



**HAL**  
open science

# The voltage sensitivity of G-protein coupled receptors: Unraveling molecular mechanisms and physiological implications

Marin Boutonnet, Moritz Bünemann, Julie Perroy

## ► To cite this version:

Marin Boutonnet, Moritz Bünemann, Julie Perroy. The voltage sensitivity of G-protein coupled receptors: Unraveling molecular mechanisms and physiological implications. *Pharmacology and Therapeutics*, 2024, 264, pp.108741. 10.1016/j.pharmthera.2024.108741 . hal-04770181v2

**HAL Id: hal-04770181**

**<https://hal.science/hal-04770181v2>**

Submitted on 12 Nov 2024

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution 4.0 International License



# The voltage sensitivity of G-protein coupled receptors: Unraveling molecular mechanisms and physiological implications

Marin Boutonnet<sup>a</sup>, Moritz Bünemann<sup>b</sup>, Julie Perroy<sup>a,\*</sup>

<sup>a</sup> IGF, University of Montpellier, CNRS, INSERM, Montpellier, France

<sup>b</sup> Department of Pharmacology and Clinical Pharmacy, Philipps-University Marburg, Marburg, Germany

## ARTICLE INFO

### Article history:

Received 16 July 2024

Received in revised form 11 September 2024

Accepted 29 October 2024

Available online 01 November 2024

Associate editor: M. Curtis

### Keywords:

G-protein coupled receptors

Voltage sensitivity

Voltage sensor

Biased signaling

Functional selectivity

## ABSTRACT

In the landscape of proteins controlled by membrane voltage ( $V_m$ ), like voltage-gated ionotropic channels, the emergence of the voltage sensitivity within the vast family of G-protein coupled receptors (GPCRs) marked a significant milestone at the onset of the 21st century. Since its discovery, extensive research has been devoted to understanding the intricate relationship between  $V_m$  and GPCRs. Approximately 30 GPCRs out of a family comprising more than 800 receptors have been implicated in  $V_m$ -dependent positive and negative regulation. GPCRs stand out as the quintessential regulators of synaptic transmission in neurons, where they encounter substantial variations in  $V_m$ . However, the molecular mechanism underlying the  $V_m$  sensor of GPCRs remains enigmatic, hindered by the scarcity of mutant GPCRs insensitive to  $V_m$  yet functionally intact, impeding a comprehensive understanding of this unique property in physiology. Nevertheless, two decades of dedicated research have furnished numerous insights into the molecular aspects of GPCR  $V_m$ -sensing, accompanied by recently proposed physiological roles as well as pharmacological potential, which we encapsulate in this review. The  $V_m$  sensitivity of GPCRs emerges as a pivotal attribute, shedding light on previously unforeseen roles in synaptic transmission and extending beyond, underscoring its significance in cellular signaling and physiological processes.

© 2024 The Author(s). Published by Elsevier Inc. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

## Contents

1. Introduction: voltage sensitivity of G-protein coupled receptors . . . . .	2
2. Molecular mechanisms of GPCR voltage sensing . . . . .	2
3. The role of GPCR voltage sensitivity . . . . .	10
CRedit authorship contribution statement . . . . .	12
Declaration of competing interest . . . . .	12
Acknowledgments . . . . .	12
References . . . . .	12

**Abbreviations:** 5HT<sub>1A-2A</sub>, 5-Hydroxytryptamine receptor 1A and 2A;  $\alpha_{2A-\beta 1-2}$ -AR, Adrenergic receptor  $\alpha_{2A}$ ,  $\beta_1$  and  $\beta_2$ ; A1AR, Adenosine receptor 1; ACh, Acetylcholine; AP, Action potential; ATP, Adenosine triphosphate; AR, Ca<sup>2+</sup>, Calcium ion; CB<sub>1</sub>, Cannabinoid receptor 1; D<sub>1-4</sub>, Dopamine receptor subtype 1 to 4; EP EPSP, Excitatory postsynaptic potential; FP, Prostaglandin F receptor; FRET, Fluorescence Resonance Energy Transfer; GIRK, G-protein-activated inwardly rectifying potassium channel; GPCRs, G-protein coupled receptors; GRK, GPCR Kinases; GTP $\gamma$ S, Guanosine triphosphate  $\gamma$ S; H<sub>1-4</sub>, Histamine receptor 1 à 4; HEK 293 T, Human embryonic kidney 293 T cells; IKACH, Acetylcholine-gated inward rectifier potassium current; IP, Prostacyclin receptor; IP<sub>3</sub>, Inositol trisphosphate; LPA, Lysophosphatidic acid receptor; M<sub>1-5</sub>, Muscarinic acetylcholine receptor 1 to 5; mAChR-A, Muscarinic acetylcholine receptor A; mGlu1–5, Metabotropic glutamate receptor 1 to 5; Na<sup>+</sup>, Sodium ion; NAM, Negative allosteric modulator; NMDA, N-methyl-D-aspartate; NT, Neurotransmitter; P2Y<sub>1-12</sub>, Purinergic receptor P2Y1 and P2Y12; PAM, Positive allosteric modulator; PTX, Pertussis toxin; TM, Transmembrane; TP, Thromboxane receptor; TRPC6, Transient receptor potential canonical 6;  $V_m$ , Membrane voltage.

\* Corresponding author.

E-mail address: [julie.perroy@igf.cnrs.fr](mailto:julie.perroy@igf.cnrs.fr) (J. Perroy).

## 1. Introduction: voltage sensitivity of G-protein coupled receptors

G-protein coupled receptors (GPCRs), also known as metabotropic receptors, serve as mediators of the majority of intracellular signaling pathways in response to external stimuli, including light, odorant molecules, hormones, and neurotransmitters (NTs). They constitute the primary targets of pharmacological drugs currently under development (Sutkevičiute & Vilardaga, 2020; Weis & Kobilka, 2018). Structurally, GPCRs consist of seven transmembrane (TM) domains interconnected by three intracellular and three extracellular loops, and they are categorized into different classes, including classes A, B, C, and F (Sutkevičiute & Vilardaga, 2020; Wootten et al., 2018).

