further distinction. **Abbreviations:** RhoA: Ras homolog gene family, member A; Cdc42: cell division control protein 42; Rac1: Ras-related C3 botulinum toxin substrate 1; cADPR: cyclic adenosine diphosphoribose; PLD: phospholipase D; NOS: nitric-oxide synthase; cGMP: cyclic guanosine monophosphate; cPLA2: cytosolic phospholipases A2.
a pseudogene in humans according to EntrezGene and the related protein is absent in UniProt.

Many papers have addressed G-protein combinations with 5-HT1A, 5-HT1B, 5-HT2A and 5-HT2C while only one or two papers refer to 5-HT1E, 5-HT1F or 5-HT6. The experimental models usually involve transfection of human genes into Spodoptera frugiperda Sf9 cells, since they express low levels of mammalian G-proteins, thus avoiding competition with endogenously expressed G-proteins in [35S]GTPγS binding assay [16].

However, to extend these findings to humans is not necessarily correct. For example, the poor coupling of Gaq to 5-HT1A and 5-HT2C could be due to a large portion of the expressed but inactive Gaq [84], maybe because Gaq is not post-translationally modified by palmitoylation in Sf9 as in humans [85]. Other authors have used hamster, mouse and human cells transfected with rat, mouse and bovine constructs. The main methods used to assess the compositions of the heterotrimers are immunoprecipitation and western blot analysis, the binding with radio-ligands and FRET ( Förster Resonance Energy Transfer) by using fluorescent ligands.

For the majority of serotonergic receptors, coupling data are available only in relation to Ga family proteins without specifying which Gβ and Gγ were coupled. However, the few data on Gβ and Gy only concerned Gβ1 with Gy2. Ga proteins coupled to receptors are the most commonly studied G-proteins, because they are held to indicate the pathway activated by receptor stimulation. In contrast, Gβ and Gy proteins are believed merely to play a structural role, that is to stabilize the receptor complex, but they actively participate in signal transduction by activating specific pathways.

We also annotated the second messengers activated downstream G-proteins, when these data were available in the related paper, since they allow to take into account the converging effect of various Ga proteins and the antagonistic/additive effects of Gβγ.

Investigation methods for the assessment of G-protein activation

We have shown that G-protein heterotrimers recruited by serotonin receptors have been evaluated experimentally. This dearth of data is mainly due to the cumbersome methods used to identify the heterotrimers involved in the effects of ligands and to some technical limitations. In fact, to assess the receptor-mediated G-protein activation, both indirect and direct assays are available [16]. Indirect methods, in spite of their good sensitivity, are focused on measuring concentrations of second messengers but the evaluation of this data can be complicated since most receptors can activate different G-proteins.

In particular, since Gas and Gai/o proteins activate or inhibit AC respectively, their activation can be indirectly detected determining intracellular adenosine triphosphate (ATP) conversion into cAMP. It is measured using [α-32P]ATP as the enzyme substrate or using cAMP antibodies. These methods cannot follow quick fluctuations as they are based on static measurements after cell lysis. In the case of the AC inhibiting Gai/o proteins, another problem regards the too low dynamic ranges of inhibition detection. To deal with this specific problem, chimeric Gai/o proteins were developed, but they do not exactly mimic the natural G-proteins.

To test ligand efficacy on Gaq/11-coupled receptors, [3H]IP3 concentration as product of PLC activity can be measured using [3H]PIP2 (phosphatidyl inositol 4,5-bisphosphate) substrate. Alternatively, antibodies can be used, but, since IP3 has a short half life, it is preferred to detect its stable metabolite inositol-1-phosphate (IP1), although this is a more downstream product. Also Ca2+ concentration, by dyes generating fluorescence upon binding of free Ca2+, can be determined to assess Gaq/11 activation, although these probes can influence calcium levels and kinetics. Moreover, Ca2+-sensitive photoproteins, as aequorin, can detect calcium in specific cell compartments by fusion with targeting sequences. This approach is not so sensitive and consists of laborious procedures, such as fusion protein production, transfection and assay calibration.

Gα12/13 activation can be assessed by determining Rho guanine nucleotide exchange factors (RhoGEFs) by immunoblotting, a not highly sensitive technique. Moreover, since RhoGEFs are activated also by Gaq/11, there are crosstalk problems that can be partially overcome by small interfering RNA (siRNA) knockdown.

