Signaling Via G-Protein-Coupled Receptors

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Abstract

This article first reviews the basic functions of G proteins and G protein-coupled receptors (GPCRs), followed by the signaling cascades induced by major neurotransmitters, including norepinephrine, acetylcholine, glutamate, dopamine and serotonin (5-HT). The exact signaling pathways depend on GPCR subtypes. This article also discusses the G protein-gated inwardly rectifying potassium (GIRK) channels and explain how alcohol reduces conscious level.

Introduction

Neurotransmitters may act on two distinct classes of receptors to transmit signals: ligand-gated ion channels and G-protein coupled receptors. The latter is discussed in this article, with focus on norepinephrine, acetylcholine, glutamate, dopamine and serotonin (5-HT). For ion channels, the binding of neurotransmitters (ligands) results in the opening of channels, allowing ions to pass through. In this case, signals are transmitted via ions, particularly the calcium ions that control all kinds of enzymes. For G-protein coupled receptors, signals are transmitted via G proteins.

G Proteins

G-protein coupled receptors do not possess a pore for ion passage. They mediate cell signaling via the G protein, which consists of three subunits: α, β and γ. Before activation, these subunits are linked together. G_β and G_γ are tightly bound, whereas G_α may dissociate from $G_{\beta\gamma}$, depending on whether it is bound by GDP (guanosine diphosphate) or GTP (guanosine triphosphate). In the resting state, G_α is bound by GDP, facilitating the assembly of three subunits. After activation, the GDP bound to G_α will be replaced by GTP, promoting dissociation of G_α from $G_{\beta\gamma}$. The separated G_α and $G_{\beta\gamma}$ can then act on specific targets, known as effectors. The GTP on G_α cannot last long, because G_α has the enzymatic activity to hydrolyze it into GDP, thereby returning to the resting state (Figure 1).

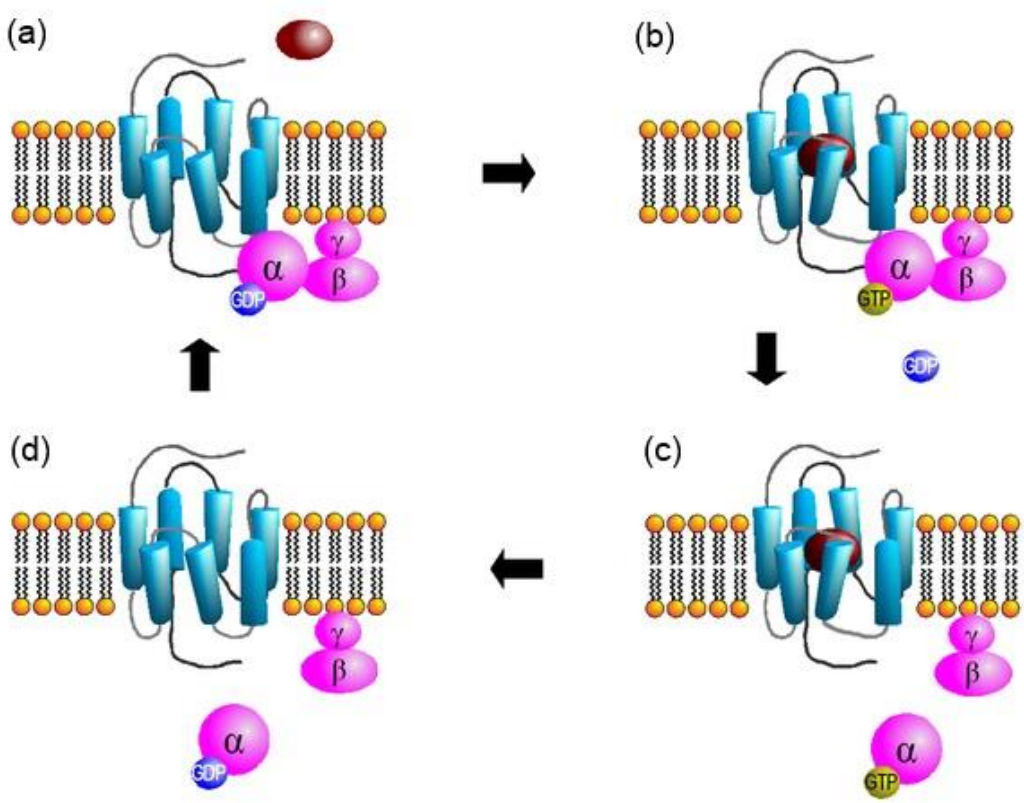

Figure 1. The G protein cycle. (a) In the resting state, G_α is bound by GDP, facilitating the assembly of three subunits. (b) The G-protein coupled receptor is activated by its agonist, resulting in the substitution of G_α-bound GDP by GTP. (c) G_α dissociates from $G_{\beta\gamma}$. (d) GTP is hydrolyzed by G_α to GDP, returning to the resting state. [Source: Wikipedia]

G_α has several isoforms, including $G_{\alpha s}$ (stimulatory), $G_{\alpha i}$ (inhibitory), $G_{\alpha o}$ (other) and $G_{\alpha q}$. They play distinct roles in cell signaling by acting on different effectors.

G Protein-coupled Receptors

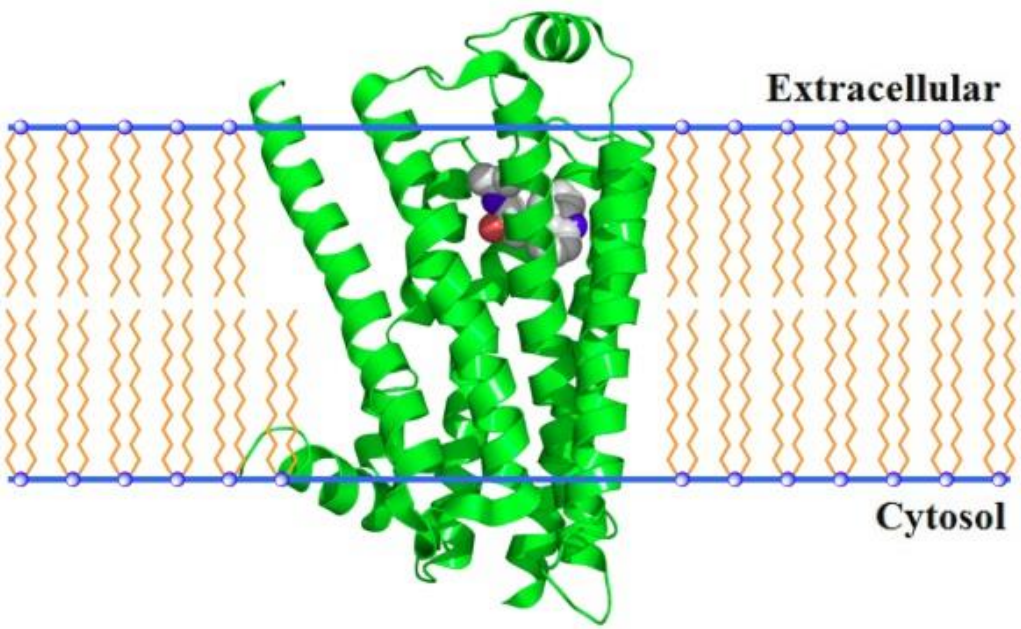

Figure 2. A G protein-coupled receptor is characterized by seven transmembrane α helices. [Source: Wikipedia]

