Physiology of GPCRs in the nervous system and the contribution of orphan GPCRs

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Abstract

G protein-coupled receptors (GPCRs) are a large family of cell surface receptors that play a critical role in nervous system function by transmitting signals between cells and their environment. They are involved in many, if not all, nervous system processes, and their dysfunction has been linked to various neurological disorders representing important drug targets. In this review, we will first discuss the role of the nervous system GPCRs in the modulation of tripartite synapse function and how GPCRs control energy metabolism in the brain. We will then discuss the (patho)physiology and pharmacology of opioid, cannabinoid, acetylcholine, chemokine, and melatonin GPCRs in the nervous system. Furthermore, we will briefly report on adhesion GPCR function in nervous tissues. Finally, we will address orphan GPCRs, their implication in the nervous system function and disease, and the challenges that need to be addressed in the future to deorphanize them.

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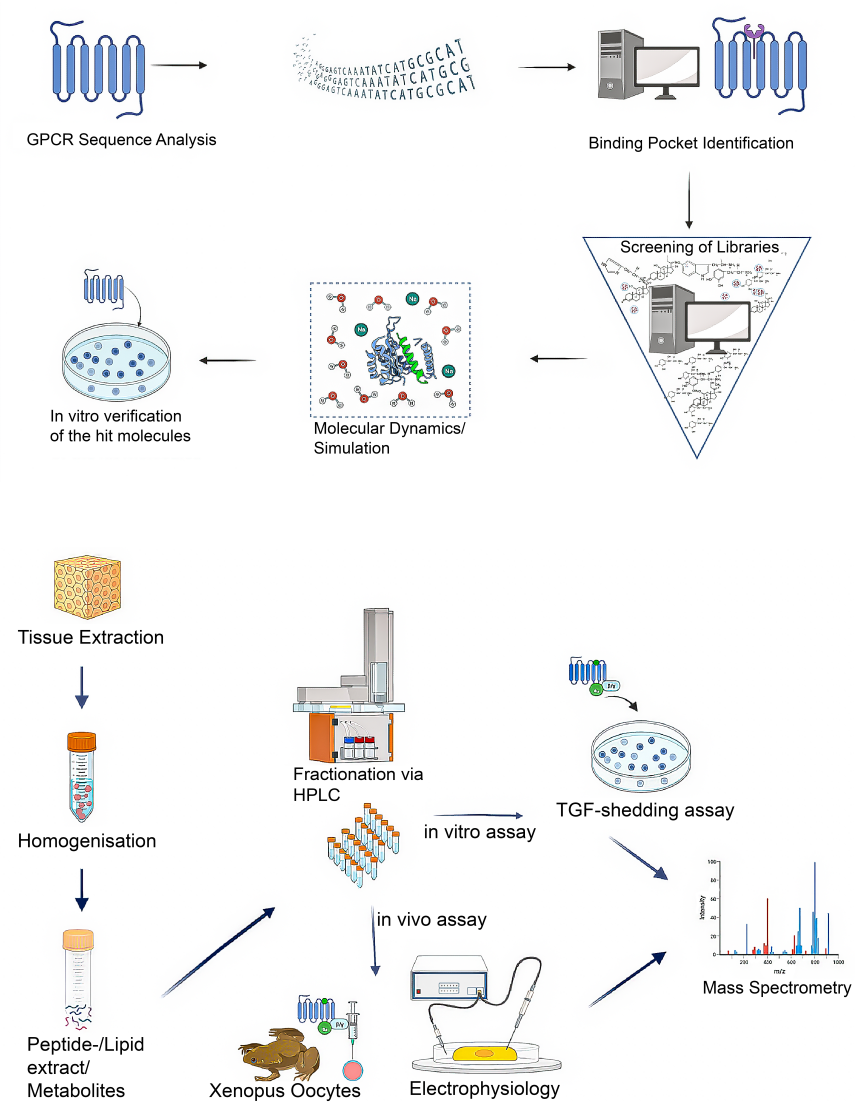

Physiology of GPCRs in the Nervous System and the Contribution of Orphan GPCRs

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Abstract

G protein-coupled receptors (GPCRs) are a large family of cell surface receptors that play a critical role in nervous system function by transmitting signals between cells and their environment. They are involved in many, if not all, nervous system processes, and their dysfunction has been linked to various neurological disorders representing important drug targets. In this review, we will first discuss the role of the nervous system GPCRs in the modulation of tripartite synapse function and how GPCRs control energy metabolism in the brain. We will then discuss the (patho)physiology and pharmacology of opioid, cannabinoid, acetylcholine, chemokine, and melatonin GPCRs in the nervous system. Furthermore, we will briefly report on adhesion GPCR function in nervous tissues. Finally, we will address orphan GPCRs, their implication in the nervous system function and disease, and the challenges that need to be addressed in the future to deorphanize them.

Keywords: G protein-coupled receptors, nervous system, neuron, glia, signalling, physiology, nervous system disorders, orphan G protein-coupled receptors, deorphanization

Abbreviations

2-AG: 2-Arachidonylglycerol
5-HTRs: 5-hydroxytryptamine or serotonin receptors
7TM: Seven α-helical transmembrane domain, seven-transmembrane
7TMD: Heptahelical transmembrane domain, seven-transmembrane domain
AC: Adenylate cyclase
ACKR: Atypical chemokine receptors
AD: Alzheimer's disease
AEA: Anandamide
aGPCR: Adhesion G protein-coupled receptors
ALS: Amyotrophic lateral sclerosis
AMPA: α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid
AraG: N-Arachidonoylglycine
AraS: N-Arachidonoylserine
ARs: Adenosine receptors
BBB: Blood-brain barrier
BCP: (−)−β−caryophyllene
BMI: Body mass index
BQCA: Benzyl quinolone carboxylic acid
Ca^{2+}: Calcium
cAMP: Cyclic adenosine monophosphate
CARTp: Cocaine- and amphetamine-regulated transcript peptide
CBD: Cannabidiol
CBR: Cannabinoid receptors
CHCL: CXC chemokine ligand
CHCR: CXC chemokine receptor
CNS: Central nervous system
COST: European cooperation in science and technology
CREB: cAMP response element-binding protein
CTF: C-terminal fragments
CXC: CXC chemokine subfamily
DAG: diacylglycerol
DCs: Dendritic cells
DOR: Delta opioid receptor
ECD: Extracellular domain
ECL: Extracellular loop
ECS: Endocannabinoid system
ERK: Extracellular signal-regulated kinases
ERK1/2: Extracellular signal-regulated kinase 1/2
FDA: Food and drug administration
GABA: γ-Aminobutyric acid
$GABA_BRs$: γ-Aminobutyric acid B receptors
GAIN: GPCR autoproteolysis–inducing
GDP: Guanosine diphosphate
GIRKs: G protein-coupled inwardly rectifying potassium channels
GLP-1: Glucagon-like peptide l
GPCRs: G protein-coupled receptors
GPR: G protein-coupled receptors, usually still orphan
GTP: Guanosine triphosphate

HCAR1: Hydroxy-carboxylic acid receptor 1
HD: Huntington's disease
HMEC-1: Human microvascular endothelial cells
I-TAC: IFN-inducible T cell α chemoattractant
ICD: Intracellular domain
ICL: Intracellular loop
IFN: Interferon
IL: Interleukin
IP-10: IFN-γ inducible protein-10
IP3: inositol (1,4,5) triphosphate
JAK: Janus kinase
JAKS: Janus kinases
kDa: Kilodalton
KO: Knockout
KOR: Kappa opioid receptor
LC: Locus coeruleus
LDs: Lipid droplets
LLRs: L-Lactate-sensitive receptors
mAb: Monoclonal antibody
mAChRs: Muscarinic acetylcholine receptors
MAPK: Mitogen-activated protein kinase
MD: Multiple sclerosis
MDL29,951: 2-carboxy-4,6-dichloro-1H-indole-3-propionic acid
mGluRs: Metabotropic glutamate receptors
MIG: Monokine induced by interferon-γ
MOR: Mu opioid receptor
MT1: Melatonin receptor 1
MT2: Melatonin receptor 2
NAAAs: N-Acyl amino acids
NAc: Nucleus accumbens
NAEs: N-Acylethanolamines
NAMs: Negative allosteric modulators
NF-κB: Nuclear factor kappa B
NFEPP: N-(3-fluoro-1-phenetylpiperidin-4-yl)-N-phenylpropionamide)
NK: Natural killer
NMDA: N-methyl-D-aspartate
NPC: Neural progenitor cells
NTF: N-terminal fragments
NTS: Nucleus tractus solitarius
OEA: N-Oleoylethanolamide
oGPCRs: Orphan G protein-coupled receptors
Olig1: Oligodendrocyte transcription factor 1
OP: Oligodendrocyte progenitor
OPCs: Oligodendrocyte precursor cells
OR: Opioid receptor
P2Y: Purinergic receptors
PAMs: Positive allosteric modulators
PD: Parkinson's disease
PEA: N-Palmitoylethanolamide
PF-4: Platelet factor-4

PI3K: Phosphoinositide 3-kinases
PIP2: Phosphatidylinositol 4,5-bisphosphate
pKa: Acid dissociation constant at logarithmic scale
PKA: Protein kinase A
PKC: Protein kinase C
PLC: Phospholipase C
POMC: Pro-opio-melanocortin
PTSD: Post-traumatic stress disorders
RGS4: Regulator of G protein signalling 4
RMS: Rostral migratory stream
SC: Neural stem cell
STAT5B: Signal transducers and activators of transcription 5B
Th1: T helper type 1
THC: Tetrahydrocannabinol
MT1: Melatonin receptor 1
MT2: Melatonin receptor 2
VFM: "Venus flytrap" module
VGCCs: Voltage gated Ca^{2+} channels
VTA: Ventral tegmental area
δ-OR: Delta opioid receptor
κ-OR: Kappa opioid receptor
μ-OR: Mu opioid receptor

1 Introduction

There are >800 human GPCRs. Around half of them (~400) have sensory functions mediating olfaction, taste, light perception, and pheromone signalling. The remaining GPCRs (~370) are non-sensory, of which >90% are expressed in the nervous system, and respond to hormones and neurotransmitters, regulating many physiological processes, such as synapse function, brain metabolism, pain perception, mood, behaviour, memory, and learning, immune response, neuroprotection, mechanosensation, myelination, cell differentiation, cell migration, nervous system development, axon guidance, dendritogenesis, and vascularization.

