



REVIEW

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# 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-HT<sub>3</sub> 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: G $\alpha$ , G $\beta$  and G $\gamma$ ; they are located on the inner plasma

membrane, from which they induce GPCR activation. The G $\beta$  and G $\gamma$  subunits form an inseparable complex, the  $\beta\gamma$  complex [2]. In the absence of receptor stimulation the G $\alpha$  subunit binds guanosine diphosphate (GDP) and the  $\beta\gamma$  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 G $\alpha$  protein exchanges GDP for guanosine triphosphate (GTP), causing dissociation of the GTP-bound  $\alpha$ -subunit from the  $\beta\gamma$  complex and their separation from the activated receptor. G $\alpha$  and  $\beta\gamma$  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 G $\alpha$  [3].

Various G $\alpha$  families have been described: they can activate different pathways or even exert opposite effects on the same pathway. In general, the 5-HT<sub>1</sub> (1A, 1B, 1D, 1E, 1F) receptor family and 5-HT<sub>5</sub> receptors couple with G $\alpha$ i/o protein family to inhibit adenylate cyclase (AC) activity, reducing the intracellular cyclic adenosine

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monophosphate (cAMP) level whilst the  $G_{\alpha s}$  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  $G_{\alpha q/11}$  proteins and stimulate the activity of phospholipase C (PLC) increasing the intracellular inositol trisphosphate (IP3), diacyl-glycerol (DAG) and  $Ca^{2+}$  levels [1]. However  $G_{\alpha q}$  can also indirectly alter cAMP production, by decreasing  $G_{\alpha s}$  protein abundance [4] or by activating adenylate cyclase 8 (ADCY8) by the PLC/ $Ca^{2+}$ /calmodulin pathway [5]. Moreover, the  $G_{\alpha i/o}$  family induces a decrease in intracellular cAMP levels through AC inhibition.

The  $G_{\beta}$  and  $G_{\gamma}$  subunits are closely associated forming a  $\beta\gamma$  complex that can be separated only by denaturation, except in cases when the complex involves  $\beta_5$ , whose bond to  $\gamma$  subunits is much weaker. At variance with previous studies, the  $\beta\gamma$  complex does not remain inert after dissociation from the  $\alpha$  subunit, but plays a key role both in the inactive and in the active receptor state [6]. The  $\beta\gamma$  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  $G_{\alpha}$  subunit involved [10]. The role of  $\gamma$  subunits is to transport the  $\beta\gamma$  complex from the endoplasmic reticulum to the plasma membrane. Although all  $\gamma$  proteins share this property, translocation kinetics differs widely among subunits, ranging from 10 sec of the fastest,  $\gamma_9$ , to several minutes of the slowest,  $\gamma_3$  [11]. It may be hypothesized that the  $\gamma$  subunits allowing fast translocation are associated with human serotonin receptors with a quicker turnover.

## Review

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

We have explored UniProt ([www.uniprot.org](http://www.uniprot.org)) and Entrez Gene ([www.ncbi.nlm.nih.gov/gene](http://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  $G_{\alpha i2}$  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:  $G_{\alpha}$ ,  $G_{\beta}$ , and  $G_{\gamma}$  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