At the close of the 20th century, several research groups began observing the membrane voltage ( $V_m$ ) regulation of certain G-protein coupled receptors (GPCRs) functions. This exploration commenced with investigations into the impact of electrical pulses on the binding of antagonists to muscarinic receptors in synaptosomes (Luqmani et al., 1979). Subsequent years unveiled  $V_m$ 's regulatory influence on various GPCR signaling pathways, including the activation of G-proteins in synaptosomes (Anis et al., 1999; Cohen-Armon & Sokolovsky, 1993) and pancreatic cells (Ong et al., 2001); inositol trisphosphate ( $IP_3$ ) synthesis in mesenteric artery cells (Itoh et al., 1992); and the muscarinic receptor-induced elevation of cytoplasmic  $Ca^{2+}$  levels in lacrimal glands (Marty & Tan, 1989). Further investigations into pre-synaptic muscarinic receptor signaling (Ilouz et al., 1999; Slutsky et al., 1999, 2002) and purinergic receptors in megakaryocytes (Mahaut-Smith et al., 1999; Mason et al., 2000; Mason & Mahaut-Smith, 2001) yielded similar findings, prompting some research groups to scrutinize the nature of  $V_m$  effects on these receptors. In 2003, Hanna Parnas' group asserted, for the first time, the direct sensitivity of GPCRs to  $V_m$  while examining muscarinic receptor-induced currents (Fig. 1A). Through comprehensive dissection of each signaling step, they demonstrated that depolarization positively modulates ( $M_1$ ) or negatively modulates ( $M_2$ ) these receptors, thereby influencing ligand binding and receptor signaling pathways (Ben-Chaim et al., 2003). Martyn P. Mahaut-Smith's team corroborated these findings for the  $P2Y_1$  receptor by investigating  $P2Y_1$ -induced intracellular  $Ca^{2+}$  variations (Martinez-Pinna et al., 2004; Martinez-Pinna et al., 2005). The presence

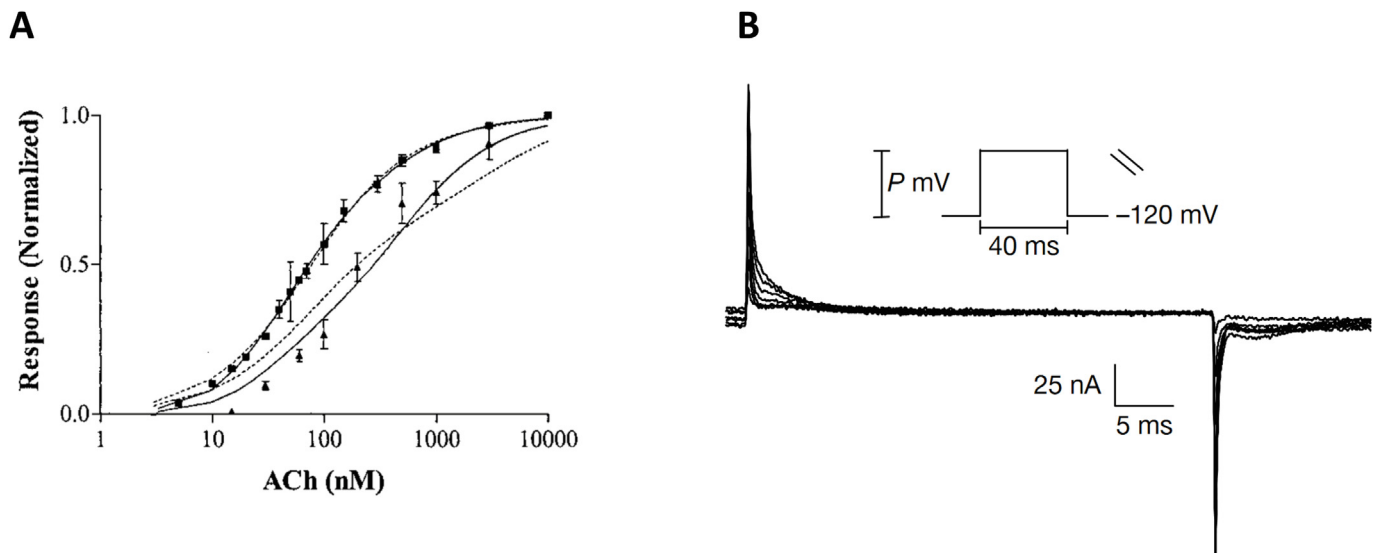
of a  $V_m$  sensor within these receptors was unequivocally demonstrated by recording a gating current reflecting the movement of gating particles along the  $V_m$  within the  $M_2$  receptor, indicating a charge movement of approximately 0.5–0.85 elementary charges per receptor (Fig. 1B, (Ben-Chaim et al., 2006; Navarro-Polanco et al., 2011)). In subsequent years, an increasing number of GPCRs were found to be sensitive to  $V_m$  (Table 1), prompting the scientific community to investigate the  $V_m$ -sensitive region of the receptor and the role of this sensitivity.

## 2. Molecular mechanisms of GPCR voltage sensing

The quest to identify the  $V_m$  sensor within GPCRs has sparked considerable debate in recent years (Bezanilla, 2008; David et al., 2022; Mahaut-Smith et al., 2008; Vickery, Machtens, & Zachariae, 2016) with no definitive consensus on its precise nature thus far. Indeed, GPCR  $V_m$  sensitivity appears to be either a highly receptor-specific mechanism or an extremely difficult to study conserved mechanism, and as of yet, it remains challenging to synthesize all findings into a unified model. Nonetheless, multiple lines of evidence have been advanced to elucidate the operation of the  $V_m$  sensor within GPCRs, which we will delineate subsequently.

### 2.1. Voltage-dependent intramolecular movements

The regulatory influence of  $V_m$  on ligand efficacy prompted early investigations into the  $V_m$  sensitivity of GPCRs, suggesting a  $V_m$ -mediated effect on agonist-induced receptor conformational changes (Navarro-Polanco et al., 2011). Subsequent research utilizing real-time monitoring techniques directly demonstrated  $V_m$ -dependent alterations in receptor conformation upon activation, particularly in response to agonist stimulation. For instance, studies employing site-directed fluorescence labeling with tetramethylrhodamine maleimide revealed that  $V_m$  imposes a distinct conformational state on GPCRs, even in the absence of ligands, implicating  $V_m$  in influencing receptor conformation upstream of agonist binding (Barchad-Avitzur et al., 2016; Dekel et al., 2012). Investigations employing FRET sensors to monitor the conformational dynamics of the  $\alpha_{2A}$  adrenergic receptor



**Fig. 1.** First evidence of GPCR voltage-sensitivity - The voltage-dependence of the  $M_2$  receptor.

A: Dose-response curves of ACh-evoked GIRK currents obtained at  $-60$  mV and  $+40$  mV show a reduced potency of the ACh at depolarized potentials. The lines were generated by two different fitting equations assuming there is two affinity state (high and low affinity) with one binding site (dashed line) or two binding sites (solid lines). Results from (Ben-Chaim et al., 2003).

B: Gating currents elicited by 40 ms depolarizing pulses (P) with 20-mV steps from a holding potential of  $-120$  mV on  $M_2$  transfected oocytes. Inset: the pulse protocol. Adapted from (Ben-Chaim et al., 2006).