The dysfunction of GPCRs in the nervous system has been linked to many neurological deficits, including neurodegeneration, psychiatric disorders, epilepsy, cancer, and addiction (Wacker et al., 2017). The development of GPCR-based drugs, modulating canonical and noncanonical GPCR signalling, for treating neurological disorders is increasing as human GPCRs are the most common target for therapeutic drugs. In 2017 it was accounted that ~34% of all FDA-approved drugs are GPCR-based drugs, acting as antagonists or agonists of different GPCRs (Hauser et al., 2017). Moreover, many GPCR-based drugs are currently in clinical trials (Shimada et al., 2019). As new functions for GPCRs are discovered, particularly for the ~100 orphan GPCRs (oGPCRs; Watkins and Orlandi, 2021) for which endogenous ligands and their role in the cellular processes are currently

unknown, the number of drugs targeting GPCRs is expected to increase. Resolving the GPCR structures and development of novel GPCR ligands is essential to further understand the role of individual GPCR types in the nervous system function and beyond.

This review will first discuss the general structural and signalling characteristics of the GPCR families commonly found in the nervous system. We will then focus on the nervous system GPCRs involved in regulating tripartite synapse function and brain and systemic energy metabolism. We will also discuss the (patho)physiology and pharmacology of opioid, cannabinoid, muscarinic acetylcholine, chemokine, and melatonin receptors. These receptors have been shown to play a role in many nervous system deficits and are important drug targets. In addition, we will discuss adhesion GPCRs and their emerging role in the nervous system function. Finally, we will address orphan GPCRs (oGPCRs) in the nervous system function and disease with the remaining challenges in structural biology that need to be addressed in the future to deorphanize oGPCRs and to fully understand the molecular mechanisms underlying the (patho)physiology of GPCRs in the nervous system.

2 GPCR Structure and Signalling Pathways

GPCRs can transduce a signal across the cell membrane due to their ability to change conformation. Binding of ligands to the extracellular side of the GPCRs promotes conformational changes in the GPCRs allowing attachment of G proteins, arrestins, and other signalling proteins to the intracellular side of a GPCR and signal transduction. GPCRs generally consist of a conserved heptahelical transmembrane domain (7TMD), an N-terminal extracellular (ECD) and a C-terminal intracellular domain (ICD). 7TM helices span the cell membrane and are connected by alternating three intracellular (ICL1-3) and three extracellular loops (ECL1-3). The ligand-binding site ("binding pocket") is usually located within the transmembrane region, which also plays a critical role in mediating a GPCR's interactions with its intracellular binding partners, such as G proteins and arrestins (Wacker et al., 2017).

GPCRs can be classified into six main families based on their sequence similarities: class A (rhodopsin-like), B (secretin (B1) and adhesion (B2)), C (metabotropic glutamate/pheromone), D (fungal mating pheromone), E (cAMP), and F (frizzled/smoothened) families. GPCRs of classes A, B, and C are the most common in the nervous system. They share the same 7TM helix structure but differ in their ECDs, ICDs, and activation mechanisms. These structural differences contribute to the specific ligand recognition and signalling properties of each GPCR class.

Class A GPCRs (rhodopsin-like receptors) are the most abundant and diverse group, accounting for ~80% of all GPCRs, which includes hormone, neurotransmitter, and light receptors. Class A GPCRs exhibit the most typical GPCR structure, characterised by an extracellular N-terminus, followed by 7TMDs and an intracellular C-terminus.

In contrast to the class A GPCR family, class B1 GPCRs (secretin-like receptors) are defined by a large, glycosylated N-terminal ECD. The native ligands of class B1 GPCRs are long peptide hormones (e.g., glucagon-like peptide l (GLP-1)) with specific functional sites at the two termini. The C-terminal part of the peptide ligand forms high-affinity interactions with the ECD and drives receptor binding, while the N-terminal part of the peptide interacts with the 7TMD and triggers receptor activation ("two-domain binding model"), often involving proteolytic cleavage of the receptor's N-terminus (Cong et al., 2022; de Graaf et al., 2017). Class B2 GPCRs (adhesion GPCRs (aGPCRs)) are distinguished not only by their large ECDs that contain a wide variety of adhesive subdomains but also by the highly conserved GPCR autoproteolysis–inducing (GAIN) domain. In most aGPCRs, the GAIN domain constitutively self-cleaves the receptors into two fragments (holoreceptor form). Dissociation of the two fragments stimulates the G protein signalling through the action of the tethered-peptide agonist occluded within the GAIN domain (Vizurraga et al, 2020).

Class C GPCRs (metabotropic glutamate/pheromone receptors) have a huge N-terminal ECD that contains the ligand-binding site. It consists of a cysteine-rich region and a "Venus flytrap" module (VFM) with two lobes (lobe 1 and lobe 2) separated by a cleft as an orthosteric site and undergoes conformational changes upon ligand binding. The 7TMD in class C GPCRs only have allosteric sites. The other unique characteristic of class C GPCRs is their mandatory dimerization, either as homodimers or heterodimers (Chun et al., 2012).

GPCRs transduce extracellular signals into intracellular signals by coupling to heterotrimeric G proteins and arrestins. According to the G protein, they either activate or inhibit the downstream signal transduction pathway. G proteins are heterotrimeric proteins composed of α, β, and γ subunits bound to the guanine nucleotide. The α subunit is classified into four distinct families: $G\alpha_s$, $G\alpha_{i/o}$, $G\alpha_{q/11}$, and $G\alpha_{12/13}$. Most GPCRs can activate more than one α subunit but show greater affinity for one α subunit. Since β and γ subunits are closely interacting, they can be described as a whole functional βγ unit (Odoemelam et al., 2020).

The activity of G proteins is regulated by guanosine triphosphate (GTP) and its inactive form, guanosine diphosphate (GDP). At rest, the inactive α subunit remains bound to GDP, but the conformational changes that occur upon agonist binding increase the affinity of the receptors for G proteins, resulting in G protein binding. As a result of this binding, GDP is released from the α subunit. The α subunit has a conserved GTPase region in all GPCRs and a unique helix region. This GTPase domain hydrolyses GTP, creating a binding surface for βγ dimers, GPCRs, and effector proteins (Calebiro et al., 2021)

GTP-bound α subunits and βγ dimers regulate the activities of specific effectors. The effector of $G\alpha_s$ and $G\alpha_{i/o}$ is the adenylate cyclase (AC), which converts ATP to cyclic adenosine monophosphate (cAMP). On the other hand, $G\alpha_{q/11}$ cleaves membrane-bound phosphatidylinositol 4,5-bishosphate (PIP2), inositol (1,4,5) triphosphate (IP3), and diacylglycerol (DAG) using phospholipase C-β (PLC-β) as an effector. This pathway causes an increase in intracellular Ca^{2+} levels. $G\alpha_{12/13}$ effectors are RhoGEF proteins. β-arrestins are ubiquitously expressed and categorised as visual (arrestin1 and 4) and non-visual (arrestin2 and 3) cytosolic adaptor proteins. They were initially discovered due to desensitisation and internalisation in GPCR signalling. They can cause internalisation by clathrin-coated pits after dephosphorylation of the receptor and recycling or lysosomal degradation (Jean-Charles et al., 2017). Furthermore, the binding of β-arrestins to GPCRs can activate signal transduction pathway independent of G protein signalling by scaffolding mitogen-activated protein kinase (MAPK) and extracellular signal-regulated kinase (ERK)) signalling cascade (Jain et al., 2018).

3 GPCRs in the Nervous System (Patho)Physiology

3.1 GPCRs in the Tripartite Synapse Function

Tripartite synapse represents a functional unit within the CNS where bidirectional communication between pre- and postsynaptic neurons and astrocytes takes place, a process crucial for the regulation of synaptic activity. The concept was first demonstrated in glutamatergic neurotransmission, which involves ionotropic (N-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors) and metabotropic glutamate receptors (mGluRs; Araque et al., 1999). Since then, several other GPCRs have been identified to modulate the function of the tripartite synapse, including metabotropic γ-aminobutyric acid (GABA), serotonin, purinergic, dopamine, opioid (section 3.3), and cannabinoid receptors (section 3.4).

3.1.1 Metabotropic Glutamate Receptors (mGluRs)

mGluRs are class C GPCRs divided into three groups: stimulatory group I ($mGluR_1$ and $mGluR_5$), coupled to $G\alpha_q$ proteins, and inhibitory groups II ($mGluR_2$ and $mGluR_3$) and III ($mGluR_4$, $mGluR_6$, $mGluR_7$, and $mGluR_8$) coupled to $G\alpha_{i/o}$ proteins (**Table 1**). Except for $mGluR_6$, they are abundantly expressed in neuronal and glial cells throughout the brain. The endogenous mGluR ligand is glutamate, the primary excitatory brain neurotransmitter, whose precursor, glutamine, is produced exclusively in astrocytes (Huang & Thathiah, 2015).

Increased activity of glutamatergic neurons activates astroglial $mGluR_5$, resulting in Ca^{2+}-dependent glutamate release from astrocytes and feedback regulation of synaptic activity (Araque et al., 1999). Astrocytic glutamate activates presynaptic neuronal $mGluR_1$s and Ca^{2+}-signals causing short-term potentiation of neurotransmitter release at hippocampal synapses, increasing synaptic strength. This transient potentiation of glutamate release, coupled with postsynaptic depolarization can cause long-term effects on synaptic transmission (Perea & Araque, 2007). In contrast, activation of presynaptic group II and III mGluRs by astroglial-derived glutamate causes heterosynaptic depression (Andersson et al., 2007), likely resulting in reduced neurotransmitter release and a negative effect on synaptic strength. The mGluR-mediated effects of glutamate on synaptic activity are achieved largely by modulation of ion channels (Reiner & Levitz, 2018).

3.1.2 γ-Aminobutyric Acid B Receptors ($GABA_BRs$)

GABA B receptors ($GABA_BRs$) are class C GPCRs. Two subtypes of $GABA_BRs$ exist in the nervous system: $GABA_{B1}$ and $GABA_{B2}$ receptors. They are enriched in cortical, hippocampal, and cerebellar brain regions. $GABA_BRs$ are functional only after heterodimerization of both subtypes. They are coupled to $G\alpha_{i/o}$ proteins (**Table 1**) and exhibit inhibitory effects on neurotransmission (Huang & Thathiah, 2015). The endogenous ligand GABA, the principal inhibitory brain neurotransmitter, is produced in GABAergic interneurons from astrocyte-derived glutamine or released by astrocytes. The GABAergic system allows precise control of the excitatory level of neuronal networks. Astrocyte-derived GABA acting on $GABA_BRs$ in GABAergic interneurons has a disinhibitory effect on hippocampal excitatory synapses of dentate granule neurons (Yarishkin et al., 2015). Stimulation of GABAergic interneurons can also trigger $GABA_BR$-dependent Ca^{2+} increase and glutamate release from astrocytes, resulting in enhanced $mGluR_1$-dependent excitatory glutamatergic neurotransmission (Perea et al., 2016). In this way, astrocytes can convert inhibitory signals into

excitatory ones, adding another layer of complexity to understanding the physiology of brain excitability.