**Table 1 G-protein isoforms**

Protein name	Gene name	Validated at protein level	Validated at transcript level
<b>G alpha proteins</b>			
Gai1	GNAI1	2	-
Gai2	GNAI2	4	6
Gai3	GNAI3	1	-
Ga11	GNA11	1	-
Ga12	GNA12	1	5
Ga13	GNA13	1	2
Ga14	GNA14	-	1
Ga15 (or Ga16)	GNA15	1	2
Gaolf	GNAL	3	1
Gao	GNAO	2	1
Gaq	GNAQ	1	2
Gas	GNAS	7	4
Gat1	GNAT1	1	-
Gat2	GNAT2	1	-
Gat3	GNAT3	-	1
Gaz	GNAZ	-	2
<b>G beta proteins</b>			
$G_{\beta 1}$	GNB1	1	2
$G_{\beta 1}$ -like	GNB1L	1	1
$G_{\beta 2}$	GNB2	1	2
$G_{\beta 2}$ -like	GNB2L1	1	17
$G_{\beta 3}$	GNB3	1	3
$G_{\beta 4}$	GNB4	1	1
$G_{\beta 5}$	GNB5	3	3
<b>G gamma proteins</b>			
$G_{\gamma 1}$	GNGT1	-	2
$G_{\gamma 2}$	GNG2	1	5
$G_{\gamma 3}$	GNG3	-	1
$G_{\gamma 4}$	GNG4	1	-
$G_{\gamma 5}$	GNG5	1	-
$G_{\gamma 7}$	GNG7	1	1
$G_{\gamma 8}$	GNG8 (alias GNG9)	-	1
$G_{\gamma 10}$	GNG10	1	-
$G_{\gamma 11}$	GNG11	1	-
$G_{\gamma 12}$	GNG12	1	-
$G_{\gamma 13}$	GNG13	-	1
$G_{\gamma t2}$	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 Ga15 and the human Ga16 [13].

the reference isoforms, so they could have a reduced functional activity. In particular, the shorter  $G\alpha$  subtype isoforms lack GTP domains, the  $G\beta$ s lack WD domains whereas the  $G\gamma$ s 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  $G\alpha q$  protein” it is unclear whether they mean *GNAQ* ( $G\alpha q$ ) or the entire family including also *GNAI1* ( $G\alpha q11$ ), *GNAI4* ( $G\alpha q14$ ), or *GNAI5* ( $G\alpha q15$  also  $G\alpha q16$ ); similarly, “ $G\alpha i$  protein” may indicate *GNAI1* ( $G\alpha i1$ ), *GNAI2* ( $G\alpha i2$ ), or *GNAI3* ( $G\alpha i3$ ). The same applies to “ $G\beta\gamma$ ” [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-HT<sub>1A</sub> 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  $G\alpha$  proteins have multiple effects. For example, some  $G\alpha i$  or family proteins inhibit AC leading to intracellular cAMP reduction, whereas others can also inhibit  $Ca^{2+}$  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* Sf9 cells [7]. Such affinity also depends on heterotrimeric composition; for example, coupling of  $G\alpha i3$  to 5-HT<sub>1A</sub> or 5-HT<sub>1B</sub> receptor was more effective than that of  $G\alpha i2$  and  $G\alpha o$  in enhancing agonist [<sup>3</sup>H]-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  $G\alpha$  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  $G\alpha z$  to 5-HT<sub>1A</sub> receptor, but not to the other 5-HT<sub>1</sub> 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-HT<sub>1p</sub> and 5-HT<sub>3</sub> 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-HT<sub>5B</sub> is