A G protein-coupled receptor (GPCR) is characterized by seven transmembrane α helices (Figure 2). It does not form an ion-conducting pore, but may act on ion channels via separated G_α or

$G_{\beta\gamma}$. In addition to ion channels, the G-protein can also act on enzymes. For instance, $G_{\alpha s}$ stimulates the production of cyclic AMP (cAMP) from ATP by directly activating the enzyme adenylate cyclase (also known as adenylyl cyclase). $G_{\alpha i}$ inhibits the production of cAMP by inactivating adenylate cyclase.

Another important signaling pathway is mediated by $G_{\alpha q}$ which can activate phospholipase C (PLC) to cleave a phospholipid, phosphatidylinositol 4,5-bisphosphate (PIP2), into diacyl glycerol (DAG) and inositol 1,4,5-trisphosphate (IP_3) (Figure 3). DAG may activate protein kinase C while IP_3 can induce the release of Ca^{2+} ions from the intracellular store (Figure 4). The GPCRs coupled to $G_{\alpha q}$ include group I metabotropic glutamate receptors (mGluR1 and mGluR5), dopamine's D1 receptor, muscarinic acetylcholine receptors (M1, M3 and M5) and α1-adrenergic receptor.

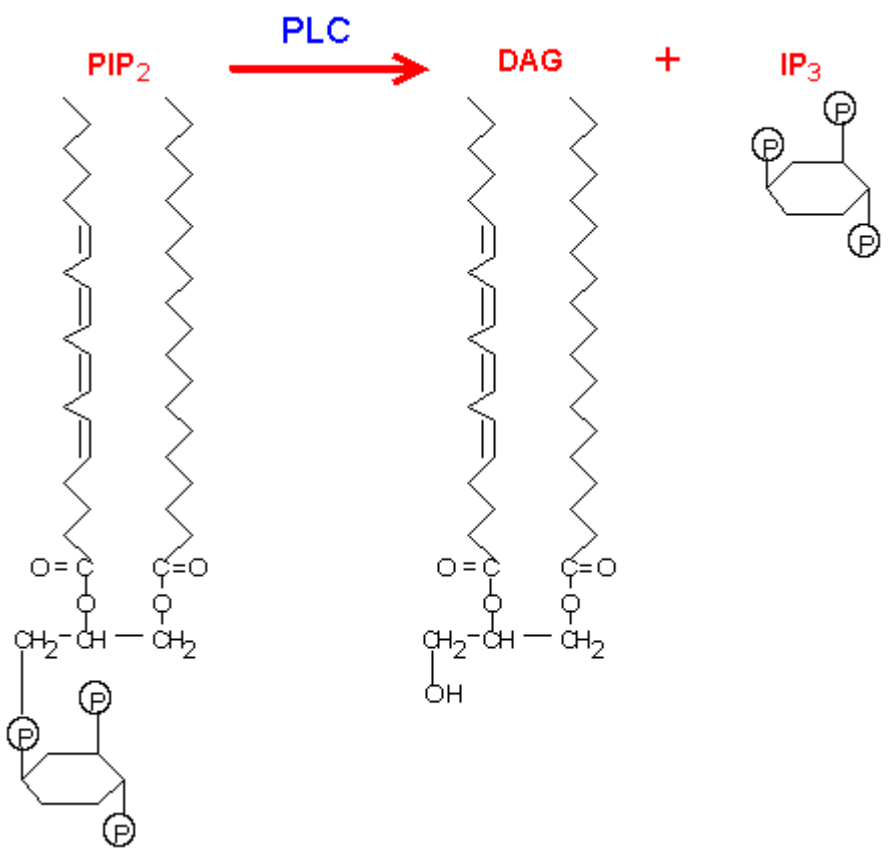

Figure 3. PLC catalyzes the conversion of PIP_2 into DAG and IP_3.

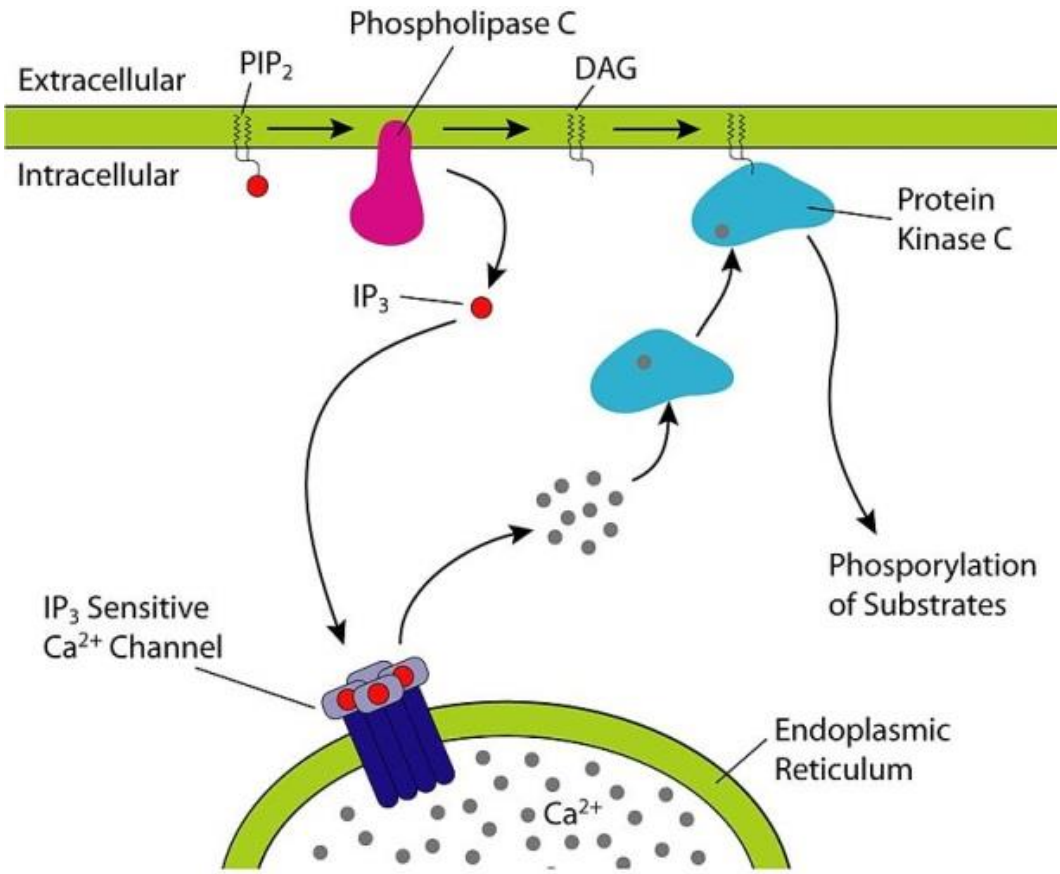

Figure 4. Signaling cascades induced by the activation of PLC. DAG may activate protein kinase C while IP_3 triggers the release of Ca^{2+} ions from the intracellular store (endoplasmic reticulum). [Source: Wikipedia]