3.1.3 Serotonin or 5-hydroxytryptamine Receptors (5-HTRs)

The majority (13 of 14) of serotonin or 5-hydroxytryptamine (5-HT) receptor (5-HTRs) subtypes are class A/19 rhodopsin-like GPCRs. They are classified into six types: 5-HT_1, 5-HT_2, 5-HT_4, 5-HT_5, 5-HT_6 and 5-HT_7 receptors. Endogenous ligand serotonin is produced by serotonergic neurons, originating mainly in the raphe nuclei, which innervate virtually all brain regions. 5-HTRs are mostly postsynaptic and possess differential (even opposing) effects on neurotransmission. Depending on the subtype, 5-HTRs are coupled to $G\alpha_q$, $G\alpha_s$ or $G\alpha_{i/o}$ proteins (**Table 1**) (Celada et al., 2013).

The most prevalent and researched are 5-HT_{1A} and 5-HT_{2A} receptors which exert homeostatic control over serotonergic neuron firing rates controlling serotonin efflux (Celada et al., 2013). However, much less is known about the neuron-glia relationship within the serotonergic system. A recent study by (Müller et al., 2021) showed that astroglial 5-HT_4R-RhoA signalling regulates cell morphology, leading to changes in glutamatergic neurotransmission, which presents a novel functional regulation of excitatory synapses.

3.1.4 Metabotropic Purinergic Receptors

Purinergic signalling in the nervous system is mediated by two subtypes of purinergic receptors: P1 and P2 purinergic receptors that bind adenosine or ATP/ADP/AMP, respectively. P2 receptors are further divided into ionotropic P2X and metabotropic P2Y receptors. ATP in the nervous system can act as an energy substrate and a signalling molecule released from neurons and neuroglia to the extracellular space with Ca^{2+}-regulated exocytosis or via hemichannels, ABC transporters, maxi-ion channels, and P2X7 receptors. When released, ATP is rapidly degraded by extracellular ectonucleotidases to ADP, AMP, and adenosine, which act as purinergic agonists and can have opposite effects on target cells (Abbracchio et al., 2009; Burnstock, 2006; Burnstock, 2018). We will only discuss the role of metabotropic P1 and P2Y GPCRs in synaptic transmission.

P1 (Adenosine) Purinergic Receptors

Purinergic P1 receptors, also named adenosine receptors (ARs), are class A/18 rhodopsin-like GPCRs with four subtypes (A_1, A_{2A}, A_{2B}, and A_3). All subtypes of ARs are expressed in the nervous tissue, both in neurons and glia (astrocytes, microglia, oligodendrocytes) (Abbracchio et al., 2009; Verkhratsky & Nedergaard, 2018) and respond to adenosine (Boison, 2007). AR subtypes can form

heteromers and oligomerize with other GPCRs. A_1Rs and A_3Rs are coupled to $G\alpha_{i/o}$ proteins, whereas A_{2A}Rs and A_{2B}Rs are coupled to $G\alpha_s$ proteins. ARs can also regulate PLC and IP3 production and MAPK signalling pathway (**Table 1**) (Abbracchio et al., 2009; Verkhratsky & Nedergaard, 2018).

A_{1A}Rs and A_{2A}Rs are the main brain ARs, while A_{2B}Rs and A_{3A}Rs are expressed at low levels. A_{1A}R is abundantly expressed in the cortex, hippocampus, and cerebellum, while A_{2A}R is found in the striatum and olfactory bulb. Both A_1Rs and A_{2A}Rs are located in the synapse, mainly in excitatory (glutamatergic). Activating A_1Rs by astrocytic adenosine inhibits excitatory transmission, while A_{2A}Rs facilitate excitatory transmission (Kofuji & Araque, 2021). Adenosine *via* A_1Rs reduces i) presynaptic Ca^{2+} influx and glutamate release, ii) activation of postsynaptic ionotropic glutamate receptors, and voltage-sensitive Ca^{2+} channels, and iii) hyperpolarizing dendrites *via* regulation of K^+ channels. In contrast, A_{2A}R enhances the evoked presynaptic glutamate release and the function of postsynaptic ionotropic glutamate receptors. A_{2A}R activation also decreases the efficiency of presynaptic A_1R- and CB_1R-mediated inhibitory systems, switching presynaptic modulation from inhibitory to facilitatory. A_{2A}R located in astrocytes and microglia regulates glial Na^+/K^+-ATPase, glutamate uptake, and proinflammatory cytokine production, which may contribute to the A_{2A}R-mediated control of synaptic plasticity (reviewed in (Abbracchio et al., 2009; Cunha, 2016; Verkhratsky & Nedergaard, 2018)).

P2Y Receptors

Depending on the subtype, P2Y receptors belong to the class A/11 and A/12 rhodopsin-like GPCRs. They are divided into 8 subtypes grouped into $P2Y_1$-like receptors ($P2Y_{1,2,4,6,11}$) and $P2Y_{12}$-like receptor ($P2Y_{12\text{-}14}$) with different sensitivity to ATP, ADP, UTP/ATP, UDP, or UDP-glucose. $P2Y_1$-like receptors are preferentially coupled to $G\alpha_q$ proteins, while P2Y12-like receptors couple to the $G\alpha_i$ proteins (**Table 1;** Abbracchio et al., 2009). They can form homodimers or heterodimers with other P2Y receptors or GPCRs (e.g., A_1R).

P2Y receptors are expressed on neurons and glia (astrocytes, oligodendrocytes, microglia) (Guzman & Gerevich, 2016; Kofuji & Araque, 2021). They inhibit the presynaptic release of neurotransmitters. For example, $P2Y_4$ receptors inhibit GABA release from basket cells on Purkinje cells in the cerebellum. In the spinal cord, hippocampus, and cortex, noradrenaline release is blocked by activation of $P2Y_1$, $P2Y_{12}$, and $P2Y_{13}$ receptors. Moreover, in the cortex P2Y receptor activation decreases serotonin release. This inhibitory action of P2Y receptors has been linked to their inhibitory

effect on various types of presynaptic voltage-gated Ca^{2+} channels (VACCs) *via* Gβγ subunit (Guzman & Gerevich, 2016).

P2Y receptors can also modulate postsynaptic signalling. $P2Y_1$ was shown to inhibit the action of postsynaptic ionotropic glutamate NMDA receptors, while $P2Y_4$ was shown to enhance their activity. $P2Y_1$ also increases the sensitivity of $GABA_A$ receptors and enhances inhibitory transmission through $GABA_A$ receptors. Moreover, ionotropic P2X receptors are inhibited by $P2Y_1$ receptors. P2Y receptors were also linked to postsynaptic inhibition of L-type VACCs, voltage-gated potassium KCNQ2/3 channels, and modulation of G protein-coupled inwardly rectifying potassium (GIRK1,2,4) channels. The latter can be activated or inhibited by P2Y receptors, depending on the P2Y receptor subtype (Guzman & Gerevich, 2016).

Activation of astrocytic $P2Y_1$ receptors causes Ca^{2+} elevations in the hippocampal, olfactory bulb, and nucleus accumbens (NAc) astrocytes. This can trigger intercellular Ca^{2+} waves in the astrocytic network and Ca^{2+}-dependent glutamate release from astrocytes. Glutamate then postsynaptically activates extrasynaptic NMDA receptors generating slow inward currents that increase neuronal excitability and/or presynaptic NMDA receptors and NMDA receptor-dependent synaptic potentiation. Astrocytic $P2Y_1$ receptor activation also triggers activation of neuronal mGluRs and P2X receptors, increasing neuronal excitability (Kofuji & Araque, 2021).

3.1.5 Dopamine receptors

Dopamine receptors (DRs) are class A GPCRs abundantly expressed in the nervous system. They are divided into i) DR-like 1 family (D_1R and D_5R receptors), which is coupled to $G\alpha_s$ signalling and ii) DR-like 2 family (D_2R, D_3R, and D_4R receptors), which is coupled to $G\alpha_{i/o}$ signalling (**Table 1**) (Beaulieu & Gainetdinov, 2011). Dopamine can trigger intracellular Ca^{2+} elevations in hippocampal astrocytes *via* D_1R activation *in situ* (Jennings et al., 2017). This has been confirmed also at the level of a tripartite synapse. Namely, it has been shown that synaptically-released dopamine through activation of astrocytic D_1Rs and signalling cascade involving IP3 triggers intracellular Ca^{2+} mobilisation in astrocytes of the NAc *in situ* and *in vivo*. The latter depresses synapse function, most likely by subsequent Ca^{2+}-triggered astrocytic ATP/adenosine release and activation of presynaptic adenosine A_1Rs. This contrasts the view that receptors from the D_1R-like family are only coupled to $G\alpha_s$ proteins and cAMP production (Corkrum et al., 2020). In contrast, activation of D_2Rs in astrocytes can decrease intracellular Ca^{2+} levels, which has been linked to the suppression of αβ-

crystallin-mediated neuroinflammation *in vivo* (Kofuji & Araque, 2021). The role of astroglial D_2R activation at the level of neuron-glia communication is not yet clear.

3.2 Nervous System GPCRs in the Regulation of Brain and Systemic Energy Metabolism

Although it represents only 2% of the body mass, the brain consumes 20-25% of the body's energy to maintain its normal function. It requires large amounts of energy to restore neuronal ion gradients and sustain synaptic transmission and neurotransmitter uptake and recycling (Sokoloff, 1960). To preserve its normal function during increased neuronal activity, the delivery of nutrients from the bloodstream and the brain energy metabolism must be tightly regulated at different levels involving various GPCRs on the surface of neurons, glial, and endothelial cells. Moreover, nervous system GPCRs were also linked to the regulation of systemic energy metabolism.

3.2.1 Adrenergic Receptors

Adrenergic receptors belong to the class A/17 rhodopsin-like GPCR family (Wu, Zeng, & Zhao, 2021). There are three types of adrenergic receptors, further subdivided into nine subtypes: α_1- (α_{1A}, α_{1B}, α_{1D}), α_2- (α_{2A}, α_{2B}, α_{2C}), and β-adrenergic receptors (β_1, β_2, β_3). Upon binding of endogenous ligands (noradrenaline/adrenaline), each of these types preferentially couples to a different G protein type (**Table 1**), resulting in distinct physiological effects (Pupo & Minneman, 2001).