To directly and quantitatively assess the Ga protein activation, [35S]GTPγS binding assay is employed. Upon Ga subunit activation, it binds the mimic substrate, so remaining blocked in the active form as it cannot hydrolyze this substrate. The blocked Ga can be measured after isolation and it can be immunoprecipitated to identify the specific Ga subunit. However, this approach is mainly suitable to evaluate Gai/o-coupled receptor activation. This assay can be effectively combined with the use of drugs stimulating or inhibiting specific G-proteins, for example, Pertussis toxin (PTX), Mastoparan, Mastoparan-S, Cholera toxin, Suramin, Pasteurella multocida toxin (PMT). Alternatively, it is possible to use G-protein-deficient mice or gene silencing by siRNA, although studies have to take into account the cellular compensatory mechanisms that alter the expression level of other G-proteins. An exhaustive review of these and other techniques was made by Denis et al. [16].

Moreover, cause of GTPase-accelerating proteins (GAPs) that accelerate GTPase activity of Ga-protein subunits, the measuring of GTPase activity in vivo and in vitro differ. In addition, for in vivo studies, methods having a
subsecond time resolution for GTP hydrolysis must be adopted [86,87].

Heterotrimers activation effects

It would be important to consider the synergic effects of the entire activated heterotrimer in order to evaluate ligand effects, drug efficacy and side effects such as hallucination onset. For this reason, we annotated also the second messengers during literature revision. Unfortunately, as can be seen in Table 2, few studies assessed the coupling of all the heterotrimer subunits and few of them assessed the second messengers. Regarding these cases, only Gβ1γ2 were present, so it was not possible to verify if and how different Gβγ combinations can affect Gα induced pathways. In general, the physiological significance of the different Gβγ pairs is unclear, since they participate in complex interactions with receptors, Gα subunits and effectors [6].

A more detailed description of the pathway downstream G-proteins was performed by Millan et al. [88], however it should be taken into account that the signalling downstream a receptor is ligand-dependent. For example, some agonists of 5-HT2A can induce hallucinations but other structurally related ones do not [89].

Expression analysis in human brain tissues

We believe that our collected data can be used for guiding experiments which seek new couplings. However, we verified if it was possible to reduce the number of combinations by filtering out those not allowed in a particular neural tissue due to one or more components that are not expressed. In Additional file 2, we report the expression profiles of Gα, Gβ, Gγ and the serotonin receptor by using three proteomic databases according to a previous work [90]: Human Protein Reference Database (www.hprd.org); Human Proteinpedia (www.humanproteinpedia.org) and Human Protein Atlas (www.proteinatlas.org). We also used expression data obtained from three transcriptomic databases: Human Transcriptome Map (http://bioinfo.amc.uva.nl/HTMseq), Cancer Gene Anatomy Project database (http://cgap.nci.nih.gov) and Allen Brain Atlas database (www.brain-map.org). An issue that arose in the course of this investigation was the partial conflict between microarray and RNA-Seq data retrieved from Allen Brain Atlas and, to a lesser extent, protein and transcript expression data. Generally, these discrepancies could be resolved by relying on proteomic data, which, if present, are usually more dependable than transcriptomic data. For example, regarding GNG1, it seems to be absent in all tissues and their sub-tissues assessed by Allen Brain Atlas RNA-seq. Instead, according to Allen Brain Atlas microarray data, GNG1 expression results as being very variable among sub-tissues of each tissue. Since in the cerebellar cortex also Protein Atlas data are available, they solve this contradiction claiming GNG1 absence. According to our expression data, most G-proteins are expressed in the majority of brain tissues, thus confirming the possible existence of a big number of heterotrimer combinations in nearly all neural tissues. Of course, co-expression of a receptor and G-proteins in a brain tissue does not imply that they are functionally coupled to each other. However, the available databases, being manually annotated, do not contain the all expression data reported in the literature, so the Additional file 2 may be incomplete.