**Table 2 Assessed couplings between G-proteins and serotonin receptors**

Coupling G subunits	Not coupling G subunits	Notes	Second messengers	References
<b>5-HT1A receptor</b>				
Gas-?-?		In guinea pig hippocampus	-	(Shenker, 1987) [28]
Gai1-?-?				
Gai2-?-?	No(Gas-?-?)	Human receptor and bovine G-proteins, in vitro reconstitution into E. coli membranes. Affinity order is Gai3 > Gai1 > Gai2 > Gao.	-	(Bertin, 1992) [29]
Gai3-?-?				
Gao-?-?				
Gai1-?-?				
Gai2-?-?	No(Gas-?-?)	Human receptor transfected in human HeLa and hamster CHO cells; affinity order is Gai3 > Gai2 > Gai1	cAMP	(Raymond, 1993) [30]
Gai3-?-?				
Gai2-?-?		Human receptor transfected in hamster CHO cells	cAMP	(Gettys, 1994) [31]
Gai3-?-?				
	No(Gas-?-?)			
Gao-?-?	No(Gai1-?-?) No(Gai2-?-?) No(Gai3-?-?)	Human receptor transfected in insect Sf9 cells	-	(Mulheron, 1994) [17]
Gai1-Gβ1-Gγ1				
Gai1-Gβ1-Gγ2				
Gai1-Gβ1-Gγ3				
Gai1-Gβ1-Gγ5				
Gai1-Gβ1-Gγ7	No(Gas-Gβ1-Gγ2)	Human receptor, rat Gas, Gai1, Gai2, Gai3, Gao, murine Gaq, human Gaz, bovine Gβγ transfected in insect Sf9 cells. Gγ1 less effective than Gγ2, Gγ3, Gγ5, Gγ7.	-	(Butkerait, 1995) [7]
Gai2-Gβ1-Gγ2	No(Gaq-Gβ1-Gγ2)			
Gai3-Gβ1-Gγ2				
Gao-Gβ1-Gγ2				
Gaz-Gβ1-Gγ2				
Gaq-Gβ1-Gγ2	No(Ga12-Gβ1-Gγ2)			
Gaz-Gβ1-Gγ2	No(Ga13-Gβ1-Gγ2) No(Gas-Gβ1-Gγ2)	Human receptor, rat Gas, murine Gaq, human Gaz, bovine Gβγ transfected in insect Sf9 cells; weak coupling with Gaq	-	(Barr, 1997) [27]
Gai1-?-?				
Gai2-?-?				
Gai3-?-?		Human receptor and G-proteins transfected in hamster CHO cells, affinity order is Gai2 > Gai3, Gai1, Gao > Gaz	-	(Garnovskaya, 1997) [32]
Gao-?-?				
Gaz-?-?				

**Table 2 Assessed couplings between G-proteins and serotonin receptors (Continued)**

Gai1-?-?				
Gai2-?-?		Human receptor and rat Gas transfected in insect Sf9 cells	-	(Clawges, 1997) [14]
Gai3-?-?				
Gao-?-?				
Gaz-Gβ1-Gy2		Human receptor and G-proteins transfected in Sf9 cells	-	(Barr, 1997) [33]
Gai1-?-?		Rat receptor and G-proteins transfected in rat GH4C1 cells.	cAMP	(Liu, 1999) [34]
Gai2-?-?		Human receptor and G-proteins transfected in human HeLa cells, affinity order is Gai1 > Gai2 >> Gai3	-	(Lin, 2002) [35]
Gai3-?-?				
Gai2-?-?	No(Gai1-?-?) No(Gai3-?-?) No(Gao-?-?)	Rat receptor and G-proteins transfected in human HEK293 cells	cAMP	(Albert, 1999) [36]
Gaz-?-?		In rat hypothalamic paraventricular nucleus	-	(Serres, 2000) [37]
Gai/o-?-?		Human receptor transfected in human HEK293 cells.	cAMP	(Malmberg, 2000) [38]
Gas-?-?				
Gai3-?-?		Human receptor co-expressed with rat G-protein in monkey COS-7 cells.	-	(Dupuis, 2001) [39]
		Human receptor transfected in hamster CHO cells.	-	(Newman-Tancredi, 2002) [40]
Gai1-?-?	No(Gat-?-?)	Reconstitution in insect Sf9 cell expressing receptor and rat Gai1 and bovine Gat	-	(Slessareva, 2003) [41]
Gaq-Gβ1-Gy2		Recombinant human receptor, mouse Gaq, rat Gai2, bovine Gβy co-expressed in insect Sf9. Strong coupling with Gai2, weak with Gaq.	-	(Okada, 2004) [42]
Gai2-Gβ1-Gy2				
Gai/o-?-?	No(Gaq-?-?)	In situ reconstitution in insect Sf9 cells with purified human receptor, squid Gaq, bovine Gai and Gao	-	(Okada, 2004) [42]
Gao-?-?	No(Gai1-?-?)			
Gai3-?-?	No(Gai2-?-?) No(Gas-?-?) No(Gaz-?-?)	In rat cortex	-	(Mannoury la Cour, 2006) [43]
	No(Gao-?-?)			
Gai3-?-?	No(Gai1-?-?) No(Gai2-?-?) No(Gas-?-?) No(Gaz-?-?)	In rat anterior raphe area	-	(Mannoury la Cour, 2006) [43]
Gao-?-?	No(Gas-?-?)			
Gai1-?-?	No(Gaz-?-?)	In rat hippocampus	-	(Mannoury la Cour, 2006) [43]
Gai3-?-?	No(Gai2-?-?)			