**Table 1**  
Voltage sensitivity of GPCRs.

GPCR family	GPCR	Ligands	Evidences	Cell type	References
Serotonine	5HT <sub>1A</sub>	5-Hydroxytryptamine, Tansospirone	↘ Agonist potency for receptor-induced GIRK currents	Xenopus Oocyte	Tauber et al. 2022
		Buspirone	→ Agonist potency for receptor-induced GIRK currents	Xenopus Oocyte	
	5HT <sub>2A</sub>	5-Hydroxytryptamine	↗ Receptor-induced Ca <sup>2+</sup> release	Megakaryocyte	Martinez-Pinna et al. 2005
Adenosine	A1AR	Adenosine	↘ Agonist potency for receptor-induced IKAch currents	Cardiomyocyte	López-Serrano et al. 2022
Adrenergic	α <sub>2A</sub> -AR	Clonidine	↘ Agonist efficacy for induced intramolecular conformational changes and β-arrestine2-receptor interaction	HEK293	Rinne et al. 2013
		Noradrenaline	↘ Agonist potency and efficacy for induced intramolecular conformational changes, Gi-protein-receptor and β-arrestine2-receptor interactions. ↘ Receptor-induced GIRK currents	HEK293	
	β <sub>1</sub> -AR	Isoprenaline and Adrenaline	↘ Agonist affinity and efficacy for induced intramolecular conformational changes, receptor-Gs-protein interaction and receptor-β-arrestine3 interaction.	HEK293	Birk et al. 2015
		Noradrenaline	↘ Agonist efficacy for induced receptor-β-arrestine3 interaction	HEK293	
	β <sub>2</sub> -AR	Isoprenaline	↘ Agonist-induced β-arrestin3-receptor interaction and receptor-induced cAMP increase	HEK293	Birk et al. 2015; Kurz et al. 2020
Cannabinoid	CB <sub>1</sub>	Anandamide	→ Agonist potency for receptor-induced GIRK currents	Xenopus Oocyte	Goldberger et al. 2022
		2-Arachidonoylglycerol, Tetrahydrocannabinol	↗ Agonist potency and ↘ deactivation time for receptor-induced GIRK currents	Xenopus Oocyte	
Dopamine	D <sub>1</sub>	Dopamine	↘ Agonist potency for receptor-induced GIRK currents	Xenopus Oocyte	Ågren & Sahlholm 2020
	D <sub>2L</sub>	Dopamine	↘ Agonist potency for receptor-induced GIRK currents	Xenopus Oocyte	Sahlholm et al. 2008c
	D <sub>2s</sub>	(S)-AT, Rotigotine, 3-MT, Apomorphine, NPA	→ Agonist potency and efficacy for receptor-induced GIRK currents	Xenopus Oocyte	Sahlholm et al. 2011
		Dopamine	↘ Agonist potency for receptor-induced GIRK currents and Gi-protein activation	HEK293	Sahlholm et al. 2011; 2008a
		N-dipropyl-m-tyramine, Bromocriptine, Pergolide, Piribedil	↘ Agonist potency and efficacy for receptor-induced GIRK currents	Xenopus Oocyte	Sahlholm et al. 2011
		p-Tyramine, m-Tyramine, β-Phenethylamine, Catechol	→ Agonist potency for receptor-induced GIRK currents	Xenopus Oocyte	Sahlholm et al. 2011; 2008b
		S-5-OH-DPAT, R-5-OH-DPAT, R/S-DPAT, R-7-OH-DPAT N-dipropyl-dopamine, (S)-5-OH-AT, Lisuride, Pramipexole, Ropinirole	↘ Agonist potency for receptor-induced GIRK currents	Xenopus Oocyte	Sahlholm et al. 2011; 2008b
		D <sub>3</sub>	Dopamine	→ Agonist potency for receptor-induced GIRK currents	Xenopus Oocyte
	D <sub>4.4</sub>	Dopamine	↘ Agonist potency for receptor-induced GIRK currents	Xenopus Oocyte	Sahlholm et al. 2008a
	D <sub>5</sub>	Dopamine	↘ Agonist potency for receptor-induced GIRK currents	Xenopus Oocyte	Ågren & Sahlholm 2020

<b>Muscarinic</b>	<b>M<sub>1</sub></b>	<b>Acetylcholine</b>	↗ Agonist potency for receptor-induced Ca <sup>2+</sup> -activated Cl <sup>-</sup> currents, agonist binding, agonist-induced β-arrestin3-receptor interaction and Gq-protein-receptor interaction.	Xenopus Oocyte and HEK293T	Ben-Chaim et al. 2003; Rinne et al. 2015; Hoppe et al. 2018
		<b>Carbachol</b>	↗ Agonist potency for intramolecular conformational changes. Increase of receptor-induced Ca <sup>2+</sup> release and induced Gq-protein-receptor interaction	HEK293	Rinne et al. 2015
		<b>Oxotremorine-M</b>	↗ Agonist-induced intramolecular conformational changes	HEK293	Tateyama & Kubo 2013
	<b>M<sub>2</sub></b>	<b>Acetylcholine</b>	↘ Agonist affinity for receptor-induced GIRK currents, agonist binding and increase of dissociation rate. ↘ Agonist potency for receptor-induced IKAch currents and agonist-induced intramolecular conformational changes	Xenopus Oocyte Atrial myocyte	Ben-Chaim et al. 2003, 2006, 2013; Navarro-Polanco et al. 2011; Barchad-Avitzur et al. 2016; Dekel et al. 2012; Moreno-Galindo et al. 2016; Agren et al. 2018; Sahlholm et al. 2008b
		<b>AF-DX 384</b>	→ Antagonist potency for receptor-induced GIRK currents (in presence of Ach or Pilocarpine)	Xenopus Oocyte	Hazan et al. 2024
		<b>Atropine</b>	↘ Antagonist potency for receptor-induced GIRK currents (in presence of Ach or Pilocarpine)	Xenopus Oocyte	Hazan et al. 2024
		<b>Bethanechol</b>	→ Agonist-induced IKAch current	Atrial Myocyte	Moreno-Galindo et al. 2016
		<b>Carbachol</b>	↘ Acetylcholine release	Xenopus Oocytes and frog NMJ	Kupchik et al. 2011
		<b>Carbachol</b>	↘ Agonist potency for induced Gq-protein-receptor interaction	HEK293	Rinne et al. 2015; Hoppe et al. 2018
		<b>Iperoxo</b>	↘ Agonist potency for IKAch currents activation	Atrial Myocyte	López-Serrano et al. 2020
		<b>Oxotremorine</b>	↘ Agonist potency for receptor-induced GIRK currents and decrease of agonist binding	Xenopus Oocyte	Ben-Chaim et al. 2003
		<b>Pilocarpine, Choline</b>	↗ Agonist efficacy and potency for IKAch currents activation and receptor-evoked GIRK currents	Atrial Myocyte	López-Serrano et al. 2020; Barchad-Avitzur et al. 2016; Navarro-Polanco et al. 2013; Moreno-Galindo et al. 2016
		<b>Scolapine</b>	↘ Antagonist potency for receptor-induced GIRK currents (in presence of Ach or Pilocarpine)	Xenopus Oocyte	Hazan et al. 2024
		<b>Without ligand</b>	↘ GIRK currents constitutive activation	Xenopus Oocyte	Ben-Chaim et al. 2019
		<b>Without ligand</b>	Conformational changes upon depolarization	Xenopus Oocyte	Ben-Chaim et al. 2006; Dekel et al. 2012; Barchad-Avitzur et al. 2016
	<b>M<sub>3</sub></b>	<b>Acetylcholine</b>	↘ Agonist-induced β-arrestin3-receptor interaction	HEK293	Hoppe et al. 2018
		<b>Acetylcholine, Carbachol</b>	↘ Agonist-induced Gq-receptor interaction	HEK293	Rinne et al. 2015
	<b>M<sub>5</sub></b>	<b>Pilocarpine</b>	↗ Agonist-induced Gq-protein-receptor interaction	HEK293	Rinne et al. 2015
	<b>m-AChR-A</b>	<b>Acetylcholine</b>	↗ Agonist potency for receptor-induced GIRK currents	Xenopus Oocyte	Rozenfeld et al. 2021
		<b>Muscarine</b>	↗ Agonist potency for muscarine-induced currents	Antennal lobes local neurons in vivo	Rozenfeld et al. 2021
			Receptor Vm-sensitivity is required for synaptic potentiation during high frequency stimulation protocol, nicotinic receptor activation and odor habituation		