Conclusion

The large number of human G-proteins that our searches found demonstrates that a very large amount of possible heterotrimers can be formed but unfortunately only a few have been assessed. Naturally, a limitation of the studies carried out in vitro is that the reported couplings do not always match to the couplings found in vivo [91]. However, knowledge of all the G-proteins that bind to each receptor would allow linking each receptor to all the possible activated pathways. Association of a receptor with multiple G-proteins would also highlight activation of different pathways in different tissues. This is important, because G-protein gene mutations or polymorphisms could alter transduction efficacy, thus explaining the non-activation of a pathway despite the presence of the right ligand and the absence of nucleotide variation in the receptor. Therefore precise knowledge of the role and distribution of G-proteins would greatly contribute to the evaluation of G-protein gene polymorphisms and to the development of drugs targeting specific G-proteins.

Finally, since receptor binding to a G-protein considerably modifies receptor behaviour, it could be that the G-proteins define many receptor subtypes. For this reason it is more appropriate to consider a receptor not individually but in association to each permitted heterotrimer. This also suggests that a number of experiments should be performed again, like the biochemical studies exploring the affinity constants between ligands and a receptor not considering if, and which, G-proteins were associated.

Additional files

**Additional file 1:** Detailed information about G-protein isoforms.
In this table all G-protein isoforms along with annotations extracted from UniProt and EntrezGene are shown.

**Additional file 2:** G-protein and serotonin receptor expression data in brain sub-tissues.
In this table G-protein and serotonin receptor expression data extracted from different transcriptomic and proteomic databases are shown.

**Abbreviations**
5-HT: Serotonin; GPCRs: G-protein-coupled receptors; GDP: Guanosine diphosphate; GTP: Guanosine triphosphate; AC: Adenylate cyclase; cAMP: Cyclic adenosine monophosphate; PLC: Phospholipase C; DAG: Diacyl-
glycerol; IP3: Inositol trisphosphate; PKC: Protein kinase C; ADCY8: Adenylate cyclase 8; TSS: Transcription start sites; FRET: Förster resonance energy transfer; ATP: Adenosine triphosphate; PIP2: Phosphatidylinositol 4,5-bisphosphate; IP1: Inositol-1-phosphate; RhGKF: Rh quanine nucleotide exchange factor; siRNA: Small interfering RNA; PTX: Pertussis toxin; PMT: Pasteurella multocida toxin; GAP: GTPase-accelerating protein.

Competing interests
The authors declare that they have no competing interests.

Authors’ contributions
MG, VW and FP performed the analyses and wrote the manuscript. GP, CB and BN conceived of the study, and participated in its design and coordination. All authors read and approved the final manuscript.