**Table 2 Assessed couplings between G-proteins and serotonin receptors (Continued)**

Gao-?-?				
Gai1-?-?	No(Gas-?-?)	In rat hypothalamus	-	(Mannoury la Cour, 2006) [43]
Gai3-?-?	No(Gai2-?-?)			
Gaz-?-?				
Gao-?-?				
Gai3-?-?	No(Gai1-?-?)	In rat hippocampus	-	(Martel, 2007) [44]
Gas-?-?				
Gaq-?-?				
Gai2-?-?	No(Gas-?-?)			
Gai3-?-?	No(Gaz-?-?)	In rat dorsal raphe nucleus	cAMP	(Valdizán, 2010) [45]
Gao-?-?	No(Gai1-?-?)			
Gai2-?-?		Human receptor transfected in hamster CHO cells	cAMP	(Rauly-Lestienne, 2011) [46]
Gai3-?-?				
<b>5-HT1B receptor</b>				
Gai1-?-?				
Gai2-?-?		Human receptor and rat Ga transfected in insect Sf9 cells	-	(Clawges, 1997) [14]
Gai3-?-?				
Gao-?-?				
Gai1-?-?	No(Gat-?-?)	Reconstitution in insect Sf9 cell expressing receptor and rat Gai1 and bovine Gat	- - -	(Bae, 1997; Bae, 1999; Slessareva, 2003) [41,47,48]
Gai2-?-?		Human receptor and rat G-proteins transfected in human HEK293 cells	cAMP	(Albert, 1999) [36]
Gai-?-?	No(Gas-?-?) No(Gaq/11-?-?) No(Ga13-?-?) No(Gao-?-?)	Chimpanzee receptor transfected in human HEK293 cells	-	(Alberts, 2000) [49]
Gai1-Gβ1-Gγ2		Human receptor, rat Ga and bovine Gβγ proteins transfected in insect Sf9 cells	-	(Brys, 2000) [50]
Gai1-?-?				
Gai2-?-?		Human receptor and G-proteins transfected in human HeLa cells, affinity order is Gai1 > Gai2 >> Gai3	-	(Lin, 2002) [35]
Gai3-?-?				
Gai3-?-?		Human receptor transfected in hamster CHO cells	-	(Newman-Tancredi, 2003) [51]