Glutamate	mGlu1	Glutamate	↗ Agonist potency for receptor-induced Ca <sup>2+</sup> -induced Cl <sup>-</sup> currents and agonist binding	Xenopus Oocyte	Ohana et al. 2006
	mGlu3	Glutamate	↘ Agonist potency for receptor-induced GIRK currents and decrease of max. binding	Xenopus Oocyte	Ohana et al. 2006
		LY341495	→ Antagonist potency for receptor -induced GIRK currents	Xenopus Oocyte	
	mGlu5	DHPG	↘ Agonist efficacy for induced Gq activation, TRPC6 & NMDAR gating	HEK293 and Hippocampal neurons	Boutonnet et al. 2024
		LY341495	↗ Antagonist potency for induced receptor intramolecular changes (with residual agonist)		
Quisqualate		↘ Agonist efficacy for induced receptor intramolecular conformational changes and receptor-induced Ca <sup>2+</sup> release			
Purinergic	P2Y <sub>1</sub>	ADP, 2MeSADP, ATP	↗ Receptor-induced Ca <sup>2+</sup> release	Megakaryocyte	Martinez-Pinna et al. 2004, 2005; Gurung et al. 2008; Tateyama & Kubo 2013
		MRS2179, A3P5PS	↗ Antagonist-induced calcium release (without agonist)	Megakaryocyte	
	P2Y <sub>12</sub>	2MesADP	↘ Receptor (Gi3q)-induced Ca <sup>2+</sup> release	HeLa	Zhang et al. 2020
		ADP	↘ Agonist potency for receptor-induced GIRK currents	HEK293	
		ATP	↘ Receptor-induced tonic block of catecholamine release	Chromaffin cell from surrenal gland	
Histamine	H <sub>3</sub> 445	Histamine, (R)- $\alpha$ -methylhistamine	↘ Agonist potency and deactivation for receptor-induced GIRK currents	Xenopus Oocyte	Sahlholm et al. 2012
	H <sub>3</sub> 365	Histamine	↘ Agonist potency and deactivation for receptor-induced GIRK currents	Xenopus Oocyte	
	H <sub>4</sub>	(R)- $\alpha$ -methylhistamine	→ Agonist potency and receptor deactivation for receptor-induced GIRK currents	Xenopus Oocyte	
		Histamine, VUF 8430	↘ Agonist potency and deactivation for receptor-induced GIRK currents	Xenopus Oocyte	
Opioid	$\mu$ -opioid	Buprenorphine, pethidine, etorphine, tramadol, PZM21	↗ Receptor-induced Gi activation	HEK293	Kirchhofer et al.2023
		DAMGO	↗ Agonist potency and efficacy for receptor-induced Kir3.X currents, induced receptor-Gi protein and receptor- $\beta$ -arrestine3 interactions	HEK293	Ruland et al. 2020; Kirchhofer et al. 2023
		DAMGO	→ Agonist-induced receptor-GRK2 interaction	HEK293	Ruland et al. 2020
		Fentanyl	↘ Agonist efficacy for receptor-induced Kir3.X currents, induced Gi-protein-receptor, GRK2-receptor and $\beta$ -arrestin3-receptor interactions.	HEK293	Ruland et al. 2020; Kirchhofer et al. 2023
		Met-Enkephalin	→ Receptor-induced Kir3.X currents, induced Go-receptor and $\beta$ -arrestine3-receptor interactions	HEK293	Ruland et al. 2020
		Methadone, meptazinol, loperamide, TRV130	↘ Receptor-induced Gi activation	HEK293	Kirchhofer et al.2023
		Morphine	↗ Agonist efficacy for receptor-induced Kir3.X currents induced receptor-Gi protein, GRK2-receptor and $\beta$ -arrestine3-receptor interactions	HEK293 and Locus Coeruleus neurons	Ruland et al. 2020; Kirchhofer et al. 2023
		Naloxone	Antagonist converted into an agonist for receptor-induced GIRK current and Gi-protein activation	HEK293	Kirchhofer et al.2023
		SR17018	→ Receptor-induced Gi activation	HEK293	Kirchhofer et al.2023
Prostannoid	LPA	Lysophosphatidic Acid	↗ Receptor-induced Ca <sup>2+</sup> release	Xenopus Oocyte	Martinez-Pinna et al. 2010
	FP	U46619	↗ Agonist induced Gq-protein-receptor interaction	HEK293	Kurz et al. 2020
	TP	1-BOP, 8-iso PGE2, 8-iso PGE1, PGE2 Ethanolamide, PGE2 methyl ester, 15-Keto-PGE2, 15(S)-15-methyl PGE2	↗ Receptor-induced G13-protein activity (G13-p115 interaction)	HEK293	Kurz et al. 2020
		U46619	↗ Receptor-induced Ca <sup>2+</sup> release. ↗ Agonist potency for induced intramolecular conformational changes, G13-receptor / G13-p115 interactions and Gq activation.	HEK 293 Megakaryocyte	Martinez-Pinna et al. 2005; Kurz et al. 2020
	IP	Isopreterenol, Iloprost	→ Receptor-induced cAMP increase	HEK293	Kurz et al. 2020
	EP <sub>3</sub>	Iloprost	↗ Receptor-induced GIRK current	HEK293	Kurz et al. 2020

The ligands used for revealing V<sub>m</sub>-sensing effects and functions are displayed in blue (agonists) and red (antagonists). The polarity of depolarization-induced receptor variations of activity is indicated by colors, rather negative (red), positive (green) or absent (gray). Only functional evidences of V<sub>m</sub> sensitivity are highlighted in this table (the gating current measurements or molecular dynamic simulations results are omitted). Supplementary sources: (Sahlholm, Nilsson, Marcellino, Fuxe, & Arhem, 2008); (Sahlholm, Marcellino, Nilsson, Fuxe, & Arhem, 2008) c; (Ben Chaim et al., 2013); (Ågren & Sahlholm, 2020); (Martinez-Pinna et al., 2010).

similarly indicated changes in conformation following membrane depolarization, albeit exclusively during agonist binding and receptor activation (Rinne et al., 2013). Analogously, observations in  $\beta$ -adrenergic receptors as well as thromboxane receptors also suggest  $V_m$  influences receptor function predominantly during the activation process (Birk et al., 2015), although gating charges may undergo movements within receptors even in the absence of ligands, as evidenced in the  $M_2$  receptor (Ben-Chaim et al., 2006; Navarro-Polanco et al., 2011). Thus,  $V_m$  appears to modulate receptor conformation both in the presence and absence of ligands, indicating its role in regulating overall receptor flexibility.