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References
1. Filip M, Bader M: Overview on 5-HT receptors and their role in physiology and pathology of the central nervous system. Pharmaco Rev 2009, 61:761–777.
2. Hurovitz EH, Melnyk JM, Chen YJ, Kouros-Mehr H, Simon MI, Shizuya H: Galphaq reduces cAMP synthesis by Gi-coupled receptors upon ablation of distinct Galphai and gamma subunit genes. DWA Res 2000, 7:111–120.
3. Oldham WM, Hamm HE: Heterotrimic G protein activation by G-protein-coupled receptors. Nat Rev Mol Cell Biol 2008, 9:60–71.
4. Tang T, Gao MH, Miyahara A, Hammond HK: Galphag reduces cAMP production by decreasing Galphag protein abundance. Biochim Biophys Acta 2008, 377:679–684.
5. Twery EN, Raper JA: SDF1-induced antagonism of axonal repulsion requires multiple G-protein coupled signaling components that work in parallel. PLoS ONE 2011, 6:e18866.
6. Smrcka AV: G protein beta-gamma subunits: central mediators of G protein-coupled receptor signaling. Cell Mol Life Sci 2008, 65:2191–2214.
7. Butkerait P, Zheng Y, Hallak H, Graham TE, Miller HA, Burris KD, Molinoff PB, Manning DR: Expression of the human 5-hydroxytryptamine1A receptor in Sf9 cells. Reconstitution of a coupled phenotype by co-expression of mammalian G protein subunits. J Biol Chem 1995, 270:18961–18969.
8. Kisselov G, Gauthier N: Specific interaction with phosphodiesterase 5 is dependent on the gamma subunit type in a G protein. J Biol Chem 1993, 268:23059–23062.
9. Kleus C, Schenull H, Hescheler J, Schultz G, Wittig B: Selectivity in signal transduction determined by gamma subunits of heterotrimic G proteins. Science 1993, 259:832–834.
10. Raymond JR, Mukhin YV, Gettys TW, Gamovskaya MN: The recombinant 5-HT1A receptor: G protein coupling and signalling pathways. Br J Pharmacol 1999, 127:1751–1764.
11. Ajith Karunarathne WK, O'Neill PR, Martinez-Espinosa PL, Kalyanaraman V, Gauthier N: All G protein beta-gamma complexes are capable of translocation on receptor activation. Biochem Biophys Res Commun 2012, 421:605–611.
12. Glass M, Northup JK: Agonist selective regulation of G proteins by cannabinoid CB(1) and CB(2) receptors. Mol Pharmacol 1999, 56:362–369.
13. Wilkie TM, Gilbert DJ, Olsen AS, Chen XN, Amatudia TT, Koenenberg JR, Trask BJ, de Jong P, Reed RR, Simon MI, et al: Evolution of the mammalian G protein alpha subunit multigene family. Nat Genet 1992, 185–91.
14. Clawges HM, Depree KM, Parker EM, Graber SG: Human 5-HT1 receptor subtypes exhibit distinct G protein coupling behaviors in membranes from Sf9 cells. Biochemistry 1997, 36:1230–1239.
15. Johnson RG, Fiorella D, Winter JC, Rabin RA: [3H]OH-DPAT labeling of 5-HT sites coupled to inhibition of phosphoinositide hydrolysis in the dorsal raphe. Eur J Pharmacol 1997, 329:99–106.
16. Denis C, Sauliere A, Galandrin S, Senard JM, Gales C: Probing heterotrimic G protein activation: applications to biased ligands. Curr Pharm Des 2012, 18:125–144.
17. Muhren JC, Casanovas SJ, Arthur JM, Gamovskaya MN, Gettys TW, Raymond JR: Human 5-HT1A receptor expressed in insect cells activates endogenous G (o)-like G protein(s). J Biol Chem 1994, 269:12954–12962.
18. Parker EM, Grisel DA, Iben LG, Nowak HP, Mahle CD, Yocca FD, Gaughan GT: Characterization of human 5-HT1 receptors expressed in Sf9 insect cells. Eur J Pharmacol 1994, 268:32–33.
19. Piva F, Giulietti M, Armeni T, Principato G: Cross-link immunoprecipitation data to detect polymorphisms lying in splicing regulatory motifs: a method to refine single nucleotide polymorphism selection in association studies. Psychiatr Genet 2012, 22:88–91.
20. Piva F, Giulietti M, Baldelli L, Nardi B, Bellantuono C, Armeni T, Saccucci F, Principato G: Bioinformatic analyses to select phenotype affecting polymorphisms in HTR2A gene. Hum Psychopharmacol 2011, 26:365–372.
21. Nardi B, Turchi C, Piva F, Giulietti M, Castellucci G, Arimata E, Rochetti D, Rocchetti G, Principato G, Tagliabucci A, Bellantuono C: Searching for a relationship between the serotonin receptor 2A gene variations and the development of inward and outward personal meaning organizations. Psychiatr Genet 2011, 21:269–270.
22. Piva F, Giulietti M, Nardi B, Bellantuono C, Principato G: An improved in silico selection of phenotype affecting polymorphisms in SLC6A4, HTR1A and HTR2A genes. Hum Psychopharmacol 2010, 25:153–161.
23. Nardi B, Piva F, Turchi C, Giulietti M, Castellucci G, Arimata E, Rochetti D, Rocchetti G, Principato G, Tagliabucci A, Bellantuono C: HTR2A gene polymorphisms and inward and outward personal meaning organizations. Acta Neuropsychiatria 2012, 24:336–343.
24. Blasi G, De Virgilio C, Papazacharias A, Taurisano P, Gelao B, Fazio L, Ursini G, Sinibaldi L, Andriola I, Maselli R, Romano R, Rampino A, Di Giorgio A, Lo Bianco L, Caforio G, Piva F, Polipozlo T, Bellantuono C, Todarello O, Kleinman JE, Gadaleta G, Weinberger DR, Bertolino A: Converging evidence for the association of functional genetic variation in the serotonin receptor 2A gene with prefrontal function and olanzapine treatment. JAMA Psychiatry 2013, 70:621–930.
25. Galeazz R, Massuccco L, Piva F, Principato G, Laudadio E: Insights into the influence of 5-HT2C aminoacidic variants with the inhibitory action of serotonin inverse agonists and antagonists. J Mol Model 2014, 20:2120.
26. Blasi G, Napolitano F, Ursini G, Di Giorgio A, Caforio G, Taurisano P, Fazio L, Gelao B, Atterto MT, Colagiglio L, Todarello C, Piva F, Papazacharias A, Maselli R, Mancone M, Porcelli A, Romano R, Rampino A, Quarto T, Giulietti M, Lipisa BK, Kleinman JE, Polipolzo T, Weinberger DR, Usellio A, Bertolino A: Association of GSK-3beta genetic variation with GSK-3beta expression, prefrontal cortical thickness, prefrontal psychology, and schizophrenia. Am J Psychiatry 2013, 170:868–876.
27. Barr AJ, Brass LF, Manning DR: Reconstitution of receptors and GTP-binding regulatory proteins (G-proteins) in Sf9 cells. A direct evaluation of selectivity for the association of functional genetic variation in the serotonin receptor 2A gene with prefrontal function and olanzapine treatment. JAMA Psychiatry 2013, 70:621–930.
28. Senker A, Maayani S, Weisbtein H, Green JP: Pharmacological characterization of two 5-Hydroxytryptamine receptors coupled to adenylyl cyclase in guinea pig hippocampal membranes. Mol Pharmacol 1987, 31:357–367.
29. Berrin B, Freismuth M, Breyer HM, Schuzt W, Strossberg AD, Malullo S: Functional expression of the human serotonin 5-HT1A receptor in Escherichia coli. Ligand binding properties and interaction with recombinant G protein alpha-subunits and lack of coupling to Gs alpha. Biochemistry 1993, 32:1066–11073.
30. Gettys TW, Fields TA, Raymond JR: Selective activation of inhibitory G-protein alpha-subunits by partial agonists of the human 5-HT1A receptor. Biochemistry 1994, 33:2823–4200.
31. Gamovskaya MN, Gettys TW, van Biesen T, Prpic V, Chuprin JK, Raymond JR: 5-HT1A receptor activates Na+/H+ exchange in CHO-K1 cells through Galpha2 and Galpha3. J Biol Chem 1997, 272:7770–7776.
32. Barr AJ, Manning DR: Agonist-independent activation of G by the 5-hydroxytryptamine1A receptor co-expressed in Spodoptera frugiperda cells. Distinguishing inverse agonists from neutral antagonists. J Biol Chem 1997, 272:32979–32987.
33. Liu YF, Ghairehmani MH, Rasenick MM, Jakobs KH, Albert PR: Stimulation of cAMP synthesis by Gi-coupled receptors upon ablation of distinct GalphaI protein expression. G subtype specificity of the 5-HT1A receptor. J Biol Chem 1997, 272:16444–16450.
35. Lin SL, Setya S, Johnson-Farley NN, Cowen DS. Differential coupling of 5-HT(1) receptors to G proteins of the Gi/o family. Br J Pharmacol 2002, 136:1072–1078.