All types of adrenergic receptors are expressed in the brain (Hertz et al., 2010). Stress response release of neuromodulator noradrenaline from the *locus coeruleus* (LC) noradrenergic neurons, which among others, activates memory formation and learning, results in the activation of brain metabolism (O'Donnell et al., 2012). Astrocytes rich in adrenergic receptors are key brain cells maintaining brain metabolic homeostasis. α_1- and β-adrenergic receptor-mediated Ca^{2+} and cAMP signalling (Horvat et al., 2016) facilitate glucose uptake, glycogenolysis, and aerobic glycolysis with L-lactate production in astrocytes (Fink et al., 2021; Horvat et al., 2021a). L-lactate is released from astrocytes and may enter hyperactive neurons during stress response *via* lactate transporters or channels, where it is oxidised in mitochondria and used as a metabolic fuel. Astrocyte-neuron lactate shuttle was shown to be crucial for memory formation and learning (Magistretti & Allaman, 2018). Extracellular lactate can also act as a signalling molecule, activating brain L-lactate-sensitive receptors (Horvat et al., 2021b). Moreover, activation of β- and α_2-adrenergic receptors also affects lipid metabolism, particularly triggering lipid droplet (LD) accumulation in astrocytes. LDs and β-oxidation of free fatty

acids can support astroglial energy needs to spare glucose for neurons and may even have neuroprotective effects preventing lipotoxicity during stress (Smolič et al., 2021).

3.2.2 L-Lactate-sensitive Receptors

L-lactate-sensitive receptors (LLRs) were recently identified, and their physiological functions remain understudied. Among these, the $G\alpha_{i/o}$ protein coupled hydroxy-carboxylic acid receptor 1 (HCAR1), previously known as GPR81, was the first GPCR described to bind L-lactate (Liu et al., 2009). HCAR1 is a class A/11 rhodopsin-like GPCR. Activation of HCAR1 downregulates cAMP production in brain tissue slices (Lauritzen et al., 2014) and inhibits neuronal activity (Vaccari-Cardoso et al., 2022).

Besides $G\alpha_{i/o}$-coupled HCAR1, two $G\alpha_s$-coupled LLRs are expressed in neurons: olfactory receptor Olfr78 (Conzelmann et al., 2000) and GPR4 (Hosford et al., 2018), which are type A/unclassified and A/15 rhodopsin-like GPCRs, respectively (**Table 1**). Moreover, LC neurons and cortical astrocytes likely express other, yet uncharacterized, $G\alpha_s$ coupled LLR(s) (**Table 1**) (Horvat et al., 2021b). In LC neurons, acute stimulation with L-lactate triggers cAMP-dependent noradrenaline release (Tang et al., 2014), whereas in astrocytes, it upregulates aerobic glycolysis with L-lactate production (D'Adamo et al., 2021; Vardjan et al., 2018), suggesting an autocrine regulation of astroglial L-lactate production (metabolic excitability). Moreover, chronic L-lactate exposure facilitates astroglial lipid metabolism, manifested as LD accumulation (Smolič et al., 2021), likely *via* a $G\alpha_s$ coupled receptor-mediated mechanism. As relatively high L-lactate levels are required to facilitate astroglial metabolism, LLR-mediated signalling in astrocytes may be particularly relevant in brain pathologies and putative L-lactate microdomains.

3.2.3 GLP-1 Receptor

The glucagon-like peptide-1 (GLP-1) receptor (GLP-1R) belongs to the class B (secretin) family of GPCRs and is part of the glucagon receptor subfamily and is coupled to $G\alpha_s$ protein (**Table 1**) (Zhang et al., 2017).

Although GLP-1R is best known for its role in the regulation of glucose metabolism in the periphery, it is found expressed in key brain regions controlling energy balance, including the hypothalamus, brainstem, and nuclei in the mesolimbic reward system, including ventral tegmental area (VTA) and NAc. Activation of GLP-1R in those brain regions modulates neuronal electrophysiological properties and suppresses feeding behaviours (food intake) (Chen et al., 2021; Trapp & Brierley,

2022). Not only neurons but also astrocytes within different brain regions express GLP-1R. Astrocytic GLP-1R signalling decreases glucose uptake and glycolytic capacity but facilitates mitochondrial β-oxidation of fatty acids in astrocytes and maintains mitochondrial integrity. Moreover, in animals with deficient astrocyte-specific GLP-1R signalling, brain glucose availability, and memory formation are enhanced, and systemic glucose metabolism is improved (Reiner et al., 2016; Timper et al., 2020), indicating that brain GLP-1R is involved in the regulation of local and systemic energy metabolism.

3.3 Opioid Receptors

Opioid receptors (ORs) are class A/4 rhodopsin-like GPCRs expressed throughout the mammalian nervous system on neurons and glial cells. They are divided into three main subtypes: mu (μ-OR or MOR), delta (δ-OR or DOR), and kappa (κ-OR or KOR), which preferentially bind the endogenous opioid peptides (opioids) β-endorphin, enkephalins, and dynorphins, respectively. Both the ORs and their endogenous peptide ligands have preferential anatomical distributions in the brain, which associate with specific physiological roles (Valentino &Volkow, 2018). ORs couple to pertussis toxin sensitive and insensitive G proteins to inhibit AC, regulate PLC, MAPK, and a variety of ion channels (**Table 1**). Postsynaptically ORs trigger activation of GIRKs, causing neuronal hyperpolarization, thereby preventing neuronal excitation and/or propagation of action potentials. ORs also modulate pre- and postsynaptic Ca^{2+} channels (e.g., N-type VGCCs), suppressing the Ca^{2+} influx and the excitability of neurons and/or the secretion of pronociceptive neuropeptides. Moreover, the recruitment of β-arrestin and OR interaction with other proteins triggers G protein-independent signalling to regulate processes related to pain perception, tolerance, dependence, stress responses, mood, and affect (Georgoussi et al., 2012, Valentino &Volkow, 2018).

(Patho)physiology and Pharmacology of Opioid Receptors

Analgesic Effects and Respiratory Depression

Activation of the μ-ORs in the midbrain triggers analgesic effects by reducing the transmission of nociceptive signals from peripheral nerves (Pathan & Williams, 2012). However, using OR ligands can also cause severe side effects, including respiratory depression. The potent μ-OR agonist fentanyl has high addictive potential and was linked to numerous overdose deaths due to respiratory depression. In contrast, its fluorinated derivative NFEPP (N-(3-fluoro-1-phenetylpiperidin-4-yl)-N-phenylpropionamide) is of significant therapeutic interest, being less toxic (Spahn et al., 2017) and whose usage does not associate with respiratory depression (Jiménez-Vargas et al., 2021). The

reduced toxicity of NFEPP is due to its reduced pKa value. NFEPP binds preferentially to μ-ORs at acidic pH typical of inflammation associated with pain (del Vecchio et al., 2017). The fact that fentanyl and NFEPP also have distinct intrinsic torsional dynamics (Giannos et al., 2021) suggests specific interactions of both ligands with μ-OR that are poorly characterised.

Besides acidic conditions, biased agonism is a central topic in μ-OR drug discovery. μ-ORs signal through either G_i protein or β-arrestin2. Adverse effects of morphine, a μ-OR agonist, were associated with the β-arrestin2 pathway and not G_i protein signalling, consequently, significant drug discovery efforts have been made to find biased agonists toward the G_i protein signalling pathway (Qu et al., 2022). However, showing the complexity of μ-OR signalling, a new hypothesis has recently emerged proposing that partial agonism and not ligand bias explain the separation of antinociception from respiratory depression (Gillis et al., 2020). Mathematical models that integrate observations from experiments and computation are crucial to tackling some of the complexity of the opioid system and may help unify the conflicting views on the molecular mechanisms of opioid-associated respiratory depression (Burgueño et al., 2017).

Addiction

Recent data also reveal that dual-target μ-OR/δ-OR ligands exhibit an improved antinociceptive profile associated with a reduced tolerance-inducing capability. The benzomorphan-based compounds LP1 and LP2 belong to this dual class of OR ligands (Pasquinucci et al., 2021). The addiction potential of opioid ligands is linked to the activation of the μ-ORs, which can be explained by the expression of μ-OR in brain structures involved in the processing of drug reinforcement reward, and threat (Le Merrer et al., 2009; Meier et al., 2021). The response to reward and threat is complex, as it is modulated by the κ-OR and δ-OR (Valentino & Volkow, 2018) and other neurotransmitter systems (Meier et al., 2021).

(Eating) Behavior

Opioid agonists can increase food intake, whereas naloxone, a universal opioid antagonist, reduces the intake of food and the intake of high-sucrose solutions in animal behavioural studies, suggesting an involvement of the opioid reward system (King et al., 1979; Cleary et al., 1996). In morbidly obese patients, a high body mass index (BMI) is associated with low availability of the μ-OR in areas related to reward processing (Karlsson et al., 2015). Important questions remain about the neurobiological mechanisms by which opioids influence food intake and side effects, and the effectiveness of opioids for long-term body-weight control. A therapy that combines the opioid antagonist naltrexone with the antidepressant bupropion appeared promising in recent phase III clinical trials for long-term weight

loss (le Roux et al., 2022). κ-OR antagonists such as nor-BNI exert anxiolytic effects, possibly through autophagy regulation (Karoussiotis et al., 2022) as well, aticaprant is of direct interest in tackling anxiety disorders, which is in phase III clinical trials (https://clinicaltrials.gov/ct2/show/NCT05455684).

Ischemia

The density of the δ-OR decreases rapidly during the acute phase of ischemia (Boutin et al., 1999). It has been shown that δ-OR activation is associated, e.g., with a reversal of ischemia-induced reduction of the cell signalling pathway (Sheng et al., 2018), and that δ-OR activation enhances neuronal cell survival by limiting apoptosis due to cellular ischemic death. At the same time, intracerebroventricular administration of δ-OR agonists increases cell proliferation and neural differentiation in the hippocampus leading to memory-enhancing changes in ischemic animal models (Wang et al., 2016). In this respect, δ-OR agonists exert a neuroprotective role *via* a signalling complex composed of $G\alpha_{i/o}$, signal transducers and activators of transcription 5B (STAT5B), and the regulator of G protein signalling 4 (RGS4) protein (Georganta et al., 2013; Pallaki et al., 2017). Based on this knowledge, it should be possible to verify specific hypotheses as to which signal supports desired or undesired side effects and contributes to the rational design of more effective drugs for therapeutic use.