While it is evident that GPCRs are directly impacted by  $V_m$ , two fundamental questions persist: How do GPCRs perceive changes in  $V_m$ ? Which critical components of GPCRs are involved in this process? Among the protein families previously identified as sensitive to  $V_m$ , a consensus  $V_m$  sensor sequence emerges between ion channels and phosphatases, the S4 segment (Bezaniilla, 2008). Upon analyzing the protein sequences of GPCRs, no domain analogous to the S4 segment of  $V_m$ -gated channels was identified, indicating a distinct  $V_m$  sensor (Mahaut-Smith et al., 2008).

## 2.2. 1st model: GPCR G-protein binding domains

The first determinant that could be considered as a  $V_m$  sensor in GPCRs is the G-binding domain. Indeed, the  $M_2$  receptor demonstrates sensitivity to  $V_m$  only in the presence of a G protein, as its agonist binding remains unaffected by  $V_m$  changes following treatment with pertussis toxin (PTX), which catalyzes adenosine diphosphate ribosylation of the  $\alpha$  subunits of  $G_i$  proteins, thereby inhibiting their coupling to the receptor (Ben-Chaim et al., 2003; Katada, 2012). Hence, early hypotheses proposed a  $V_m$  sensor within the G-protein coupling region (Ben-Chaim et al., 2003), a notion supported by subsequent evidence. Notably, the polarity of  $V_m$ 's effect appears to be specific to the type of G protein. For instance,  $G_i$ -coupled receptors are primarily inhibited by depolarization upon binding to their endogenous agonists (e.g.,  $M_2$ , mGlu3,  $D_2$ ,  $D_4$ , P2Y<sub>12</sub>, 5HT<sub>1A</sub>, H<sub>3</sub>, H<sub>4</sub>, and  $\alpha_{2A}$ ), whereas the majority of  $V_m$ -sensitive  $G_q$ -coupled receptors are potentiated by depolarization (e.g.,  $M_1$ , mGlu1, LPA, P2Y<sub>1</sub>, 5HT<sub>2A</sub>, TP, FP receptors), suggesting a potential role for G-protein coupling in the polarity of  $V_m$ 's effect (refer to Table 1). Consistently, G-protein recruitment by many GPCRs, such as the dopamine  $D_2$  receptor, is shown to be  $V_m$ -dependent (Sahlholm et al., 2011). Moreover, critical residues for G-protein coupling, including charged amino acid moieties (KKDKK) in the intracellular loop 3 of the  $M_2$  receptor, appear to be necessary for  $V_m$  sensitivity (Ben-Chaim et al., 2006). Similarly, alterations in the intracellular loops required for G protein-receptor coupling could lead to a reversal in the polarity of  $V_m$  sensitivity for mGlu receptors (mGlu1/3) in response to changes  $V_m$  (Ohana et al., 2006).

Aligned with the ternary model of GPCR activation (De Lean et al., 1980; Grishina & Berlot, 2000), a model elucidating these varied results was proposed by Hanna Parnas' group in the early 2000s, wherein  $V_m$  influences G-protein coupling, subsequently modulating the affinity state of membrane receptors. However, studies from the late 2000s onwards have presented results inconsistent with this model. Notably, the polarity of  $V_m$ 's effect varies among muscarinic receptors despite being coupled to  $G_q$  ( $M_1$ ,  $M_3$ , and  $M_5$ , (Rinne et al., 2015)). Additionally, PTX treatment has no effect on the  $V_m$  sensitivity of the  $\alpha_{2A}$  receptor, unlike the  $M_2$  receptor (Rinne et al., 2013). Moreover, the addition of saturating guanosine triphosphate  $\gamma$ S (GTP $\gamma$ S), a slowly hydrolyzing GTP analogue that irreversibly activates G proteins, uncouples the receptor from the G protein activation cycle, yet it does not affect the  $V_m$  sensitivity of  $\alpha_{2A}$  and  $M_1$  receptors (Rinne et al., 2013, 2015; Tateyama & Kubo, 2013). Lastly, the observation of  $V_m$  sensitivity in arrestin binding of  $M_1$  and  $M_3$  receptors, a state considered to be in the absence of G protein, contradicts the importance of G protein presence for  $V_m$  sensitivity (Rinne et al., 2015; Shukla et al., 2013). Some of

these apparent discrepancies might be attributable to the different experimental approaches that have been taken by the different groups. In summary, the significance of G protein coupling in  $V_m$  sensitivity of GPCRs appears highly receptor-dependent and seemingly lacks universality, suggesting the existence of alternative  $V_m$  sensors within these receptors.

## 2.3. 2nd model: the orthosteric ligand binding domain

Early studies intriguingly demonstrated a correlation between the  $V_m$  dependence of charge movement and agonist affinity, suggesting a potential relationship between these phenomena (Ben-Chaim et al., 2006; Zohar et al., 2010). In essence, this implies that  $V_m$  sensitivity may be localized within the orthosteric site, where it directly influences receptor activation in accordance with variations in  $V_m$ . This theory has gradually gained traction following multiple observations made in the late 2000s.

### 2.3.1. Agonist-specific voltage sensitivity

Early investigations into the  $D_2$  receptor yielded surprising findings, indicating that the voltage dependence of this receptor varies depending on the specific agonist employed (Sahlholm et al., 2011; Sahlholm, Marcellino, Nilsson, Fuxe, & Århem, 2008). Similar observations were made regarding the  $M_2$  receptor. For instance, studies examining acetylcholine (ACh)-gated inward rectifier  $K^+$  (IKACh) currents in cardiomyocytes revealed a reduction in ACh affinity for the receptor upon depolarization; A similar observation was made regarding the relaxation gating of the IKACh, referring to the time course of opening or closing KACH channels, shown to rely mainly on  $M_2$  receptor voltage sensitivity (Moreno-Galindo et al., 2011). However, other agonists such as pilocarpine exhibited an opposed affinity, efficacy and relaxation gating kinetics under the same  $V_m$  variations (Navarro-Polanco et al., 2011; Navarro-Polanco et al., 2013). This phenomenon extends to numerous other GPCRs, exemplified by the  $\mu$ -opioid receptor, where the effects of morphine and fentanyl on G protein-activated inwardly rectifying  $K^+$  (GIRK) currents are respectively potentiated and inhibited by membrane depolarization, contrasting with the  $V_m$  independence of the endogenous agonist, metenkephalin, in inducing GIRK currents (Ruland et al., 2020). These findings have led to the concept of 'agonist-specific voltage sensitivity' proposed by several research groups (Moreno-Galindo et al., 2016; Sahlholm et al., 2011).