36. Albert PR, Sajedi N, Lemosde S, Grahmermhi M. Constitutive G(i/o) dependent activation of adenyl cyclase type II by the 5-HT(1A) receptor. Inhibition by anoxicotic partial agonists. J Biol Chem 1999, 274:35469–35474.

37. Sures F, Li J, Garcia F, Raap DK, Battaglia G, Guma NA, Van de Kar I. Evidence that G(i/o)-proteins couple to hypothyamic S-HT(1A) receptors in vivo. J Neurochem 2000, 73:3095–3103.

38. Malmberg A, Strange PG. Site-directed mutations in the third intracellular loop of the serotonin 5-HT(1A) receptor alter G protein coupling from Gi(o) to Gi(o) in a ligand-dependent manner. J Neurochem 2000, 75:1283–1293.

39. Dupuis DS, Wurz T, Tarass SV, Colpaert FC, Fauvel MI. Modulation of 5-HT(1A) receptor activation by its interaction with wild-type and mutant g (alpha3) proteins. Neuropharmacology 2001, 40:36–47.

40. Newman-Tancredi A, Cussac D, Marini L, Millan MJ. Antibody capture assay reveals bell-shaped concentration-response isotherms for 5-HT(1A) receptor-mediated Galphai3 activation: conformational selection by high-efficacy agonists, and relationship to trafficking of receptor signaling. Mol Pharmacol 2002, 62:590–601.