Norepinephrine-induced signaling

Norepinephrine (NE), also called noradrenaline, is a neurotransmitter produced mainly in the locus coeruleus (a small brain region within the brainstem). In adult humans, the locus coeruleus contains less than 50,000 noradrenergic neurons that release primarily NE. However, they project to widespread brain areas, including hippocampus, cerebral cortex, basolateral amygdala and striatum (Hansen, 2017; Ferrucci et al., 2013), where NE may bind to specific receptors, triggering a series of signaling cascades. The NE receptors are often referred to as "adrenergic receptors" (ARs).

ARs have two main groups: α and β. α is divided into two types: α_1 and α_2. β is divided into three types: β_1, β_2 and β_3. They are coupled to different G proteins:

- $G_{\alpha s}$: β_1 and β_3
- $G_{\alpha i}$: α_2 and β_2
- $G_{\alpha q}$: α_1

$G_{\alpha q}$ mediates the release of Ca^{2+} ions from the intracellular store, as described above. The signaling cascades mediated by $G_{\alpha s}$ and $G_{\alpha i}$ are illustrated in the following figure.

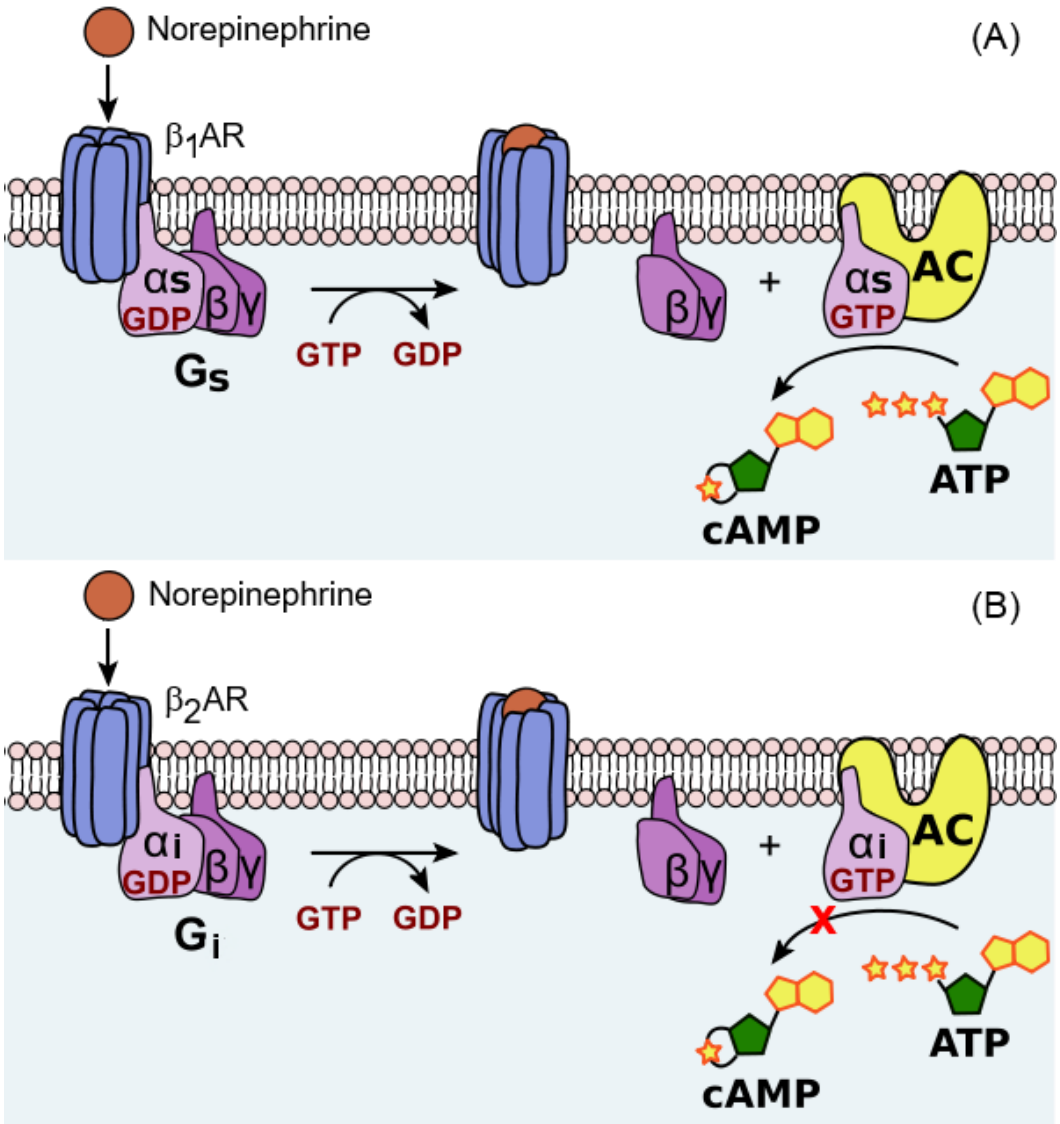

Figure 5. Norepinephrine induced signaling.
(A) Binding to β_1AR induces dissociation of $G_{\alpha s}$ from $G_{\beta\gamma}$. $G_{\alpha s}$ may activate adenylyl cyclase (AC) to increase cAMP levels.
(B) Binding to β_2AR induces dissociation of $G_{\alpha i}$ from $G_{\beta\gamma}$. $G_{\alpha i}$ may inactivate AC to reduce cAMP levels.
[Adapted from: Wikipedia]