To elucidate the divergent effects of  $V_m$  on agonist activity at the orthosteric site, one might initially consider the role of ligand charges in receptor function. However, observations indicate that the same ligand can activate different receptors while exhibiting distinct  $V_m$  sensitivity, as evidenced by ACh binding to various muscarinic receptors with non-identical  $V_m$  sensitivity (Ben-Chaim et al., 2006; Rinne et al., 2015; Stanfield, 2006). Through computational modeling of putative ligand interactions at the binding pocket of the  $M_3$  receptor, coupled with site-directed mutagenesis of orthosteric site residues, it has been proposed that agonist-dependent sensitivity may be attributed to the specific docking orientation of the ligand within the binding pocket (Rinne et al., 2015). Indeed, the initial observation demonstrated an inverse effect of membrane depolarization on receptor-mediated signaling when the receptor was bound to carbachol or pilocarpine. However, a point mutation (N508<sup>6.52</sup>Q) in the orthosteric binding site turned the receptor activation towards a predicted alternative "pilocarpine-like" binding mode for carbachol. This resulted in the fully reversed voltage sensitivity of the mutated receptor-mediated G-protein activation during carbachol agonist application, elegantly demonstrating how essential is the agonist docking in the orthosteric binding site for  $V_m$  sensitivity. Similarly, investigations employing a range of ligands for the  $\mu$ -opioid receptor have unveiled strong ligand-specific  $V_m$  sensitivity, suggesting that the unique binding mode of each ligand determines its sensitivity to  $V_m$  (Kirchhofer et al., 2023).

Consequently,  $V_m$  modulation of GPCRs appears to be contingent upon the interaction of each ligand within the orthosteric binding pocket. However, the question remains: is the  $V_m$  sensor located within the orthosteric binding site?

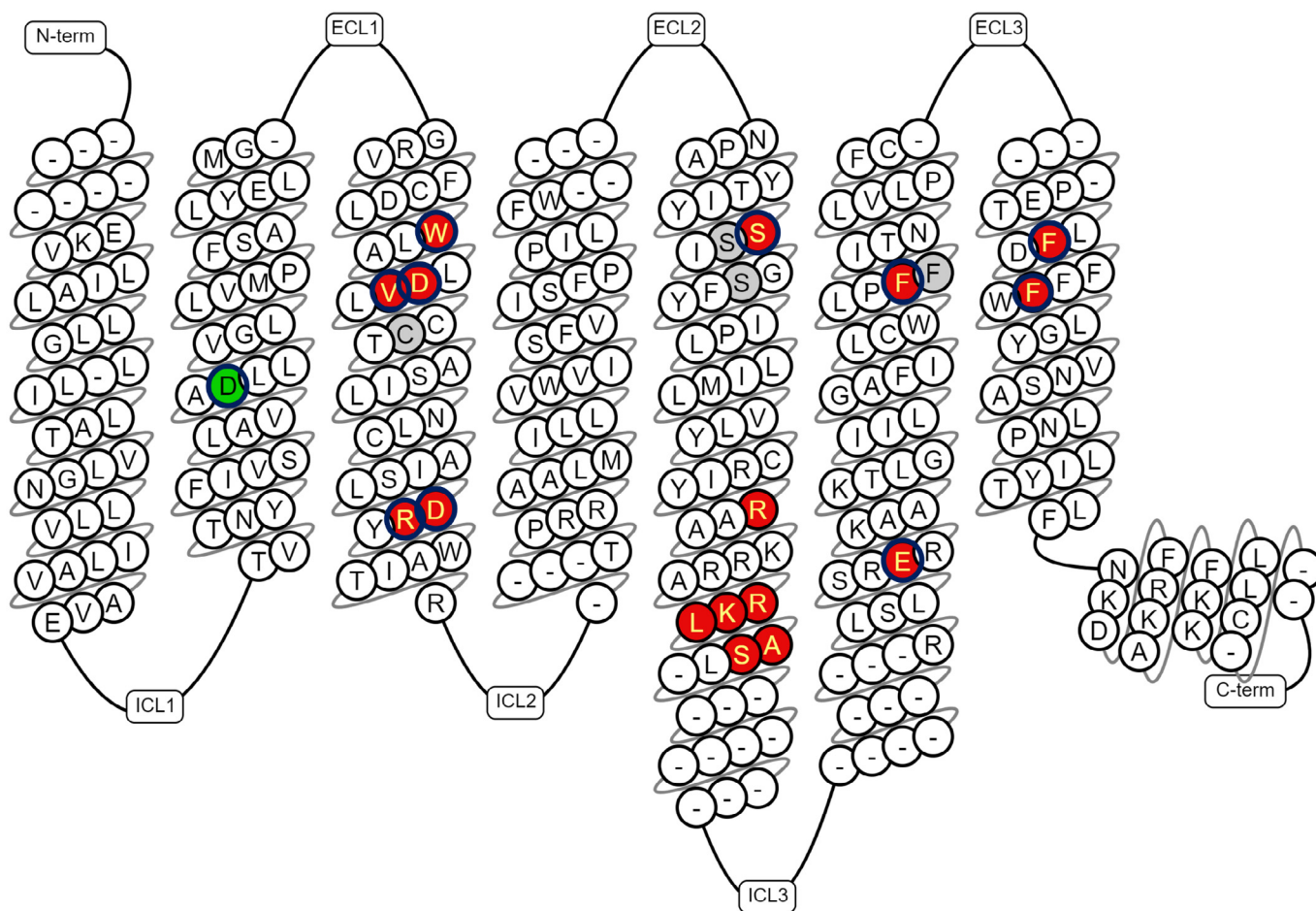
### 2.3.2. Exploring the voltage-sensor within the orthosteric binding site

Remarkably, the P2Y<sub>1</sub> receptor exhibits heightened sensitivity to  $V_m$  at low agonist concentrations (Gurung et al., 2008). Additionally, gating currents of the M<sub>2</sub> receptor diminish in amplitude with increasing concentrations of ACh or methochramine (Kupchik et al., 2011; Navarro-Polanco et al., 2011). Hence, one might postulate that binding to certain ligands either obstructs the  $V_m$  sensor or, at the very least, indicates a structural proximity between the binding site and the  $V_m$  sensor. Amino acids implicated in  $V_m$  sensitivity typically comprise charged residues strategically positioned within the TM domain relative to the electric field, notably aspartate. However, mutations affecting these residues generally do not alter the valence of the measured charges or the associated conformational changes (Barchad-Avitzur et al., 2016; Navarro-Polanco et al., 2011). Another candidate, tyrosine, potentially senses  $V_m$  variations owing to its dipole organization via its hydroxyl group (Bezanilla, 2000, 2008). Intriguingly, mutation of three tyrosines (Y104<sup>3,33</sup>, Y403<sup>6,51</sup>, Y426<sup>7,39</sup>) to phenylalanine within the orthosteric site of M<sub>2</sub>, referred to as the 'tyrosine lid', results in the loss of  $V_m$  sensitivity upon ACh binding, along with a reduction in  $V_m$ -dependent conformational changes and gating currents (Barchad-Avitzur et al., 2016; Ben-Chaim et al., 2019; Dekel et al., 2012;

Navarro-Polanco et al., 2011). This underscores the direct correlation between the disruption of gating charges by amino acid mutations in the orthosteric site and changes in  $V_m$  sensitivity. However, these observations are not universally applicable to all GPCRs. Indeed, reports suggest that M<sub>3</sub> lacking these three tyrosines remains functionally sensitive to  $V_m$ , but with a reversed effect of the  $V_m$  (Hoppe et al., 2018), and certain  $V_m$ -sensitive GPCRs, such as prostanoid and cannabinoid receptors, lack these residues (Goldberger et al., 2022; Kurz et al., 2020).