**Table 2 Assessed couplings between G-proteins and serotonin receptors (Continued)**

<b>5-HT1D receptor</b>			
Gai3-?-?		Human receptor and rat Ga transfected in insect Sf9 cells	- (Clawges, 1997) [14]
Gai-?-?	No(Gas-?-?)	Chimpanzee receptor transfected in human HEK293 cells	-
	No(Gaq/11-?-?)		
	No(Ga13-?-?)		
	No(Gao-?-?)		
Gai1-Gβ1-Gγ2	No(Gaq-Gβ1-Gγ2)	Human receptor, rat Gai/o, mouse Gaq and bovine Gβγ proteins transfected in insect Sf9 cells	-
Gai2-Gβ1-Gγ2			
Gai3-Gβ1-Gγ2			
Gao-Gβ1-Gγ2			
Gai1-?-?		Human receptor and G-proteins transfected in human HeLa cells, affinity order is Gai1 > Gai2 >> Gai3	-
Gai2-?-?			
Gai3-?-?			
<b>5-HT1E receptor</b>			
	No(Gai3-?-?)	Human receptor and rat Ga transfected in insect Sf9 cells	- (Clawges, 1997) [14]
<b>5-HT1F receptor</b>			
Gai-?-?	No(Gas-?-?)	Monkey receptor transfected in human HEK293 cells	-
	No(Gaq/11-?-?)		
	No(Ga13-?-?)		
	No(Gao-?-?)		
<b>5-HT2A receptor</b>			
Gai/o-?-?		In mouse NIH3T3 cells	cPLA2 (not PLC, IP3) (Kurrasch-Orbaugh, 2003) [52]
Ga12/13-?-?		Rat receptor and G-protein transfected in human HEK293 cells.	Inositol Phosphate (Bhatnagar, 2004) [53]
Gaq-?-?		Human receptor and G-protein transfected in human HEK293 cells.	Inositol Phosphate (Millan, 2012) [54]
		In rat cerebral cortical membranes.	- (Odagaki, 2014) [55]
Gai1-?-?		Human G-proteins transfected in rat cortex A1A1v cells	Inositol Phosphate (Shi, 2007) [56]
Gaq-?-?			
Gai1-?-?		Human G-proteins transfected in rat cortex A1A1v cells	Inositol Phosphate (Shi, 2007) [57]
Gaq/11-?-?		Human receptor transfected in hamster CHO cells.	Ca <sup>2+</sup> (Cussac, 2008) [58]
		In rat cortex.	- (Mannoury La Cour, 2009) [59]

**Table 2 Assessed couplings between G-proteins and serotonin receptors (Continued)**

<b>5-HT2B receptor</b>				
Gaq/11-Gβ1-Gγ2		Mouse receptors stably expressed in mouse fibroblast cells	Ras	(Launay, 1996) [60]
Gα13-?-?	No(Gαs-?-?) No(Gaq/11-?-?) No(Gαi-?-?)	In mouse LM6 and 1C11 and in <i>M. natalensis</i> carcinoid tumor primary cultured cells	IP3, NOS, cGMP	(Manivet, 2000) [61]
Gaq/11-?-?		Human receptor transfected in hamster CHO cells	Ca <sup>2+</sup>	(Cussac, 2008) [58]
Gaq-?-?		Human receptor and G-protein transfected in human HEK293 cells	Inositol Phosphate	(Millan, 2012) [54]
<b>5-HT2C receptor</b>				
Gαi1-?-?		Mouse receptor and rat G-proteins expressed in <i>Xenopus</i> oocytes	-	(Chen, 1994) [62]
Gαo-?-?				
Gαo-?-?	No(Gαs-?-?)			
Gaq-?-?	No(Gαolf-?-?)	Mouse receptor and G-proteins expressed in <i>Xenopus</i> oocytes	Inositol Phosphate	(Quick, 1994) [63]
Gα11-?-?	No(Gαt-?-?)			
Gaq-?-?	No(Gαt-?-?) No(Gαi/o-?-?)	Rat receptor and squid Gaq, bovine Gat, Gai/o proteins transfected in insect Sf9 cells	-	(Hartman, 1996) [64]
	No(Gαs-?-?)			
Gαi-?-?	No(Gαo-?-?) No(Gaq/11-?-?) No(Gα13-?-?)	Human receptor in human HEK293 cells	IP3, cAMP	(Alberts, 1999) [65]
Gaq-?-?	No(Gαs-?-?)	In rat choroid plexus epithelial cells	Inositol Phosphate	(Chang, 2000) [66]
Gaq-?-?*	No(Gα11-?-?)*			
Gα11-?-?#	No(Gα12-?-?)*# No(Gα13-?-?)#	Human receptor and mouse G-proteins (except human Gα16) transfected in mouse NIH3T3 cells		
Gα13-?-?*	No(Gα14-?-?)*#	* with not edited receptor form	Inositol Phosphate	(Price, 2001) [67]
Gα15-?-?*	No(Gα15-?-?)# No(Gα16-?-?)*#	# with edited receptor form		
Gαi3-?-?		Human receptor transfected in hamster CHO cells	-	(Cussac, 2002) [68]
Gaq/11-?-?				
Gaq-?-?	No(Gαs-?-?)	In rat choroid plexus epithelial cells and rat receptor transfected in mouse NIH3T3 cells	Inositol Phosphate (via PLD, not PLC)	(McGrew, 2002) [69]
Gα13-?-?				
Gaq-?-?		Human receptor and squid G-protein reconstitution in insect Sf9 cells.	Ca <sup>2+</sup>	(Okada, 2004) [70]
		Human receptor and G-protein transfected in human HEK293 cells.	Inositol Phosphate	(Millan, 2012) [54]