Acetylcholine-induced signaling

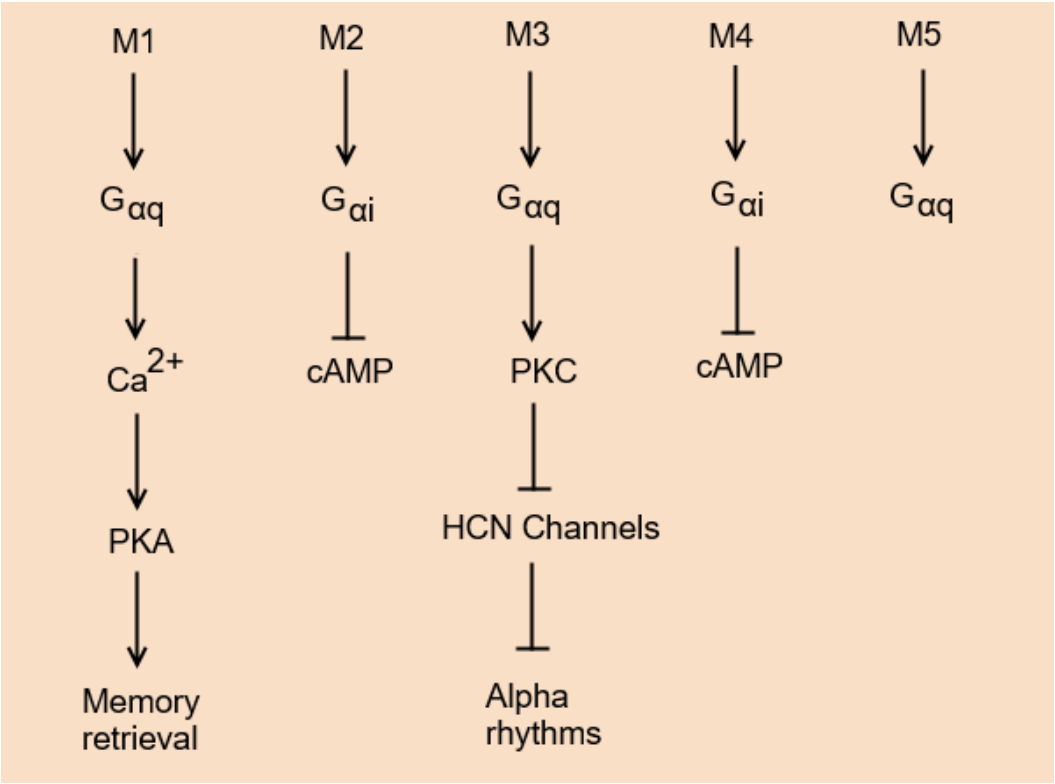

Figure 6. Acetylcholine-induced signaling. See text for detail.

Acetylcholine (ACh) has five muscarinic receptors, M1 - M5, which belong to G protein-coupled receptors. M1, M3 and M5 are coupled to $G_{\alpha q}$ while M2 and M4 are coupled to $G_{\alpha i}$. Activation of $G_{\alpha i}$ inactivates adenylyl cyclase (AC), reducing cAMP levels, thereby suppressing the activity of protein kinase A (PKA).

$G_{\alpha q}$ can activate PLC to cleave PIP2 into DAG and IP_3, thereby activating protein kinase C (PKC) and inducing the release of Ca^{2+} ions from the intracellular store. PKC has been shown to inhibit hyperpolarization-activated cyclic nucleotide-gated (HCN) channels which underlie the h current (Ih) (Williams et al., 2015). The HCN channels play key roles in regulating alpha rhythms. Consistent with these findings, ACh has been demonstrated to

induce alpha rhythms in sensory thalamic nuclei by acting on M3 receptors (Lörincz et al., 2008).

M1 receptors are highly expressed in the granule cells of dentate gyrus and the pyramidal cells of CA3 and CA1. Importantly, they are distributed preferentially on the extrasynaptic membrane of pyramidal cell dendrites and spines (Yamasaki et al., 2010), suggesting that their major targets are not located at synapses. Rather, ACh could regulate extrasynaptic NMDA receptors by acting on M1 receptors to facilitate memory retrieval.

Glutamate-induced signaling

Glutamate has eight types of metabotropic receptors that belong to G protein-coupled receptors. They are classified into three groups:

- Group I: mGluR1 and mGluR5
- Group II: mGluR2 and mGluR3
- Group III: mGluR4, 6, 7 and 8

Group I mGluRs are coupled to $G_{\alpha q}$ whereas Group II and III are coupled to $G_{\alpha i}$ and $G_{\alpha o}$. As described above, $G_{\alpha q}$ can activate PLC to cleave PIP2, producing DAG and IP_3. Subsequently, DAG may activate protein kinase C (PKC) while IP_3 induces the release of Ca^{2+} ions from the intracellular store. Coupling with $G_{\alpha i/o}$ leads to inhibition of adenylyl cyclase and other signaling pathways (Niswender and Conn, 2010).

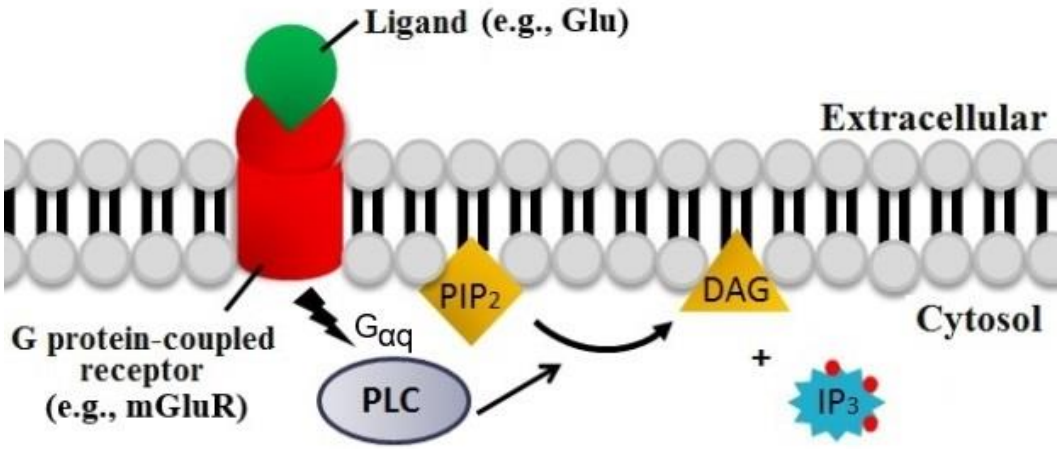

Figure 7. The binding between glutamate (Glu) and group I mGluR (mGluR1 and mGluR5) can activate PLC to cleave PIP2 into DAG and IP_3. [Source: Wikipedia]

mGluR5 plays important roles in gene transcription by regulating the transcription factors, cyclic AMP responsive element binding protein (CREB) and nuclear factor κB (NF-κB). CREB can be activated by protein kinase A (PKA) and CaMKIV, both are under the regulation of Ca^{2+} ions induced by IP_3 (Wang and Zhuo, 2012). NF-κB is regulated by PKC subtypes β and δ through PKC-associated kinase (PKK) (Muto et al., 2002; Kim et al., 2014).