In conjunction with experimental findings, molecular dynamics simulations further suggest the significance of the orthosteric binding site in  $V_m$  sensitivity. Based on simulations of the M<sub>2</sub> receptor,  $V_m$  could alter the orientation of aromatic residue side chains within the orthosteric site (such as the 'tyrosine lid'), with additional amino acids ('expandable ring') (Y177<sup>45,51</sup> & F181 of extracellular loop 2, W422<sup>7,35</sup> of TM7) regulating agonist access relative to  $V_m$  (López-Serrano et al., 2020). Collectively, these simulations underscore the critical role of the orthosteric binding site in  $V_m$  sensing. As illustrated in Fig. 2 for the aminergic consensus sequences, approximately 70 % of mutations affecting  $V_m$  sensitivity also impact ligand binding or potency, underscoring the significance of residues essential for ligand function.

Finally, it is noteworthy that the majority of research on  $V_m$  sensors in GPCRs has focused on class A receptors. However, for class C receptors in particular, the agonist binding site resides within extracellular domains (Pin & Bettler, 2016), distant from the electric field. Nevertheless, allosteric modulators binding to the TM domain effectively modulate



**Fig. 2.** Key amino acids sensing  $V_m$  in aminergic GPCRs.

This scheme displays a consensus sequence of human aminergic GPCRs, generated by [gpcrdb.org](http://gpcrdb.org). Based on site directed mutagenesis evidences, we coloured amino acids (AAs) which mutations are altering functions tuned by GPCR  $V_m$  sensitivity (red), altering only gating charges (green) or ineffective (gray). The AAs which mutation affects agonist binding/potency ([gpcrdb.org](http://gpcrdb.org)) are highlighted with a dark blue circle. Note the presence of AAs crucial for orthosteric binding site (upper part) and for G-protein coupling (lower part). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)



agonist potency and efficacy, suggesting that TM domain interactions of these molecules are adequate to regulate these receptors. Hence, it is plausible that flexibility changes within the TM domain of class C receptors induced by  $V_m$  could influence receptor activation broadly, akin to allosteric modulators. This hypothesis finds support in our recent work, demonstrating striking similarities between  $V_m$  regulation of the mGlu5 receptor and the application of negative allosteric modulators (NAM) (Boutonnet et al., 2024).

Besides, recent speculation posits that the allosteric pocket of class A receptors may also contribute to GPCR sensitivity to  $V_m$ .

#### 2.4. 3rd model: the allosteric and orthosteric pockets dialogue

Allosteric modulators, typically synthetic ligands, exert their influence on GPCRs by binding to specific pockets, modulating receptor activation (Foster & Conn, 2017; Wootten et al., 2013). These effects can either decrease (NAM) or increase (positive allosteric modulator, PAM) the affinity and/or efficacy of orthosteric ligands. Similarly, recent research highlights various ways in which  $V_m$  can also impact these parameters (affinity/efficacy) upon receptor activation, either positively or negatively.

The significance of the allosteric pocket in  $V_m$  sensing was initially suggested by studies involving the mutation of a tryptophan residue (W422<sup>7,35</sup> A) in the  $M_2$  receptor, altering its  $V_m$  (Dekel et al., 2012). Additionally, Navarro-Polanco and colleagues underscored the importance of a tryptophan residue (W99<sup>3,28</sup>) at the junction between the allosteric and orthosteric sites of the  $M_2$  receptor (Navarro-Polanco et al., 2011). Notably, the tyrosine lid (Y104<sup>3,33</sup>, Y403<sup>6,51</sup>, Y426<sup>7,39</sup>) of the  $M_2$  receptor, located at the interface between the allosteric and orthosteric sites, also suggests a role for the allosteric pocket. Furthermore, Hoppe et al., 2018 observed similar G protein recruitment induced by  $V_m$  or an allosteric modulator in  $M_1$  and  $M_3$  receptors, emphasizing the role of the allosteric site in  $V_m$  sensitivity. Remarkably, mutation of a crucial residue (W422<sup>7,35</sup>), important for allosteric modulator binding and the cooperativity of allosteric and orthosteric sites, caused the  $M_3$  receptor to lose sensitivity to gallamine (an allosteric modulator) and  $V_m$ , simultaneously.

These findings underscore the importance of the dialogue between the orthosteric and allosteric sites, potentially involving W422<sup>7,35</sup>, which may stabilize tyrosine lid conformation during  $M_2$  receptor activation, as suggested by computational simulations (Barchad-Avitzur et al., 2016; Hoppe et al., 2018; Kruse et al., 2013). Additionally, W422<sup>7,35</sup> appears crucial for controlling ligand access along with Y177<sup>45,51</sup> (another key amino acid for allosteric ligands) and F181 relative to  $V_m$  variations (López-Serrano et al., 2020). Similarly, our recent work highlights a strikingly similar effect between voltage (Boutonnet et al., 2024) and negative allosteric modulators (Bradley et al., 2011) on the mGlu5 receptor. In contrast, a recent study failed to demonstrate a link between  $V_m$  and allosteric site, as the presence of the NAM gallamine, or the PAM LY2119620, does not significantly change the depolarization-induced reduction in affinity observed during acetylcholine and pilocarpine application (Hazan et al., 2024), arguing against the universality of the role of the allosteric site in GPCR  $V_m$ -sensitivity. Moreover, although the allosteric site seems crucial for  $V_m$  sensitivity, it is not the  $V_m$  sensor itself, as demonstrated by the  $V_m$ -dependent effect of pilocarpine on mutated or chimeric receptors with altered allosteric sites (Hoppe et al., 2018).

In conclusion, an intriguing hypothesis emerges: the modulation of GPCRs by synthetic ligands occurs via allosteric pockets precisely because these locations are evolutionarily significant for  $V_m$  modulation. In other words, allosteric ligands act on GPCRs' pockets functionally influenced by  $V_m$  via key interactions with specific residues.

Finally, considering the variety of amino acids involved in  $V_m$  sensitivity, a recent model has proposed that no single amino acid acts as the  $V_m$  sensor, but rather an ion housed within GPCRs:  $Na^+$ .