3.4 Cannabinoid Receptors

Cannabinoid receptors (CBR) are class A/13 rhodopsin-like GPCRs and belong together with endogenous ligands like 2-arachidonylglycerol (2-AG) and anandamide (AEA) to the endocannabinoid system (ECS). Two types of CBR have been described, CBR type 1 (CB_1R) and 2 (CB_2R). CB_1R and CB_2R were initially described to couple to $G\alpha_{i/o}$ signalling and inhibition of AC activity and cAMP production or activation of MAPK pathway. For CB_1R, but not CB_2R, an inhibition of P/Q-type VGCC and activation of GIRKs *via* the Gβγ pathway has also been described. Under certain conditions or in some cells, CB_1R can also couple to $G\alpha_S$ and $G\alpha_q$-dependent signalling and upregulation of cAMP and Ca^{2+} signals, respectively (Ibsen et al., 2017) (**Table 1**). Pioneer structures revealed for the CB_1R (PDB:*5XRA*) a double toggle switch during activation (Hua et al., 2017). The toggle switch residue in CB_2R structure (PDB:*5ZTY*) forms interactions with antagonists (MRI2687 and AM10257), stabilizing the receptor in the inactive state (Li et al., 2019). The V-shaped loop of the CB_1R N-terminus inserts extracellularly into the ligand-binding region and acts as a plug (Hua et al., 2017), and the N-terminal domain of CB_2R forms a short helix over the orthosteric pocket without directly participating in antagonist binding (Li et al., 2019). Interestingly, similarities were

found between the agonist-bound motif in CB_1R and the antagonist AM10257-bound CB_2R structure and *vice versa* (Li et al., 2019). Notable differences in G protein binding between CB_1R and CB_2R (PDB: *6PT0*) suggest that the activation mechanisms differ (Xing et al., 2020). Lipophilic CB_2R agonists enter the membrane *via* a membrane channel within the CB_2R structure (e.g. PDB:*8GUQ*) (Li et al., 2023). The knowledge of these structures is essential for drug-targeting approaches.

(Patho)physiology of Cannabinoid Receptors

Cannabis-plant-derived tetrahydrocannabinol (THC) activates both CBRs. It induces psychoactive functions *via* the CB_1R, which is highly expressed and predominantly localised presynaptically in excitatory and inhibitory neurons and glial cells in various brain regions (Cristino et al., 2020). CB_1R suppresses neurotransmitter release and functions in long-term depression and potentiation (Castillo, 2012). Postsynaptically, CB_1R inhibits electron transport in mitochondria and affects memory and brain metabolism (Cristino et al., 2020). Astrocytic CB_1Rs respond to cannabinoids with intracellular Ca^{2+} increase and subsequent glutamate release from astrocytes stimulating neuronal synaptic transmission and plasticity (Navarrete et al., 2014). CB_2R, expressed on microglia, acts immunomodulatory and inhibits the release of pro-inflammatory cytokines in neurodegenerative diseases. Tools with low selectivity led to inconsistent data on CB_2R expression in healthy neurons (Cristino et al., 2020).

Epilepsy and Dravet Syndrome

Preclinical mouse models of epilepsy revealed anti-epileptogenic effects of CB_1R and CB_2R agonists and the CB_1R negative allosteric modulator cannabidiol (CBD) (Cristino et al., 2020), whereas pro-epileptogenic effects of CB_1R and CB_2R blockers had been described (Cristino et al., 2020). These data led to a series of placebo-controlled clinical trials to test the efficacy of CBD, showing a reduction in seizure frequency in patients with epileptic rare genetic disorders such as Dravet syndrome (Devinsky et al., 2017), Lennox-Gastaut syndrome and tuberous sclerosis complex-associated disorders (Thiele et al., 2021). Since 2018, the FDA has approved CBD (Epidiolex) for treating rare, severe forms of epilepsy.

Mental Disorders

Brain regions such as the amygdala, prefrontal cortex, and hippocampus express the CB_1R and are *i.a.* involved in controlling emotions and behaviour. Thus, activation and blockade of the CB_1R in mice affect mental conditions such as anxiety, depression, and stress (Estrada & Contreras, 2020).

Recently, a systematic review and meta-analysis of patients treated with medicinal cannabinoids reported no improvement in primary outcomes in depression or post-traumatic stress disorders and insufficient evidence for improvement in anxiety symptoms (Black et al., 2019).

Eating Disorders

In 2008, the CB_1R antagonist rimonabant, approved for treating obesity, was withdrawn from the market due to its adverse side effects associated with depression and anxiety. However, cell type-specific CB_1R knockouts revealed that hypothalamic CB_1R activation in pro-opio-melanocortin (Koch et al., 2015) and at cortical glutamatergic neurons increases and forebrain GABAergic neurons decrease food intake (Bellocchio et al., 2010).

Neuroinflammation, Pain, and Neurodegeneration

Different roles of CBRs in the brain have been identified in neuropathic pain, neuro-inflammation, and neurodegenerative diseases such as Parkinson's disease (PD), Huntington's disease, Alzheimer's disease, amyotrophic lateral sclerosis, and multiple sclerosis using animal models and human studies as well (Ferrisi et al., 2021). Studies have shown the relevance of CB_1R and CB_2R in neuroprotective therapies in *in vivo* HD models (Lastres-Becker et al., 2004). Further investigations revealed a role of CB_1R in reducing motor inhibition typical of PD patients, while CB_2R is mainly involved in homeostasis and survival in animal models of PD (Bisogno et al., 2016). In response to neuropathological conditions, CB_2R signalling can modulate the shift from neuroinflammatory (M1-type) genes to neuroprotective (M2-type) and homeostatic (M0-type) genes (Ferrisi et al., 2021). The regulatory role of CB_2R rather than CB_1R in the brain reduces the immune response to prevent over-inflammation through neuro-immune mechanisms (Ferrisi et al., 2021).

Moreover, CB_2R-mediated anti-inflammatory effects of its natural agonist (−)-β-caryophyllene (BCP) showed beneficial effects in inflammatory and neuropathic pain in animal models. Attention has recently been paid to a new category of so-called peptide (hemopressin family) CB ligands (Ferrisi et al., 2021).

3.5 Muscarinic Acetylcholine Receptors

Muscarinic acetylcholine receptors (mAChRs) are class A/18 rhodopsin-like GPCRs comprising five subtypes (M1–M5) with different G protein-coupling preferences (**Table 1**) (Felder, 1995). Except for the M3, mAChRs are expressed throughout the brain and are involved in key functions of the CNS.

The M1-mAChR, the most predominant subtype in the CNS, is critical for cognition and locomotion control and has long been a validated drug target for treating cognitive dysfunction, especially in AD (Scarpa, Hesse, & Bradley, 2020). M1-mAChR signalling exerts inherent neuroprotective effects in mice (Scarpa et al., 2021), and M1-selective agonists (PAMs) were shown to reduce pathology in AD animal models (Lebois et al., 2017; Dwomoh et al., 2022).

M2-mAChRs were found to be significantly reduced in the anterior cingulate cortex of patients with bipolar and major depressive disorders, suggesting the M2-mAChR is a potential drug target for the treatment of such psychiatric conditions (Cannon et al., 2006).

The M4-mAChR is involved in emotional and social behaviour (Koshimizu et al., 2012). Clinical trials of the M1/M4-preferring agonist xanomeline could improve cognitive impairments and psychosis-like behaviour in schizophrenic patients (Shekhar et al., 2008) and, importantly, the antipsychotic effects were proven to be primarily driven by the M4-mAChR (Woolley et al., 2009). M4-selective PAMs could induce cognitive enhancement and anti-psychotic-like effects in rodent models of schizophrenia, highlighting their promising therapeutic potential (Gould et al., 2018).

The M5-mAChR is linked to drug abuse, as its expression was up-regulated in the brains of rats following long-term alcohol consumption, and morphine-induced addiction (Basile et al., 2002). M5-selective NAMs were shown to reduce alcohol self-administration in rats significantly (Walker et al., 2021), supporting the potential of negatively modulating M5-mAChR signalling as a therapeutic avenue for drug abuse.

3.6 Chemokine Receptors

Chemokines, also known as chemotactic cytokines, are small signaling proteins (7-12 kDa) that control several processes, such as cell migration, proliferation, and differentiation (Thelen & Stein, 2008). They exert their biological effects by activating chemokine receptors, class A rhodopsin-like GPCRs. Chemokines and their receptors are widely distributed in the CNS and are expressed in neurons, glia (microglia, astrocytes, oligodendrocytes), and endothelial cells. They are involved in many (patho)physiological brain functions, including synaptic transmission, neuron-glia communication, and inflammation (Sowa & Tokarski, 2021)

Chemokine receptors are typically coupled to G_i proteins and inhibit AC activity and cAMP levels. In contrast, some can couple to G_q protein and activate PLC, PKC, and Ca^{2+} signals. They can also transduce their signals *via* distinct G proteins ($G\alpha_{11}$ and $G\alpha_{12/13}$), β-arrestins, MAPK/ERK1/2, Janus kinases (JAKS), and nuclear factor kappa B (NF-κB). Chemokine receptors can form homodimers

and heterodimers with other chemokine receptors or GPCRs (e.g., OR), thereby modifying ligand binding properties or activating different signaling pathways (Dorsam & Gutkind, 2007) (**Table 1**). Although some chemokine receptors bind only a unique chemokine, it is common that several chemokines can interact with a particular receptor (Luster et al., 1995) and *vice versa*. Many chemokines also bind to the atypical chemokine receptors (ACKR), which act mainly as scavenger receptors. Opposite to classical chemokine GPCRs, ACKRs do not promote G protein-mediated signaling upon ligand binding, while they preserve the capability to recruit β-arrestins and internalize bound ligands (Bachelerie et al., 2014). While various chemokine receptors are expressed in the brain, this review will only focus on CXCR3 and CXCR4, which belong to the CXC chemokine receptor (CXCR) family.