Like acetylcholine M3 receptors, the Group I mGluRs can also regulate alpha rhythms (Hughes et al., 2004; Ahnaou et al., 2015), possibly through the $G_{αq}$/PKC/HCN pathway (Figure 8).

mGluR5 is critical for the toxicity exerted by beta amyloid oligomers (AβOs). The signaling cascade involves Ca^{2+}-stimulated coactivation of Fyn and Pyk2, which in turn activate GSK-3β and RhoA kinase, leading to hyperphosphorylation of CRMP2 and consequently synapse loss (see this article).

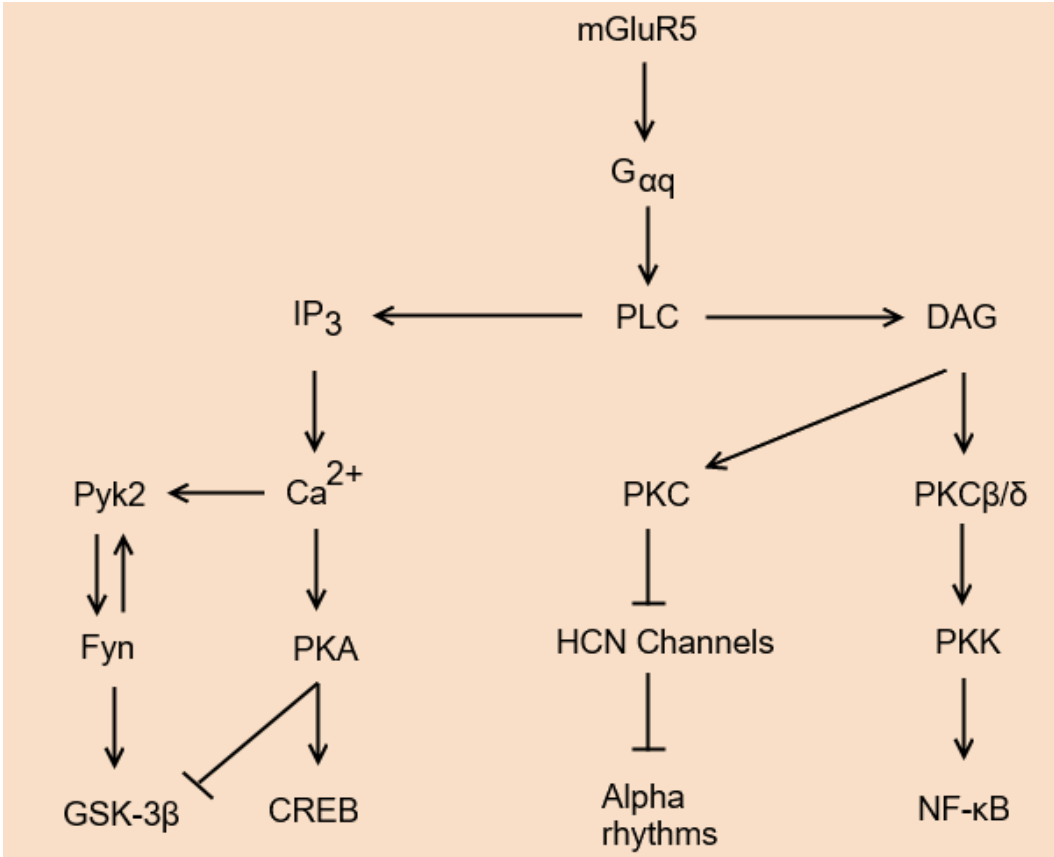

Figure 8. The mGluR5-mediated signaling pathways.

Dopamine-induced signaling

Dopamine has five known receptor subtypes, designated as D1 - D5. D1 and D5 receptors are alike, while D2, D3 and D4 receptors have similar properties. Their signaling pathways are summarized in Figure 9.

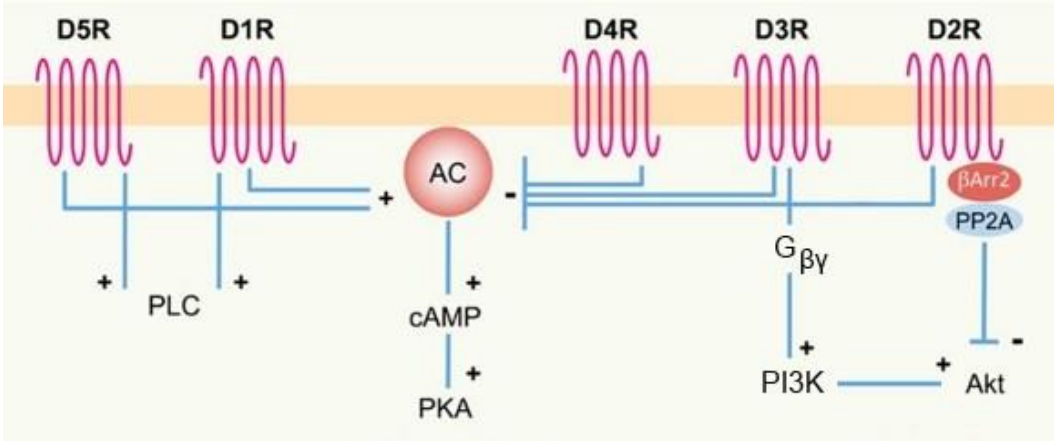

Figure 9. Dopamine-induced signaling pathways. [Adapted from Bozzi and Borrelli, 2013]

G-protein-Dependent Pathways

D1-like receptors are coupled to $G_{\alpha s}$ and $G_{\alpha q}$. D2-like receptors are coupled to $G_{\alpha i}$. Their downstream signaling has been described in previous sections. These pathways are sometimes referred to as "canonical pathways", which apply to a variety of neurotransmitters. In addition to canonical pathways, the D3 receptor (D3R) can also activate the PI3K/Akt pathway through $G_{\beta\gamma}$ (Collo et al., 2014).

G-protein-Independent Pathways

The D2 receptor (D2R) also mediates a non-canonical pathway via coupling with β-arrestin 2 (βArr2) (Bozzi and Borrelli, 2013). In this pathway, D2R stimulates the formation of a complex containing βArr2, protein phosphatase 2A (PP2A) and Akt such that PP2A can dephosphorylate and inactivate Akt (Beaulieu et al., 2005). Akt may activate mTOR which is a risk factor for various diseases, including neurodegeneration, diabetes and cancer (see this article).

D1R plays an important role in attention by regulating alpha rhythms (Granon et al., 2000; Misener et al., 2004; Kempadoo et al., 2016).