**Table 2 Assessed couplings between G-proteins and serotonin receptors (Continued)**

Gaq-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]
Gai2-Gβ1-Gγ2				
Gaq-?-?	No(Gai/o-?-?)	In situ reconstitution in insect Sf9 cells with purified human receptor, squid Gaq, bovine Gai and Gao	-	(Okada, 2004) [42]
Gaq/11-?-?		Human receptor transfected in hamster CHO cells	Ca <sup>2+</sup>	(Cussac, 2008) [58]
<b>5-HT4 receptor</b>				
	No(Gai2-Gβ1-Gγ2)			
	No(Gai3-Gβ1-Gγ2)			
Gas-Gβ1-Gγ2	No(Ga12-Gβ1-Gγ2)	Murine receptor and G-proteins transfected in insect Sf9 cells	cAMP	(Ponimaskin, 2002) [71]
	No(Gaq-Gβ1-Gγ2)			
Gas-?-?		Human receptor transfected in human HEK293 cells; 5HT4a receptor coupled only to Gas, while 5HT4b isoform coupled to Gas and Gai/o	cAMP, Ca <sup>2+</sup>	(Pindon, 2002) [72]
Gai/o-?-?			(not IP3)	
Gas-Gβ1-Gγ2	No(Gai2-Gβ1-Gγ2)			
Ga13-Gβ1-Gγ2	No(Ga12-Gβ1-Gγ2)	Murine receptor and G-proteins transfected in insect Sf9 cells	Inositol Phosphate, RhoA	(Ponimaskin, 2002) [73]
	No(Gaq-Gβ1-Gγ2)			
Gas-?-?		Human receptor transfected in monkey COS-7 cells	cAMP, Inositol Phosphate	(Pellissier, 2011) [74]
<b>5-HT5A receptor</b>				
	No(Gas-Gβ1-Gγ2)			
Gai1-Gβ1-Gγ2	No(Gaz-Gβ1-Gγ2)			
Gai2-Gβ1-Gγ2	No(Gaq-Gβ1-Gγ2)			
Gai3-Gβ1-Gγ2	No(Ga11-Gβ1-Gγ2)	Human receptor, rat Gai/o, human Gaz and Ga16, bovine Gas, mouse Gaq, Ga11, Ga12 and Ga13, bovine Gβγ reconstituted in insect Sf9 cells	-	(Francken, 2000) [75]
Gao-Gβ1-Gγ2	No(Ga12-Gβ1-Gγ2)			
	No(Ga13-Gβ1-Gγ2)			
	No(Ga16-Gβ1-Gγ2)			
Gai1-Gβ1-Gγ2				
Gai2-Gβ1-Gγ2	No(Gas-Gβ1-Gγ2)	Human receptor, rat Gai/o, human Gaz, bovine Gas and Gβγ reconstituted in insect Sf9 cells	-	(Francken, 2001) [76]
Gai3-Gβ1-Gγ2	No(Gaz-Gβ1-Gγ2)			
Gao-Gβ1-Gγ2				
Gai/o-?-?		Human receptor transfected in rat C6 glioma cells	IP3, Ca <sup>2+</sup> , cAMP, cADPR	(Noda, 2003) [77]
<b>5-HT6 receptor</b>				
		In human HEK293 cells stably expressing human receptor.	cAMP	(Baker, 1998) [78]
Gas-?-?		Human receptor and G-protein, assessed in vitro.	cAMP	(Kang, 2005) [79]