41. Slessareva JE, Ha D, Depree KM, Flood LA, Ba e, Cabrera-Vera TM, Hamm HE, Graber SG. Closely related G protein-coupled receptors use multiple and distinct domains on G-protein alpha-subunits for selective coupling. J Biol Chem 2003, 278:50530–50536.

42. Okada M, Golden D, Linnola M, Iwata N, Otsuki N, Northup JK. Comparison of G-protein selectivity of human 5-HT2C and 5-HT1A receptor, Att N Y Acad Sci 2004, 1025:570–577.

43. la Cour Mannoury C, El Mestikawy S, Hanoun N, Hamon M, Lefumey L. Regional differences in the coupling of 5-hydroxytryptamine1A-receptors to G proteins in the rat brain. Mol Pharmacol 2004, 70:1013–1021.

44. Martel JC, Omirome AM, Ledur N, Assie MB, Cussac D, Newman-Tancredi A. Native rat hippocampal 5-HT1A receptors show constitutive activity. Mol Pharmacol 2007, 71:588–643.

45. Valizadeh M, Castro E, Pazos A. G protein agonist-dependent modulation of G-protein coupling and transduction of 5-HT1A receptors in rat dorsal raphe nucleus. Int J Neuropharmacology 2010, 138:835–843.

46. Rauy-Lestienne I, Lestienne F, Alhaud MC, Binesse J, Newman-Tancredi A. Antibody capture assay reveals bell-shaped concentration-response isotherms for 5-HT(1A) receptor-mediated Galphai3 activation: conformational selection by high-efficacy agonists, and relationship to trafficking of receptor signaling. Mol Pharmacol 2002, 62:590–601.

47. Slessareva JE, Ha D, Depree KM, Flood LA, Ba e, Cabrera-Vera TM, Hamm HE, Graber SG. Closely related G protein-coupled receptors use multiple and distinct domains on G-protein alpha-subunits for selective coupling. J Biol Chem 2003, 278:50530–50536.

48. Okada M, Golden D, Linnola M, Iwata N, Otsuki N, Northup JK. Comparison of G-protein selectivity of human 5-HT2C and 5-HT1A receptor, Att N Y Acad Sci 2004, 1025:570–577.

49. J Biol Chem 2000, 75:1283–1293.

50. Manivet P, Mouflier-Richard S, Callebert J, Nebigil CG, Maroteaux L, Hosoda S, Kellermann O, Launay JM. PDZ-dependent activation of nitric-oxide synthases by the serotonin 2B receptor. J Biol Chem 2000, 275:9924–9931.

51. Chen Y, Baez M, Yu L. Functional coupling of the 5-HT2C serotonin receptor to G proteins in Xenopus oocytes. Neurosci Lett 1994, 179:100–102.

52. Hartman JL, Bixaux E, Bondoux D, Callebert J. Choi DS, Loric S, Maroteaux L: Ras involvement in signal transduction by the serotonin 5-HT2B receptor. J Biol Chem 1996, 271:1341–1347.

53. Launay JM, Bixaux E, Bondoux D, Callebert J. Choi DS, Loric S, Maroteaux L: Ras involvement in signal transduction by the serotonin 5-HT2B receptor. J Biol Chem 1996, 271:1341–1347.

54. Manivet P, Mouflier-Richard S, Callebert J, Nebigil CG, Maroteaux L, Hosoda S, Kellermann O, Launay JM. PDZ-dependent activation of nitric-oxide synthases by the serotonin 2B receptor. J Biol Chem 2000, 275:9924–9931.

55. Alhajj MM, Alhajj ML. Antibody capture assay reveals bell-shaped concentration-response isotherms for 5-HT(1A) receptor-mediated Galphai3 activation: conformational selection by high-efficacy agonists, and relationship to trafficking of receptor signaling. Mol Pharmacol 2002, 62:590–601.

56. Okada M, Golden D, Linnola M, Iwata N, Otsuki N, Northup JK. Comparison of G-protein selectivity of human 5-HT2C and 5-HT1A receptor, Att N Y Acad Sci 2004, 1025:570–577.

57. Valizadeh M, Castro E, Pazos A. G protein agonist-dependent modulation of G-protein coupling and transduction of 5-HT1A receptors in rat dorsal raphe nucleus. Int J Neuropharmacology 2010, 138:835–843.