Serotonin (5-HT)-induced signaling

There are at least 7 families of 5-HT receptors. Except for 5-HT3, all others belong to G-protein-coupled receptors (Rojas and Fiedler, 2016). This section will focus on 5-HT1 and 5-HT2 receptors, which are the most studied. 5-HT1 has five subtypes: A, B, D, E and F while 5-HT2 has three subtypes: A, B and C. Note that there is no 5-HT1C receptor because the originally nemed 5-HT1C was found to have more in common with the 5-HT2 family and thus redesignated as the 5-HT2C receptor (Wikipedia).

5-HT_{1A}R-mediated signaling

In neurons, activation of the 5-HT1A receptor (5-HT_{1A}R) stimulates $G_{\beta\gamma}$ signaling which promotes the activity of adenylyl cyclase types 2 (AC2) that in turn augments cAMP level and protein kinase A (PKA) activity (Figure 10). In non-neuronal cells, activation of 5-HT_{1A}Rs produces the opposite effect, namely, reduction of PKA activity, by stimulating the $G_{\alpha i/o}$ signaling (Rojas and Fiedler, 2016).

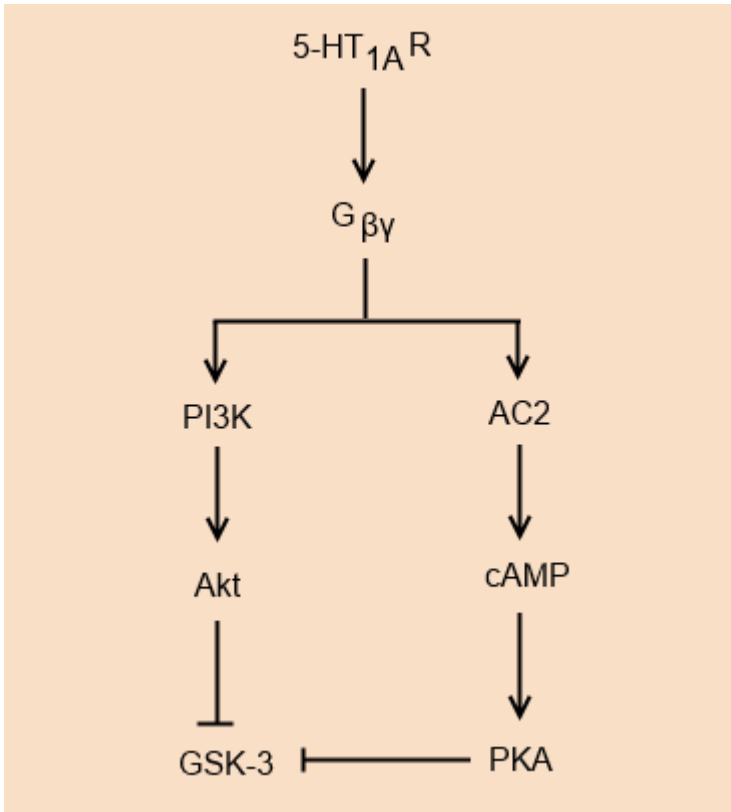

Figure 10. 5-HT_{1A}R-mediated signaling pathways in neurons.

$G_{\beta\gamma}$ also participates in the activation of the phosphoinositide-3-kinase (PI3K)-Akt pathway. Akt plays an important role in regulating the activity of glycogen synthase kinase 3 (GSK-3). Phosphorylation of GSK-3α and GSK-3β on Ser-21 and Ser-9, respectively by Akt may become inactive. GSK-3 is also under the regulation of PKA via direct and indirect pathways. In the direct pathway, PKA physically associates with and phosphorylates both forms of GSK-3, α and β on Ser-21 and Ser-9, respectively (Fang et al., 2000). In the indirect pathway, PKA may inhibit Src (a non-receptor tyrosine kinase) via phosphorylation of C-terminal Src kinase (Csk) which can inactivate Src (Trepanier et al., 2013). Importantly, Src can phosphorylate GSK-3 at Y216, leading to its activation even when Akt is active (Goc et al., 2014). Therefore, Src plays a

dominant role in GSK-3 activation. Inhibition of PKA would augment both Src and GSK-3 activities.

5-HT$_{2A}$R-mediated signaling

5-HT2A receptors (5-HT$_{2A}$Rs) play crucial roles in schizophrenia which is a mental disorder characterized by hallucination, avolition and cognitive dysfunction. They are the target of atypical antipsychotics (Beaulieu, 2012) and a class of hallucinogenic drugs called psychedelics (Nichols, 2016). Activation of 5-HT$_{2A}$R may trigger both canonical and non-canonical pathways.

In the canonical pathway, G_q stimulates phospholipase C (PLC) to cleave PIP2 into DAG and IP_3. DAG may activate protein kinase C (PKC) while IP_3 can induce the release of Ca^{2+} ions from the intracellular store. The elevated Ca^{2+} concentration in the cytosol may further activate Ca^{2+}-dependent adenylyl cyclase (AC), types 1 and 8 (Wong et al., 1999; Wang et al., 2003), to increase the production of cAMP, thereby enhancing the activity of PKA. PKC may stimulate the expression of brain-derived neurotrophic factor (BDNF) via the ERK-CREB pathway (Ferraguti et al., 1999; Wang et al., 2012).

The non-canonical pathway is mediated by β-arrestin which may recruit protein phosphatase 2A (PP2A) to dephosphorylate and inactivate Akt, thereby enhancing GSK-3 activity (Polter and Li, 2011). IP_3 activation increases intracellular Ca^{2+} level which can stimulate synergistic coactivation of Src and Pyk2 (Heidinger et al., 2002; Brody and Strittmatter, 2018), which also augment GSK-3 activity (Hartigan et al., 2001). However, Ca^{2+} may attenuate GSK-3 activity via the AC/cAMP/PKA pathway (Figure 11).