**Table 2 Assessed couplings between G-proteins and serotonin receptors (Continued)**

5-HT7 receptor				
Gas-?-?	No(Gai-?-?)	In human HEK293 cells stably expressing human receptor	cAMP, Ca <sup>2+</sup> /calmodulin	(Baker, 1998) [78]
Gas-?-?	No(Gaq/11-?-?) No(Gai-?-?)	Human receptor transfected in murine LM cells	cAMP	(Adham, 1998) [80]
Gas-?-? Gai-?-?	No(Gao-?-?) No(Ga13-?-?)	Human receptor transfected in human HEK293 cells, affinity order is Gas > Gai = Gaq/11	cAMP	(Alberts, 2001) [81]
Gaq/11-?-?				
Gas-Gβ1-Gγ2 Ga12-Gβ1-Gγ2	No(Gai2-Gβ1-Gγ2) No(Ga13-Gβ1-Gγ2) No(Gaq-Gβ1-Gγ2)	Murine receptor and G-proteins transfected in insect Sf9 cells	RhoA, Cdc42 (NOT Rac1)	(Kvachnina, 2005) [82]
Gas-Gβ1-Gγ2 Ga12-Gβ1-Gγ2	No(Gaq-Gβ1-Gγ2) No(Gai-Gβ1-Gγ2) No(Ga13-Gβ1-Gγ2)	Murine receptor and G-proteins transfected in insect Sf9 cells	cAMP	(Kvachnina, 2009) [83]

"?" means that the particular Gβ or Gγ protein has not been identified in the reference paper. "-" means that the second messenger has not been assessed in the reference paper. Note: unfortunately some papers reported Gai/o, Gai, or Gaq/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 [<sup>35</sup>S]GTPγS binding assay [16].

However, to extend these findings to humans is not necessarily correct. For example, the poor coupling of Gαq to 5-HT1A and 5-HT2C could be due to a large portion of the expressed but inactive Gαq [84], maybe because Gαq 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 Gα family proteins without specifying which Gβ and Gγ were coupled. However, the few data on Gβ and Gγ only concerned Gβ1 with Gγ2. Gα 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 Gγ 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 Gα 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 these 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-<sup>32</sup>P] 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 Gαq/11-coupled receptors, [<sup>3</sup>H]IP3 concentration as product of PLC activity can be measured using [<sup>3</sup>H]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 Ca<sup>2+</sup> concentration, by dyes generating fluorescence upon binding of free Ca<sup>2+</sup>, can be determined to assess Gαq/11 activation, although these probes can influence calcium levels and kinetics. Moreover, Ca<sup>2+</sup>-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 Gαq/11, there are crosstalk problems that can be partially overcome by small interfering RNA (siRNA) knockdown.

To directly and quantitatively assess the Gα protein activation, [<sup>35</sup>S]GTPγS binding assay is employed. Upon Gα subunit activation, it binds the mimic substrate, so remaining blocked in the active form as it cannot hydrolyze this substrate. The blocked Gα can be measured after isolation and it can be immunoprecipitated to identify the specific Gα 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 Gα-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  $G\beta 1\gamma 2$  were present, so it was not possible to verify if and how different  $G\beta\gamma$  combinations can affect  $G\alpha$  induced pathways. In general, the physiological significance of the different  $G\beta\gamma$  pairs is unclear, since they participate in complex interactions with receptors,  $G\alpha$  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-HT<sub>2A</sub> 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  $G\alpha$ ,  $G\beta$ ,  $G\gamma$  and the serotonin receptor by using three proteomic databases according to a previous work [90]: Human Protein Reference Database ([www.hprd.org](http://www.hprd.org)); Human Proteinpedia ([www.humanproteinpedia.org](http://www.humanproteinpedia.org)) and Human Protein Atlas ([www.proteinatlas.org](http://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](http://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 subtissues.**

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: Phosphatidyl inositol 4,5-bisphosphate; IP1: Inositol-1-phosphate; RhoGEF: Rho guanine 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, W 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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