CXCR3

CXCR3 is expressed on a wide variety of immune cells infiltrating the brain, such as interleukin (IL)-2 activated T helper type 1 (Th1) cells, cytotoxic $CD8^+$ T cells, natural killer (NK) cells and dendritic cells (DCs). It is also found in non-immune cells, particularly endothelial, epithelial, and smooth muscle cells (Andrews & Cox, 2015; Groom & Luster, 2011a, 2011b; Zhu et al., 2015). The human CXCR3 has three alternative splice variants: CXCR3-a, CXCR3-b, and CXCR3-alt, which differ in their carboxy- or amino-terminal domains (Lasagni et al., 2003). CXCL9, CXCL10, and CXCL11 activate CXCR3-a, whereas CXCR3-b interacts with an additional selective ligand, CXCL4 (Lasagni et al., 2003). In addition to their varying affinities for the ligands, CXCR3-a and CXCR3-b have been proposed to have distinct expressions and functions, leading to opposite cellular effects (Berchiche & Sakmar, 2016). CXCR3-a is present in the fetal and mature brain (especially in glial cells (e.g., astrocytes), Purkinje cells of the cerebellum, endothelial cells of the choroid plexus) (Van Der Meer et al., 2001). However, CXCR3-b is selectively present in endothelial cells such as the human microvascular endothelial cells (HMEC-1) (Lasagni et al., 2003).

CXCR3-a isoform has been associated (with its cognate ligands) with various neurological disorders (Dijkstra et al., 2004; Fife et al., 2001; Hamilton et al., 2002; Huang et al., 2000; Kivisakk et al., 2002; Pu et al., 2015; Rappert et al., 2004). CXCR3 and its ligand CXCL10 were shown to be crucial for microglia activation and migration to the lesion site after brain injury (Rappert et al., 2004). CXCR3 has been implicated in glioma development (Pu et al., 2015) and increased glioma cell migration from the primary tumour site (Boye et al., 2017). It has also been reported that in glioblastoma, CXCR3 signaling promotes the recruitment of specific brain resident memory T cells to tumour sites (Zhao et al., 2023).

CXCR4

CXCR4 is a critical regulator of cell migration in the context of immune surveillance and development. Human CXCR4 has two variants, CXCR4-A and CXCR4-B, with CXCR4-B being the more highly expressed (Kaiser et al., 2021). CXCR4 can couple to $G\alpha_i$, $G\alpha_q$, or $G\alpha_{12/13}$, activating various signalling pathways. This results in a complex signalling cascade involving, among others, MAPK and PI3K pathways, which ends as promoting cell migration and/or activation of adhesion molecules (Ye et al., 2021). Furthermore, CXCR4 can activate signalling independently of G protein by recruiting JAK2/3 upon receptor activation and subsequent homodimerization. In addition, CXCR4 recruits β-arrestin2 more efficiently than β-arrestin1 following C-terminal phosphorylation of the receptor upon activation (De Filippo & Rankin, 2018). As for other GPCRs, β-arresting recruitment sterically blocks other signal transduction pathways of CXCR4 and promotes receptor internalisation by facilitating the recruitment of clathrin. CXCR4 is one of the few chemokine receptors that bind exclusively to one chemokine, CXCL12, also known as stromal cell-derived factor 1 (Bachelerie, 2014).

CXCR4 in the CNS is expressed in NPCs, whereas astrocytes express CXCL12 (Murray et al 2022). CXCL12 has 6 functional transcripts variants in humans (CXCL12α, β, γ, ε, δ, and θ), with CXCL12γ having the lowest affinity for CXCR4 while CXCL12α has the highest affinity(). CXCR4 internalises only after activation by CXCL12, and the internalised receptor subsequently degrades, leading to a negative modulation of CXCR4 expression at the cell membrane. The binding between CXCR4 and CXCL12 is regulated by the scavenger activity of ACKR3, which binds CXCL12 most effectively than CXCR4 (Huynh et al., 2020). Thus, ACKR3 actively maintains CXCL12 gradients, critical for target cell migration (Huynh et al., 2020).

In the CNS, the CXCL12/CXCR4 axis has therapeutic potential in regeneration processes, inducing homing by regulating cell secretion and adhesion molecules (Bianchi & Mezzapelle, 2020). CXCL12/CXCR4 signalling influences stem cell migration from the bone marrow or niche to repair damaged tissues. CXCL12 in the damaged tissue recruits stem cells and regulates their differentiation to repair injuries in CNS. In the damaged CNS, CXCR4 *in vitro* activation promotes the differentiation of human embryonic stem cells into neural stem cells (NSCs). Consequently can be used for regenerative medicine in the future in damaged tissue in patients Depending on the CXCL12 concentration, CXCL12 can induce different cellular responses (e.g., activation, mobilisation, homing, and retention). In CNS, CXCL12 at low concentrations promotes NPC migration, while at high concentrations, it favours NPC adhesion (Bianchi & Mezzapelle, 2020).

During CNS development, the CXCR4/ACKR3/CXCL12 axis also directs neuronal migration . CXCR4 signalling induces the survival and migration of NPCs and OPCs . In addition, CXCL12 signalling regulates neuroblast migration within the rostral migratory stream. Furthermore, CXCL12 induces the migration of HSCs CXCR4 (Dillenburg-Pilla et al., 2015).

3.7 Melatonin Receptors

Melatonin receptors are type A/9 rhodopsin-like GPCRs activated by melatonin, a natural neurohormone produced primarily at night by the pineal gland. Melatonin helps regulate circadian rhythms, particularly the sleep-wake cycle. Melatonin is permeable to cell membranes and crosses the BBB to activate two types of receptors, MT1 and MT2, which are found in the brain and preferentially activate $G\alpha_{i/o}$ proteins (**Table 1**).

Although the precise effects and mechanisms of action of melatonin receptors in the brain are still not fully understood, they are gaining interest due to their diverse functions. These receptors are suggested to protect the BBB and may directly or indirectly affect neuronal functions. MT2 activation has been shown to increase neural stem cell proliferation *in vitro* and promote neurogenesis *in vivo*, improving neuronal function in a mouse model of ischemic stroke (Chern et al., 2012). Additionally, recent intriguing findings reveal that MT1 is localised and signals in neuronal mitochondria and may contribute to the neuroprotective effects of melatonin by reducing ischemic brain damage in mice (Suofu et al., 2017). In addition to their classical signalling functions, these receptors have atypical roles independent of their signalling capacity. MT1 and MT2 interact with and retain the dopamine transporter in its immature, non-glycosylated form within the ER, thereby regulating its availability at the cell membrane. This, in turn, limits the reuptake of striatal dopamine in mice, and melatonin receptor knockout (KO) mice show decreased amphetamine-induced locomotor activity (Benleulmi-Chaachoua et al., 2018).

Drugs that target these receptors, such as Ramelteon®, are prescribed for minor conditions like jet lag and insomnia. Agomelatine, a dual ligand agonist of melatonin receptors and an antagonist of serotonin 5-HT_{2C} receptors, is used for depression treatment. In mice, there is evidence of heteromeric MT2/5-HT_{2C} receptor complexes, which may also exist in humans and may contribute to the antidepressant effects of agomelatine (Gerbier et al., 2021).

Recent advances include the determination of MT1 and MT2 structures bound to various ligands (Stauch et al., 2019) or in complex with $G\alpha_{i/o}$ proteins (Wang et al., 2022). These structures are crucial

for initiating virtual screens to identify new specific modulating ligands for these receptors (Stein et al., 2020).

3.8 Adhesion GPCRs – Mechanosensors in Nervous Tissues

Adhesion GPCR (aGPCR)/class B2 receptors constitute the second largest GPCR family in mammals. Several aGPCRs are expressed in nervous tissue, shaping fundamental neurobiological processes such as synaptogenesis, axon guidance, and myelination (Liebscher et al., 2022). aGPCRs are equipped for metabotropic mechanosensing (Lin, 2022). However, knowledge of how mechanosensation through aGPCR affects nervous tissue development and function is scarce.

aGPCRs are bulky molecules with huge extracellular regions that contain multiple structural adhesive folds and a characteristic GPCR autoproteolysis-inducing (GAIN) domain. The GAIN domain harbours a cryptic tethered agonist (Stachel) relevant for receptor activation (Liebscher et al., 2014), and mediates self-cleavage of the maturing protein giving rise to heterodimeric aGPCR consisting of non-covalently associated N- and C-terminal fragments (NTF and CTF) (reviewed in (Hamann et al., 2015)). Recent work has shown that NTF-CTF dissociation can be induced both ligand- and mechano-dependently, and the resulting fragments can serve separate neural functions (Hamann et al., 2015; Petersen et al., 2015; Scholz et al., 2023). For example, a critical step during peripheral nervous system development is to choose which axons to myelinate (radial sorting; (Feltri et al., 2016). ADGRG6/GPR126 is expressed in Schwann cells, and its NTF is important to keep them immature and for radial sorting, whereas the CTF promotes axon wrapping by increasing cAMP. The latter is initiated when ADGRG6 ‘feels’ basal lamina stiffening, i.e. its ligand Laminin-221 polymerizing (Petersen et al., 2015). In the developing *Drosophila* brain, ADGRL/Latrophilin/Cirl (calcium-independent receptor for α-latrotoxin) is expressed in the neuroblast lineage and cortex glia. Glia-derived NTF controls the size of the neuroblast pool cell non-autonomously. Strikingly, the Toll-like receptor Toll-8/Tollo can promote or prevent this glia-to-neuroblast signalling, depending on its expression in *cis* or *trans* to Cirl (Scholz et al., 2023). Moreover, Cirl modulates the sensitivity of peripheral mechanosensory to touch and sound as well as nociceptive neurons to noxious mechanical stimuli (Scholz et al., 2015; Dannhäuser et al., 2020).

Mounting evidence supports the idea that cell mechanics affect neural processes (Barnes et al., 2017). Yet, more often than not it remains unknown how neural cells sense and transduce mechanical forces and how this impinges on their biochemical and genetic profile. aGPCRs seems to fit that ‘bill’. However, future investigations are required to understand aGPCR biology in more detail and to reveal if and how their function intersects with mechanobiological aspects of nervous tissues.

4 Orphan GPCRs in the Nervous System (Patho)Physiology

As extensive as GPCR research is, there are remaining GPCRs with unknown structures and functions (Rajagopal et al., 2010; Yanamala & Klein-Seetharaman, 2010). These novel GPCRs are described as orphan GPCRs (oGPCRs). Deorphanization of these oGPCRs is essential because they can be great drug targets and might help us uncover an unknown disease mechanism, we can interfere with through drug therapies (Im, 2002).