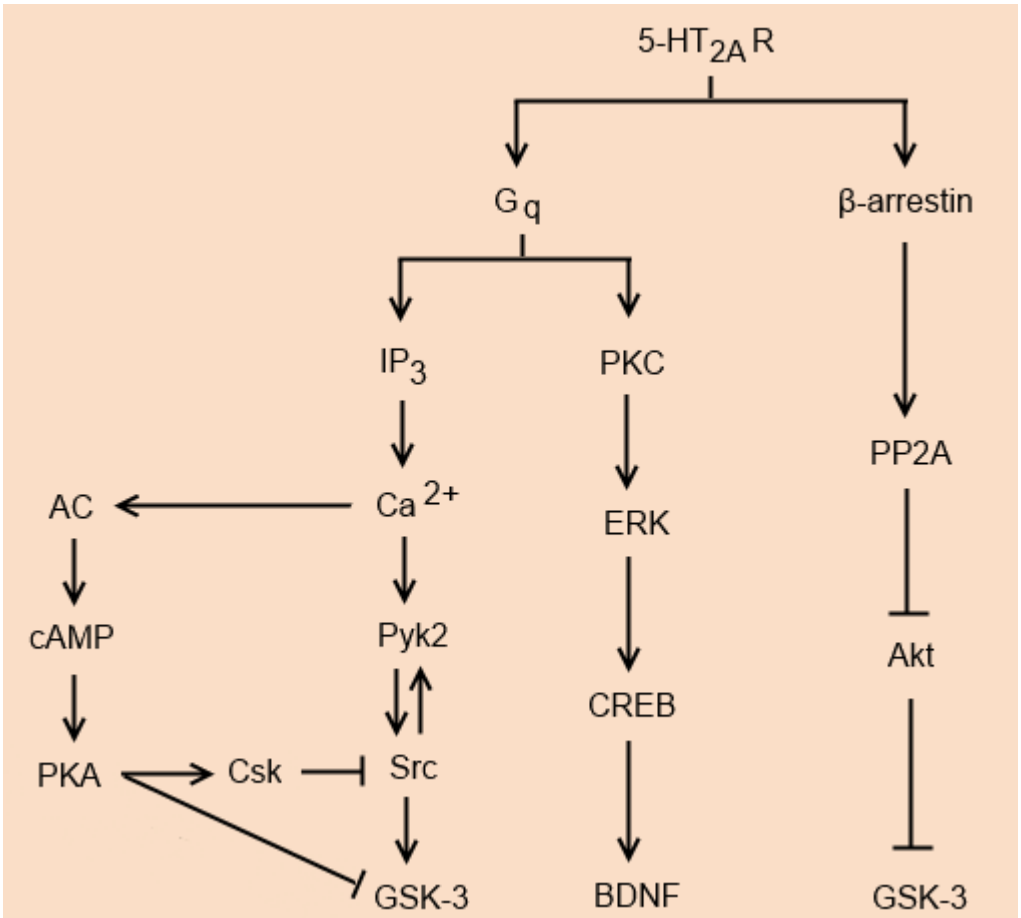

Figure 11. 5-HT_{2A}R-mediated signaling pathways. Note that 5-HT_{2A}R activation may augment GSK-3 activity via the β-arrestin and Ca^{2+}/Pyk2/Src pathway, but it can also attenuate GSK-3 activity by activating Ca^{2+}-dependent adenylyl cyclase (AC) which enhances PKA activity. Under normal physiological conditions, the GSK-3 activity could be maintained at an appropriate level. Hallucinogenic drugs have the capacity to recruit mGluR2 to induce the $G_{i/o}$ pathway (not shown), thereby reducing PKA activity and making GSK-3 hyperactive.

Coupling with GIRK Channels

A neurotransmitter receptor, whether it is a ligand-gated ion channel or G protein-coupled receptor (GPCR), can be activated by only one type of neurotransmitters. For instance, the $GABA_A$ or $GABA_B$ receptor can be activated only by GABA, while the NMDA or mGluR5 receptor responds only to glutamate. Although they could be activated by other agonists which, however, are not endogenous neurotransmitters. In contrast, a variety of neurotransmitters (adenosine, GABA, serotonin, etc.) can act on their specific GPCRs to open the G protein-gated inwardly rectifying potassium (GIRK) channels which play important roles in conscious perception as well as other physiological functions (Mayfield et al., 2015).

Basic Properties of GIRK Channels

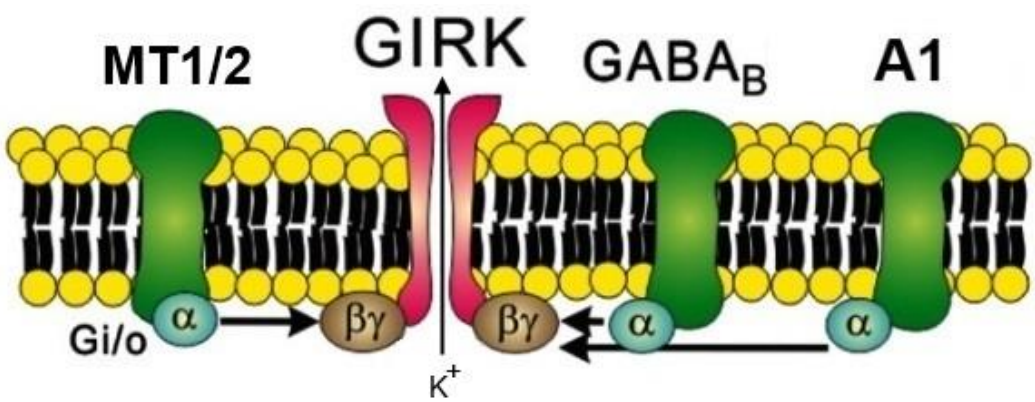

Figure 12. The GPCRs coupled with GIRK channels. Activation of GPCR can lead to the opening of GIRK channels by $G_{\beta\gamma}$. This figure shows only the adenosine's A1 receptor, melatonin's MT1 or MT2 receptor, and GABA's $GABA_B$ receptor. [Adapted from Sohn, 2013]

The G protein coupled with GIRK channels comprises $G_{\alpha i}$ (or $G_{\alpha o}$) and $G_{\beta\gamma}$. Upon activation, the $G_{\alpha i}$ subunit dissociates from $G_{\beta\gamma}$.

GIRK channels do not interact directly with G_{α}. Instead, they are activated by $G_{\beta\gamma}$ (Luscher and Slesinger, 2010). A few neurotransmitter receptors have been demonstrated to couple with GIRK channels, including adenosine's A1 receptor, melatonin's MT1 or MT2 receptor, and GABA's $GABA_B$ receptor (Figure 12). Activation of GIRK channels causes K^+ ions to flow outward, resulting in membrane hyperpolarization. Hence, the opening of GIRK channels has inhibitory effects on neuronal firing. This property can be used to suppress faster oscillations (e.g., alpha and theta rhythms) within the infra-slow oscillation (ISO).