58. Rauy-Lestienne I, Lestienne F, Alhaud MC, Binesse J, Newman-Tancredi A. Antibody capture assay reveals bell-shaped concentration-response isotherms for 5-HT(1A) receptor-mediated Galphai3 activation: conformational selection by high-efficacy agonists, and relationship to trafficking of receptor signaling. Mol Pharmacol 2002, 62:590–601.

59. J Biol Chem 2000, 75:1283–1293.

60. Launay JM, Birraux G, Bondoux D, Callebert J. Choi DS, Loric S, Maroteaux L: Ras involvement in signal transduction by the serotonin 5-HT2B receptor. J Biol Chem 1996, 271:1341–1347.

61. Manivet P, Mouflier-Richard S, Callebert J, Nebigil CG, Maroteaux L, Hosoda S, Kellermann O, Launay JM. PDZ-dependent activation of nitric-oxide synthases by the serotonin 2B receptor. J Biol Chem 2000, 275:9924–9931.

62. Hartman JL, Bixaux E, Bondoux D, Callebert J. Choi DS, Loric S, Maroteaux L: Ras involvement in signal transduction by the serotonin 5-HT2B receptor. J Biol Chem 1996, 271:1341–1347.

63. Quick MM, Simon MI, Davidson N, Lester HA, Aragay AM: Differential coupling of G protein alpha subunits to seven-helix receptors expressed in Xenopus oocytes. J Biol Chem 1994, 269:30164–30172.

64. Hartman JL, Northup JK. Functional reconstitution in situ of 5-hydroxytryptamine(2C) (5HT2C) receptors with alphaq and inverse agonism of 5HT2C receptor antagonists. J Biol Chem 1996, 271:22591–22597.

65. Albers GL, Pregenerer JF, Im WB, Zaworski PG, Gill GS. Agonist-induced GTPgammaS binding mediated by human 5-HT(2C) receptors expressed in human embryonic kidney 293 cells. Eur J Pharmacol 1999, 383:311–319.

66. Chang M, Zhang L, Tarn JP, Sanders-Bush E: Dissecting G protein-coupled receptor signaling pathways with membrane-permeable blocking peptides. Endogenous 5-HT(2C) receptors in choroid plexus epithelial cells. J Biol Chem 2000, 275:7021–7029

67. Price RD, Weiner DM, Chang MS, Sanders-Bush E: RNA editing of the human serotonin 5-HT2C receptor alters receptor-mediated activation of G13 protein. J Biol Chem 2001, 276:46663–46668

68. Cussac D, Newman-Tancredi A, Duqueyros A, Pasteau V, Millan MJ. Differential activation of Gq(11) and G(i) proteins at 5-hydroxytryptamine(2C) receptors reverses by antagonist occupancy: influence of receptor reserve and relationship to agonist-directed trafficking. Mol Pharmacol 2002, 62:578–589.

69. McGrew L, Chang MS, Sanders-Bush E: Phospholipase D activation by endogenous 5-hydroxytryptamine-2C receptors is mediated by Galphai3 and pertussis toxin-insensitive Gbetagamma subunits. Mol Pharmacol 2002, 62:1339–1347.

70. Okada M, Northup JK, Otsuki N, Russell JT, Linnola M, Golden D, Maroteaux L: Modulation of human 5-HT(2C) receptor function by Cys23Ses, an abundant, naturally occurring amino-acid substitution. Mol Psychiatry 2004, 9:55–64.

71. Ponimaskin EG, Heine M, Joubert L, Sebben M, Bickmeyer U, Richter DW, Dierichs M, Junger A, Bockaert J, Bananais J, Claeywens S: Protein activation by serotonin type 4 receptor dimers: evidence that turning on two protoners is more efficient. J Biol Chem 2011, 286:9985–9997.

72. Franken B, Joson K, Lijnen P, Juzarik M, Luyten WH, Maroteaux L: Human 5-hydroxytryptamine(3)(A) receptors activate coexpressed G(i) and
Glo) proteins in Spodoptera frugiperda 9 cells. Mol Pharmacol 2000, 57:1034–1044.

76. Francken BJ, Vanhauwe JF, Josson K, Jurzak M, Luyten WH, Leysen JE: Reconstitution of human 5-hydroxytryptamine5A receptor–G protein coupling in E. coli and Sf9 cell membranes with membranes from Sf9 cells expressing mammalian G proteins. Receptor Channels 2001, 7:303–318.