The "deorphanization" of oGPCRs is identifying highly selective ligands for these receptors. It is one of the essential missions in oGPCR research because it impacts our understanding of the organism's function (Nagata et al., 2016; Wacker et al., 2017). Deorphanization of oGPCRs has advanced in recent years, including *in silico* studies to achieve cost- and time-effective research and *in vitr*o studies to validate a specifically selected list of compounds before continuing with *in vivo* studies to uncover their potential in a disease-specific manner (Pierce et al., 2002; Tang, et al., 2012). *In silico* studies include several aspects. If the structure is unknown, the amino acid sequence is needed to model the 3D structure through various online and offline tools and available algorithms (Laschet et al., 2018; Lin & Civelli, 2004). Once the structure is known, virtual screening for extensive compound libraries can be performed (Morris & Lim-Wilby, 2008). Once there is a list of potential compounds, the *in silico* studies follow up with molecular dynamics simulations and molecular mechanics – generalised-born surface area analyses to investigate the receptor-ligand interaction stability (Hollingsworth & Dror, 2018; Yoshino et al., 2019; Zhang et al., 2017). This strategy stems from structure-based virtual screening studies (Li & Shah, 2017). Once a list of low-energy scoring compounds can be ligand candidates, *in vitro* studies validate the selected candidates. *In vitro*, studies generally investigate the GPCR activation and its activity through specific G protein couplings. General methods used to analyse these include TGFα shedding and cAMP assays, colorimetric assays (Inoue et al., 2012; Tang et al., 2012), and BRET/FRET assays utilising fluorescent dyes (Cottet et al., 2012; Okashah et al., 2019). Then the activity showing validated compounds can be tested *in vivo*. The validated compounds, described as ligand-based virtual screening, can also screen for further active compounds (Hamza et al., 2012).

It is possible to find both the native/orthosteric ligand and allosteric compounds by following the described research strategy, reverse pharmacology (Im, 2002). We will discuss some of the most exciting oGPCRs in the brain, including GPR3, GPR6, GPR12, GPR17, GPR18, GPR26, GPR55, GPR119, GPR150, and GPR160, which play a role in brain (patho)physiology.

4.1 GPR3, GPR6, and GPR12

GPR3, GPR6, and GPR12 comprise a cluster of class A oGPCRs that is phylogenetically related to receptors binding sphingosine-1-phosphate (S1P), lysophosphatidic acid (LPA), cannabinoids, and proopiomelanocortin-derived peptides (George et al., 1997). These three oGPCRs do not only share a high level of sequence similarity (about 60%) but also similar expression patterns across human tissues. Peak expression levels of GPR3/6/12 are found in the CNS, but – in contrast to GPR6 and GPR12 – GPR3 can also be detected in several peripheral tissues and cell types, including the reproductive system and adipocytes (Human Protein Atlas; proteinatlas.org; (Uhlén et al., 2015)).

All three oGPCR are associated with the brain's complex physiological and pathological processes. For instance, GPR3, 6, and 12 promote neurite outgrowth and neuronal cell survival (Geithe, et al. 1981; Masuda et al., 2022; Tanaka et al., 2014; Tanaka et al., 2022). GPR3 is further associated with the early phases of cocaine reinforcement and emotional behaviour during stress, relieving neuropathic pain (Ruiz-Medina et al., 2011; Tourino et al., 2012; Valverde et al., 2009). GPR6 expressed in striatopallidal neurons regulates instrumental conditioning (Lobo et al., 2007), and genetic mapping in outbred mice identified GPR12 as a potent modifier of short-term memory (Hsiao et al., 2020).

Under pathological conditions, GPR12 affects the response to antipsychotic drugs in patients with schizophrenia (Drago & Kure Fischer, 2018; Zhao et al., 2022), while GPR3 and GPR6 play essential roles in neurodegenerative diseases. Seminal studies in GPR3 deficient mouse models and histological analyses of post-mortem brains of diseased patients found a strong link between GPR3 and AD. Mechanistically, GPR3 coupling to β-arrestin stimulates amyloid-β generation and plaque formation through membrane-bound γ-secretase (Huang et al., 2015; Nelson & Sheng, 2013; Thathiah et al., 2013). Intriguingly, G protein-biased GPR3 mutants ameliorate AD pathology in mouse models indicating that G protein-biased agonists of this receptor could provide new cues for developing advanced AD drugs (Huang et al., 2022).

GPR6, in contrast, is linked to PD. GPR6 deficient PD model mice show higher basal locomotor activity and reduced dyskinesia – presumably due to increased striatal dopamine concentrations (Oeckl et al., 2014). These observations spurred the development of a selective GPR6 inverse agonist, CVN424 (Brice et al., 2021; Margolin et al., 2022; Sun et al., 2021). GPR6 inverse agonists like CVN424 normalise activity in basal ganglia circuitry and motor behaviour, and only recently, CVN424 completed clinical trial phase II in PD patients with motor fluctuations (ClinicalTrials.gov

Identifier: NCT04191577). This breakthrough raises hopes for developing mechanistically novel drugs for treating motor symptoms in PD.

4.2 GPR17

The mapping of 78 oGPCRs expression throughout the mouse brain allowed the identification of 25 receptors involved in neuropsychiatric disorders (Ehrlich et al., 2018). Among these oGPCRs, GPR17 is attractive regarding its localization and brain function.

GPR17 is expressed in central brain regions, such as the cortical area serving as a source of inhibitory control and decision-making over other brain areas, the nucleus accumbens (NAc) controlling motivation and reward, the habenula involved in reward valuation and decision-making, the amygdala associated with fear and negative emotions and the midbrain dopaminergic nuclei which are essential for movement and reward-related behaviours (Ehrlich et al., 2018).

Oligodendrocytes express GPR17 in the brain and are a negative regulator of oligodendrocyte transcription factor 1 (Olig1) that promotes oligodendrocyte formation, maturation, and myelination. Thus, GPR17 could be a potential therapeutic target for CNS myelin repair (Chen et al., 2009). Another study also reported that the inhibition of GPR17 signaling pathways in oligodendrocyte precursor cells (OPCs) and depletion of microglia lead to oligodendrocyte maturation and robust myelination of regenerated axons after optic nerve injury. These findings support the importance of GPR17 in oligodendrocyte maturation and its relevance as a target in the myelination process after CNS injury (Wang et al., 2020).

In 2006, Ciana and colleagues showed that GPR17, which is phylogenetically related to cysteinyl leukotriene receptors and P2Y, could be activated by both cysteinyl-leukotriene and uracil nucleotide endogenous ligands (Ciana et al., 2006). However, despite all efforts to deorphanize GPR17, this receptor is still debated in the scientific community due to contradictory findings related to the identity of its endogenous ligand (Benned-Jensen & Rosenkilde, 2010; Ciana et al., 2006; Harden, 2013; Qi et al., 2013). Therefore, determining the GPR17 structure is essential for understanding its physiology and natural ligand prevision. By combining Cryo-Electron microscopy and directed mutagenesis, it has been shown that the GPR17 receptor is auto-activated by its ECL2, which acts as a “self-agonist” by coupling the G_i. In GPR17, ECL2 occupies and covers about 40% of the orthosteric binding pocket and forms several interactions with hydrophilic residues suggesting the presence of a hydrophilic environment and, consequently, the hydrophilic nature of GPR17 endogenous ligand (Ye et al., 2022). Other brain-expressed oGPCRs, such as GPR52 and GPR21,

follow the general activation pattern by their ECL2, providing them with a high basal activity level (Lin et al., 2023; Lin et al., 2020).

4.3 GPR26

GPR26 was first described in 2000 (Lee et al., 2000) as a type A rhodopsin-like oGPCR (Chung et al., 2008). It contains 317 amino acids with conserved sequence similarity among the orthologues. More than 95% sequence identity exists between humans and rodents, indicating high phylogenetic conservation of protein structure and function. GPR26 overexpression demonstrates that it has stimulatory G protein-releasing constitutive activity resulting in increased cAMP concentration in target cells. It is predominantly expressed in brain tissues (Jones et al., 2007), e.g., amygdala, striatum, and hypothalamus, and is related to affective disorders. GPR26 KO mice show more anxiety & depression. Moreover, a two-bottle free-choice paradigm test demonstrated that GPR26 KO mice showed increased alcohol intake, which might be related to addiction (Zhang et al., 2011). Furthermore, GPR26 is involved in glioma and hepatocellular carcinoma. Both cancer types are epigenetically downregulated (Meng et al., 2020). Lastly, since targeted inactivation of GPR26 leads to hyperphagia (Chen et al., 2012), this supports the idea that it is involved in appetite. Studies related to obesity, hyperglycemia, and type-2 diabetes are undergoing (Kichi et al., 2022).

4.4 GPR50

GPR50 is an oGPCRs belonging to the melatonin receptor family with sequence homology with MT1 and MT2, with which it heterodimerises (Levoye et al., 2006). It has lost the ability to bind melatonin during evolution (Clement et al., 2018). Some mechanisms of regulation by heterodimerization with other receptors (Levoye et al., 2006; Wojciech et al., 2018) or involving its C-terminal domain (Ahmad et al., 2020) have been proposed without knowing their physiological implication. It has been almost 30 years since this GPR50 was found to be expressed in chromosome X, yet the function of this receptor, which is found in parts of the brain (Hamouda et al., 2007) is not well known. In the neuronal-like rat cell line *Neuroscreen-1* (*NS-1*), its expression has been associated with increased neurite outgrowth (Grünewald et al., 2009; Xu et al., 2022). Some sporadic studies suggest that GPR50 is involved in regulating energy homeostasis and fasting-induced torpor (Bechtold et al., 2012). In a small number of studies, GPR50 locus has been associated with psychiatric disorders, including schizophrenia and bipolar disorders (Thomson et al., 2005), but these data have not consistently been replicated. A possible association with seasonal affective disorders (Delavest et al., 2012), autism spectrum disorders, and depression has been proposed with a sex-dependent susceptibility.

4.5 GPR55, GPR18, and GPR119 as Endocannabinoid Receptors?

Some oGPCRs, such as GPR55, GPR18, and GPR119, which are type A rhodopsin-like oGPCRs, are activated by lipid molecules that can be assigned to the world of endocannabinoids known as the endocannabinoidome including N-acyl amino acids (NAAAs) and N-acylethanolamines (NAEs) (Cristino et al., 2020; Pacher et al., 2020). *In vivo,* activation of the receptors by these lipid molecules is still pending, but *in vitro,* data showed activation by N-arachidonoylserine (AraS) for the $G\alpha_{12/13}$- (and probably $G\alpha_q$-) protein-coupled GPR55 (Pacher et al., 2020). Lysophosphatidylinositol was reported to elevate synaptic drive in a CBD- and GPR55-dependent manner (Rosenberg et al., 2023). The endocannabinoid 2-AG can also act through GPR55 (Ross, 2009). For the $G\alpha_{i/o}$-coupled GPR18, several ligands such as N-arachidonoylglycine (AraG), AraS, N-palmitoylethanolamide (PEA), abnormal cannabidiol, 2-AG, resolvin D2, and THC have been mentioned (Pacher et al., 2020; Schoeder et al., 2020). GPR119 has been described as a receptor for N-oleoylethanolamide (OEA), oleoyl- and palmitoyl-lysophosphatidylcholine and is coupled to the AC-cAMP pathway (Pacher et al., 2020). About some contradictory results, it must be considered that a given ligand may have completely different effects in different signalling pathways due to biased signalling.