Suppression of Alpha Oscillations Within ISO

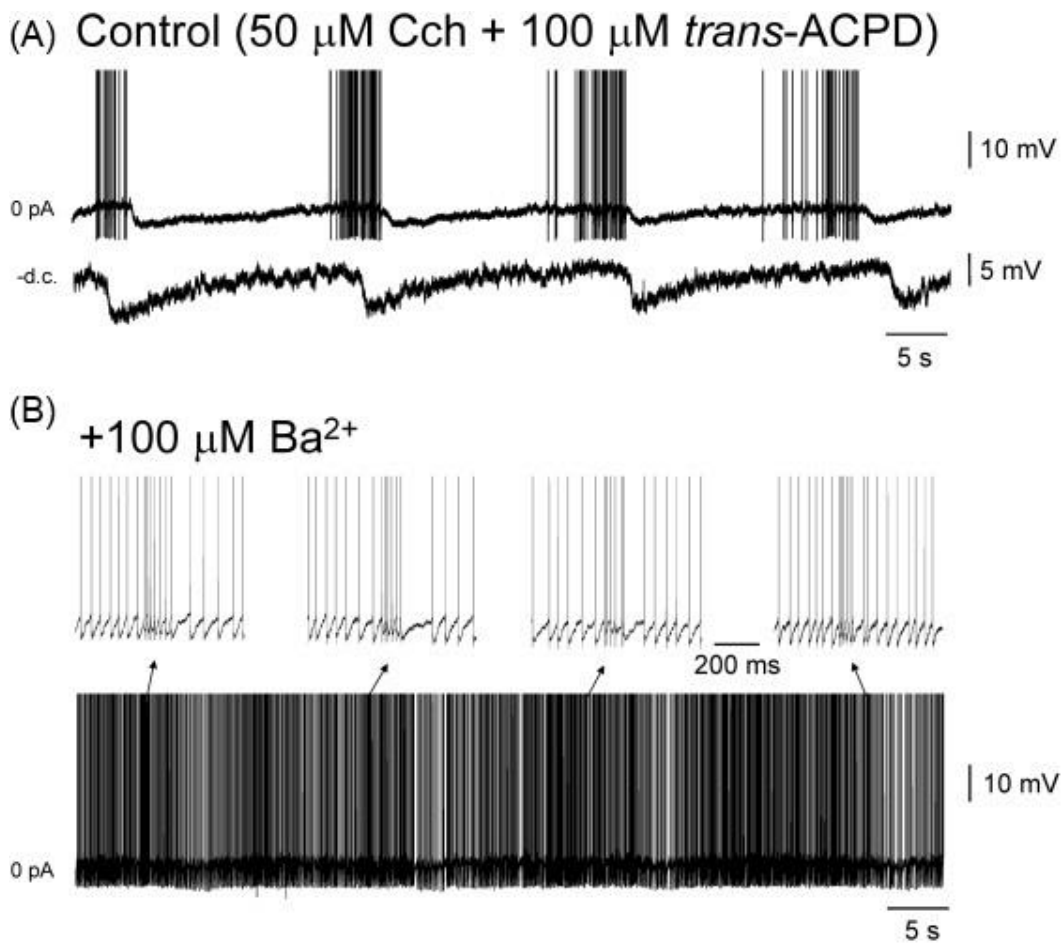

Figure 13. The GIRK channel blocker, Ba^{2+}, prevents suppression of alpha oscillations. (A) Under control conditions, ISO contains alpha oscillations and a long silence period. (B) The addition of Ba^{2+} eliminates the silence period. [Source: Lörincz et al., 2009]

The infra-slow oscillation (ISO) typically contains alpha oscillations and a long silence period. Experiments have demonstrated that this silence period is caused by GIRK channels which can reduce neuronal excitability (Figure 13).

How Alcohol Reduces Conscious Level

Alpha oscillations play a critical role in consciousness (more info). Since activation of GIRK channels can suppress the alpha oscillations nested within ISO, higher GIRK channel activity should result in lower conscious level. This simple mechanism can explain how alcohol reduces conscious level.

A GIRK channel is formed by four subunits (Figure 14). Four different GIRK channel subunits have been identified: GIRK1-GIRK4. A GIRK channel may contain four identical GIRK2, or mixed GIRK1/2, GIRK1/3, GIRK1/4, or GIRK2/3. Binding with PIP_2 is required to stabilize the GIRK channel in its open state. $G_{\beta\gamma}$ can enhance their binding, thus activating the GIRK channel. Similarly, alcohol can also interact directly with the GIRK channel to enhance its binding with PIP_2 (Bodhinathan and Slesinger, 2014). thereby increasing the activity of GIRK channels.

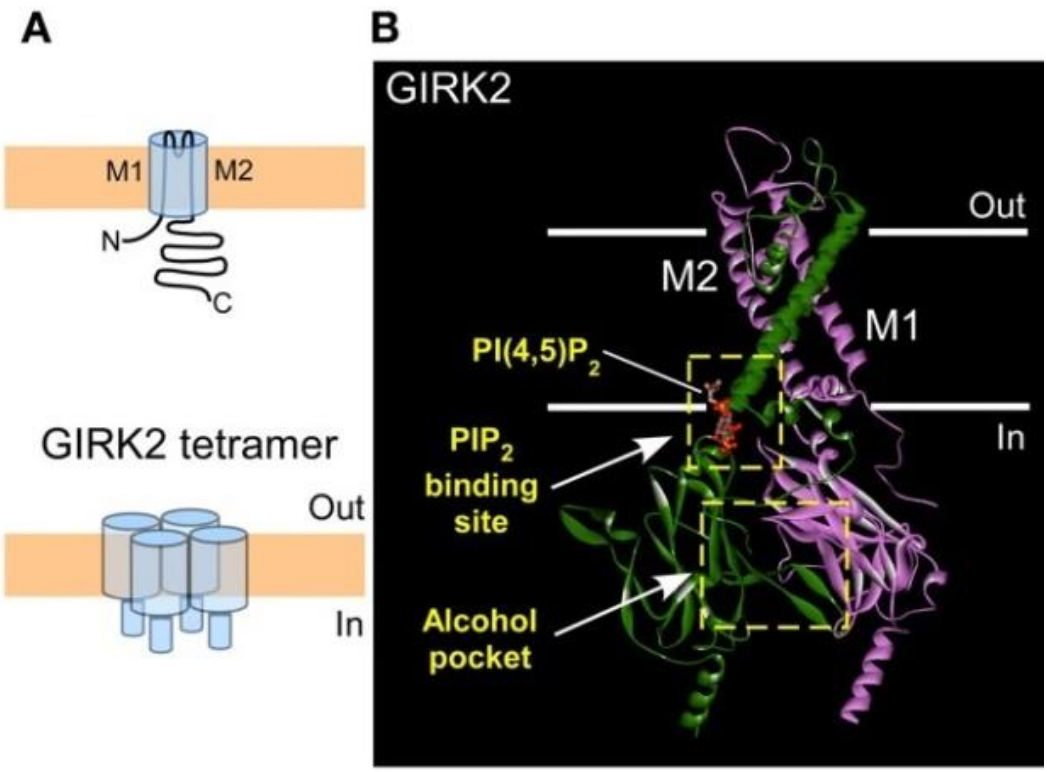

Figure 14. The structure of a GIRK channel.
[Source: Bodhinathan and Slesinger, 2014]