77. Noda M, Yasuda S, Okada M, Higashida H, Shimada A, Iwata N, Ozaki N, Nishikawa K, Shirasawa S, Uchida M, Aoki S, Wada K: Reombinant human serotonin 5A receptors stably expressed in C6 glioma cells couple to multiple signal transduction pathways. J Neurochem 2003, 84:222–232.

78. Baker LP, Nielsen MD, Impey S, Metcalf MA, Poser SW, Chan G, Obrietan K, Hamblin MW, Storm DR: Stimulation of type 1 and type 8 Ca2+-calmodulin-sensitive adenylly cyclases by the G-coupled 5-hydroxytryptamine subtype 5-HT7A receptor. J Biol Chem 1998, 273:17469–17476.

79. Kang H, Lee WK, Choi YH, Vukoti KM, Bang WG, Yu YG: Molecular analysis of the interaction between the intracellular loops of the human serotonin receptor type 6 (5-HT6) and the alpha subunit of Gs protein. Biochim Biophys Res Commun 2005, 329:684–692.

80. Adham N, Zgombick JM, Bard J, Branchek TA: Functional characterization of the recombinant human 5-hydroxytryptamine7(a) receptor isoform coupled to adenylyl cyclase stimulation. J Pharmacol Exp Ther 1998, 287:508–514.

81. Alberts GL, Chio CL, Im WB: Allosteric modulation of the human 5-HT(7A) receptor by lipidic amphipathic compounds. Mol Pharmacol 2001, 60:1349–1355.

82. Kvachnina E, Liu G, Dityatev A, Renner U, Dityateva G, Schachnir M, Voyno-Yasenetskaia TA, Ponimaskin EG: 5-HT17 receptor is coupled to G alpha subunits of heterotrimetric G12-protein to regulate gene transcription and neuronal morphology. J Neurosci 2005, 25:7821–7830.

83. Kvachnina E, Dumuis A, Wlodarczyk J, Renner U, Doucet M, Richter DW, Ponimaskin E: Constitutive Gi-mediated, but not G12-mediated, activity of the 5-hydroxytryptamine 5-HT7(a) receptor is modulated by the palmitoylation of its C-terminal domain. Biochim Biophys Acta 2009, 1793:1646–1655.

84. Houston C, Wenzel-Seifert K, Buczakstumper T, Seifert R: The human histamine H2-receptor couples more efficiently to Sf9 insect cell Gs-proteins than to insect cell Gq-proteins: limitations of Sf9 cells for the analysis of receptor/ Gq-protein coupling. J Neurosci 2002, 20:678–696.

85. Linder ME, Middleton P, Hepler JR, Taussig R, Gilman AG, Mumble SM: Lipid modifications of G proteins: alpha subunits are palmitoylated. Proc Natl Acad Sci U S A 1993, 90:3675–3679.

86. Lambart NA, Johnston CA, Cappell SO, Kuravi S, Kimpel AJ, Willard FS, Siderovski DP: Regulators of G-protein signaling accelerate GPCR signaling kinetics and govern sensitivity solely by accelerating GTPase activity. Proc Natl Acad Sci U S A 2010, 107:7066–7071.

87. Bosch DE, Zielinski T, Lowery RG, Siderovski DP: Evaluating modulators of “Regulator of G-protein Signaling” (RGS) proteins. Curr Protoc Pharmacol 2012, Chapter 25Unit2.8.

88. Millan MJ, Marin P, Buckbaet J, Mannory la Cour C: Signaling at G-protein-coupled serotonin receptors: recent advances and future research directions. Trend Pharmacol Sci 2008, 29:454–464.

89. Moya PR, Berg KA, Gutierrez-Hernandez MA, Saez-Briones P, Reyes-Parada M, Cassels BK, Clarke WP: Functional selectivity of hallucinogenic phenethylamine and phenylisopropylamine derivatives at human 5-hydroxytryptamine (5-HT)2A and 5-HT2C receptors. J Pharmacol Exp Ther 2007, 321:1054–1061.

90. Piva F, Giulietti M, Burini AB, Principato G: SpliceAid 2: a database of human splicing factors expression data and RNA target motifs. Hum Mutat 2012, 33:81–85.

91. Albert PR, Robillard L: G protein specificity: traffic direction required. Cell Signal 2002, 14:267–278.