4.6 GPR160

GPR160 is a type A rhodopsin-like oGPCRs member. The orthologues of GPR160 exist in diverse vertebrates, including mice, monkeys, cows, etc., with high sequence coverage. However, GPR160 shares at most 18% sequence identity with the members of the GPCR superfamily, complicating the structural predictions. The highest expression occurs in the small intestine, colon, bone marrow, and kidney on a tissue basis. Even though its function has not been fully elucidated, it's been implicated in apoptosis and cell cycle arrest in prostate cancer and associated with traumatic nerve injury and pain (Yosten et al., 2020; C. Zhou et al., 2016).

Recently, Yosten et al. revealed that GPR160 is upregulated in the spinal cord upon traumatic nerve injury. In addition, monoclonal antibodies (mAb) targeting GPR160 abolished the neuropathic pain, while normal pain was still observed. Cocaine- and amphetamine-regulated transcript peptide (CARTp) polypeptide may also function as a natural ligand. Supporting this, upon inhibition of CARTp, downregulation of ERK and cAMP response element-binding protein (CREB) phosphorylation is observed as same as in inhibition of GPR160 (Yosten et al., 2020).

4.7 How to Deorphanize the Orphan GPCRs (Reverse Physiology, Bioinformatics)

Deorphanization of oGPCRs is complicated due to the membrane embedded in them. In 2000, the x-ray crystal structure of the rhodopsin receptor was found for the first time (Palczewski et al., 2020). After 11 years, the structure of the adrenergic receptors has been discovered (Rasmussen et al., 2011). It can be seen from here that it takes years to solve the structure of GPCRs. In recent years, the structure of GPCRs has been studied more efficiently using bioinformatic analysis. Firstly, the sequence of the targeted GPCR must be identified, and the homology model using similar models must be constructed. If there are no comparable models and their similarity is less than 30%, creating a shared model by building multiple models and superimposing them is essential. Or Ab-initio is an *in silico* method used to model a protein given its sequence when there are no similar sequences or crystal structures to use as a template (Alford et al., 2017). The next step is to generate the orthosteric binding pocket. To verify the binding pocket, site-directed mutagenesis has to be done. Then different libraries can be screened, and hit molecules can be verified by in vitro assays (Figure 1). To find the native ligand, reverse physiology (Birgül et al., 1999) can be applied. Proteins, lipids, or metabolites can be isolated from the tissue in which the receptor is expressed. After several HPLC steps, the purified ligand can be sequenced.

With the recently developed bioinformatics programs, starting from the sequences of these oGPCRs, their structures are extracted, and a homology model is created. After completing the model, the orthosteric binding pocket is determined and conformed with site-directed mutagenesis. Afterward, different libraries can be scanned, and hit molecules that bind to the binding pocket with low energy can be verified. Finally, those hit molecules' activity can be measured by *in vitro* assays (**Figure 2**).

There are about 100 oGPCRs whose endogenous ligand and physiological roles are still unknown (Watkins & Orlandi, 2021). Considering that GPCRs are the most important drug targets, it is essential to deorphanize all oGPCRs.

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Disclosure

The authors have no multiplicity of interest to disclose.

5 Figures and Figure Legends

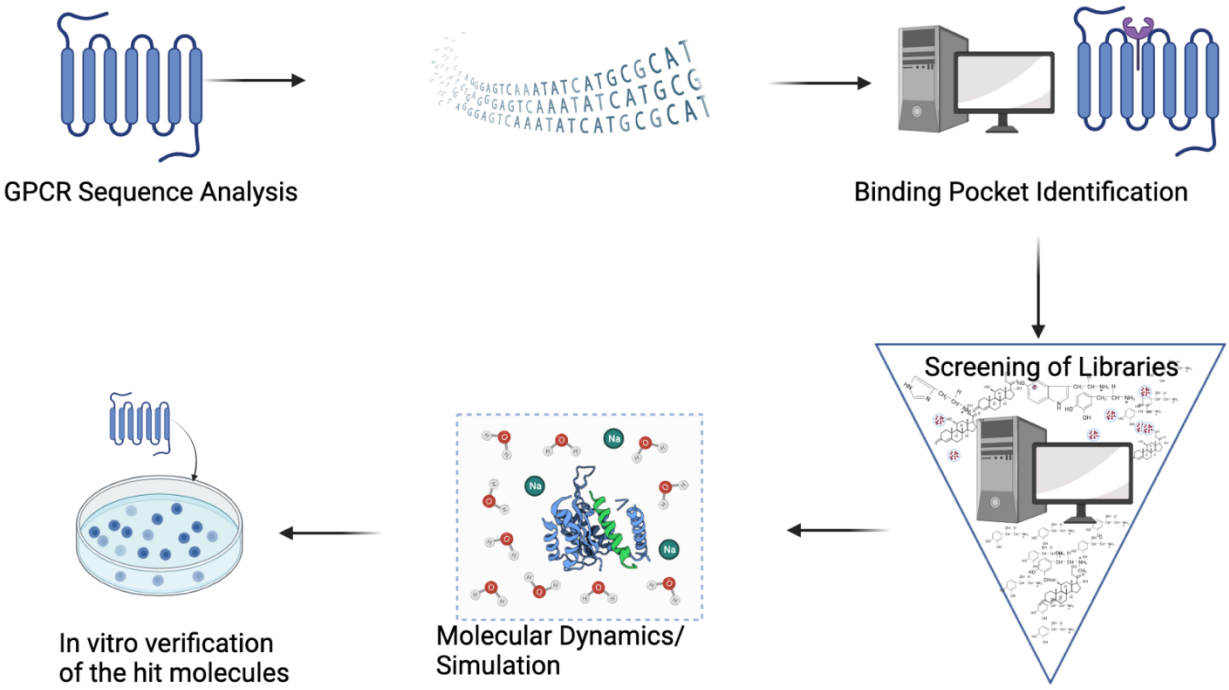

Figure 1. *In silico* strategy for deorphanization of oGPCRs. The first step is to model the 3D structure of the oGPCR through various online and offline tools and available algorithms. The second step is to find the orthosteric binding pocket and verify it by site-directed mutagenesis. The final step is to use molecular dynamics simulations of different *in silico* libraries, identify low-energy scoring compounds, and verify those by *in vitro* assays.

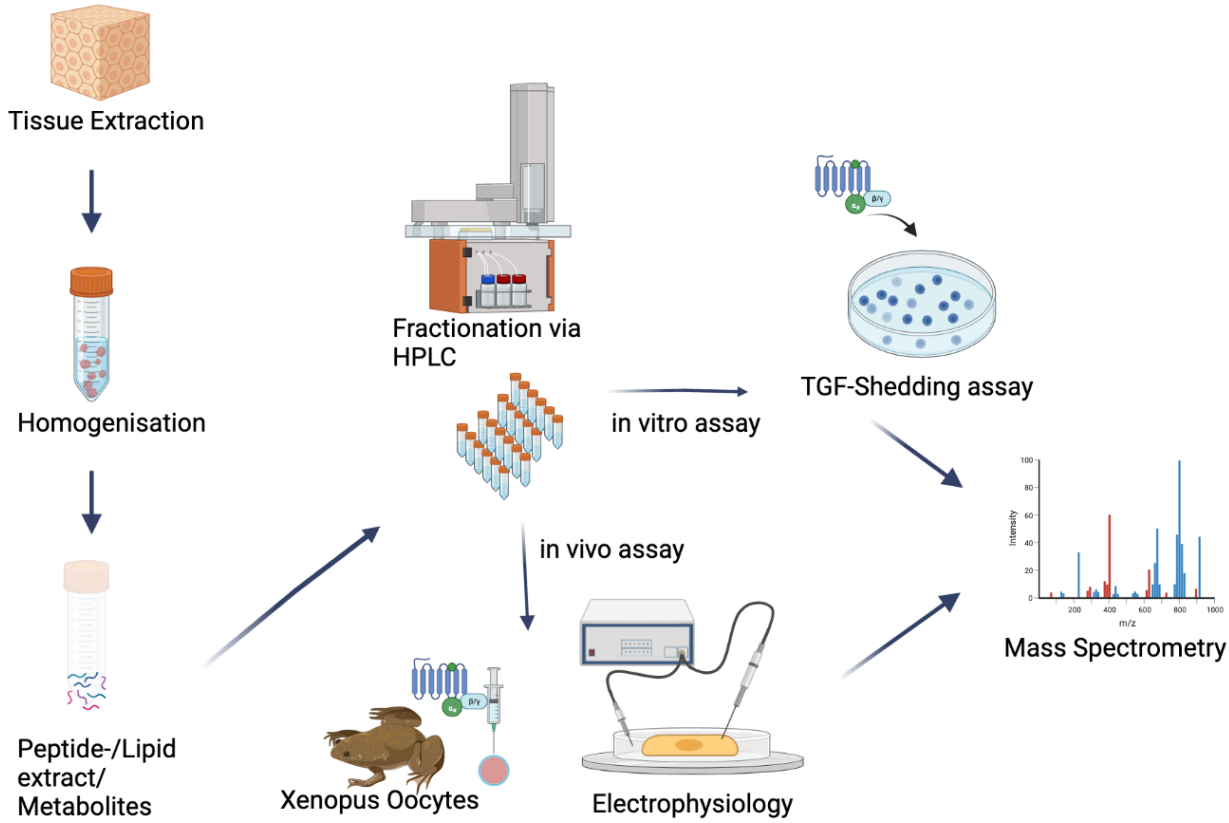

Figure 2. Isolation of the native ligand from tissue in which the oGPCR is expressed; the first step is to homogenise the tissue in which the GPCR is expressed. The second step is to undergo several fractionation steps *via* HPLC, and the biologically active fraction has to be verified by in vitro such as TGF shedding assay. The final step is *in vivo* analysis, such as electrophysiology in whole *Xenopus* oocytes.

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