#### 2.5. 4th model: the mobile $Na^+$ ion

Numerous observations underscore the significance of  $Na^+$  ions situated within the central region of TM domains of class A GPCRs in their functionality (Zarzycka et al., 2019). These ions reside within a hydrophilic pocket extending from the orthosteric ligand binding site to the G protein binding site, where they can modulate receptor activation and potentially relocate from the allosteric pocket upon GPCR activation (Vickery et al., 2018; Wootten et al., 2013). Hence, Vickery and colleagues propose, based on computer simulations of  $\delta$ -opioid and  $M_2$  receptor activation, that  $V_m$  governs the activation of class A GPCRs by inducing movement of  $Na^+$  ions within the receptor (Vickery, Machtens, Tamburrino, et al., 2016). Specifically, by applying an electric field to the  $\delta$ -opioid receptor, these simulations demonstrate that  $Na^+$  ions relocate within the hydrophilic pocket depending on the simulated potential: moving upwards into the orthosteric pocket upon depolarization or downwards towards the allosteric pocket upon hyperpolarization. Certain amino acids, such as D69<sup>2,50</sup> for the  $M_2$  receptor and N131<sup>3,35</sup> for the  $\delta$ -opioid receptor, appear crucial for  $Na^+$  movement within distinct compartments, with D<sup>2,50</sup> previously recognized for its pivotal role in  $Na^+$  binding (Zarzycka et al., 2019). Intriguingly, experimental evidence supports these simulations; for instance,  $M_2$  receptors mutated at D69<sup>2,50</sup> lose their gating charges (Barchad-Avitzur et al., 2016; Navarro-Polanco et al., 2011), aligning with the calculated gating charges during  $Na^+$  movement being consistent with experimental measurements (Ben-Chaim et al., 2006; Navarro-Polanco et al., 2011). This hypothesis suggests a plausible explanation for the disruption of  $V_m$  sensitivity observed in numerous amino acid mutations without affecting the observed charge movements within the receptor. However, experimental validation of this model is imperative. Notably, the  $M_2$  receptor retains  $V_m$  sensitivity even in the absence of extracellular  $Na^+$  ions, and its  $V_m$  sensitivity does not correlate with extracellular  $Na^+$  concentration, despite the ion's allosteric modulation of the receptor (Friedman et al., 2020), implying distinct mechanisms. Moreover, while mutation of residue D69<sup>2,50</sup> in  $M_2$  abolishes gating charges, the receptor is still functionally influenced by  $V_m$ -induced intramolecular conformational changes (Ågren et al., 2018; Barchad-Avitzur et al., 2016). Similar observations were noted for the 5HT<sub>1A</sub> receptor, which remains sensitive to  $V_m$  even without extracellular  $Na^+$  ions or the D82<sup>2,50</sup> residue, equivalent to  $M_2$ 's D69<sup>2,50</sup> (Tauber & Ben Chaim, 2022), suggesting the involvement of another ion, such as  $Ca^{2+}$ , in this model. Furthermore, unlike class A receptors, no ion was identified in the structure of the  $V_m$ -sensitive mGlu receptor from class C GPCRs (Kruse et al., 2013; Nasrallah et al., 2021; Wu et al., 2014), indicating an alternative  $V_m$ -related mode of operation for this subgroup of GPCRs.

#### 2.6. Conclusion

As demonstrated thus far, the past two decades have witnessed the formulation of multiple theories and diverse attempts aimed at elucidating the  $V_m$  sensitivity of G-protein coupled receptors (GPCRs). Some theories propose that  $V_m$  induces shifts in GPCRs towards low or high affinity states through differential coupling to G proteins, while others underscore the significance of the orthosteric site in interaction with the allosteric site. Additionally, mechanisms involving the role of  $Na^+$  ions have been postulated. It appears that there is no singular mechanism governing  $V_m$ -sensing in GPCRs, with specificity necessitating particular amino acid compositions and intramolecular configurations. Nonetheless, an endeavor to delineate general lines of evidence can be made: Among converging lines of evidence, ligand presence appears to modulate receptor flexibility concerning  $V_m$  sensitivity, contingent upon interactions with neighboring residues' side chains. Moreover, certain conserved residues, predominantly situated within orthosteric/allosteric sites, seem to play a role in  $V_m$  sensitivity across various receptors. For instance, as depicted in Table 2, notable

**Table 2**  
Site-directed mutations on  $V_m$ -sensitive GPCRs.

AAs	Receptor	Mutation	References
D69 <sup>2.50</sup>	M <sub>2</sub>	D69A	Barchad-Avitzur et al. 2016; Navarro-Polanco et al. 2011
D76 <sup>2.50</sup>	P2Y <sub>12</sub>	D76N D76E	Zhang et al. 2020
Q124 <sup>2.60</sup>	μ-opioid	Q124E	Kirchhofer et al. 2023
D124 <sup>3.19</sup>	EP <sub>3</sub>	D124R	Kurz et al. 2020
W99 <sup>3.28</sup>	M <sub>2</sub>	W99A	Dekel et al. 2012; Navarro-Polanco et al. 2011
I144 <sup>3.29</sup>	μ-opioid	I144S I144V	Kirchhofer et al. 2023
D114 <sup>3.32</sup>	D <sub>2</sub>	D114A	Sahlholm et al. 2011
Y148 <sup>3.33</sup>	μ-opioid	Y148A Y148F	Kirchhofer et al. 2023
Y106 <sup>3.33</sup>	M <sub>1</sub>	Y106A	Barchad-Avitzur et al. 2016
Y104 <sup>3.33</sup>	M <sub>2</sub>	Y104A Y104F	Barchad-Avitzur et al. 2016; Navarro-Polanco et al. 2011;
Y149 <sup>3.33</sup>	M <sub>3</sub>	Y149F	Hoppe et al. 2018
S107 <sup>3.36</sup>	M <sub>2</sub>	S107A	Navarro-Polanco et al. 2011
M151 <sup>3.36</sup>	μ-opioid	M151A	Kirchhofer et al. 2023
D127 <sup>3.49</sup>	P2Y <sub>12</sub>	D127N D127E	Zhang et al. 2020
D120 <sup>3.49</sup>	M <sub>2</sub>	D120N	Ben-Chaim et al. 2006
R121 <sup>3.50</sup>	M <sub>2</sub>	R121N	Ben-Chaim et al. 2006
D193 <sup>5.36</sup>	TP	D193E	Kurz et al. 2020
K233 <sup>5.39</sup>	μ-opioid	K233A K233E	Kirchhofer et al. 2023
S193 <sup>5.42</sup>	D <sub>2</sub>	S193A	Sahlholm et al. 2011
S194 <sup>5.43</sup>	D <sub>2</sub>	S194A	Sahlholm et al. 2011
S197 <sup>5.46</sup>	D <sub>2</sub>	S197A	Sahlholm et al. 2011
V236 <sup>5.46</sup>	μ-opioid	V236N	Kirchhofer et al. 2023
S201 <sup>5.55</sup>	TP	S201T	Kurz et al. 2020
R220 <sup>5.63</sup>	5HT <sub>1A</sub>	R220L	Tauber & Ben-Chaim 2023
K <sup>5.70</sup> -K <sup>5.71</sup> -D <sup>5.72</sup> - K <sup>5.73</sup> -K <sup>5.74</sup>	M <sub>2</sub>	K218E+ K219L + D220A + K221L + K222L	Ben-Chaim et al. 2006
D353 <sup>6.30</sup>	H <sub>3</sub>	D353A	Sahlholm et al. 2012
A298 <sup>6.30</sup>	H <sub>4</sub>	A298D	Sahlholm et al. 2012
S255T <sup>6.45</sup>	TP	S255T	Kurz et al. 2020
W293 <sup>6.48</sup>	μ-opioid	W293F	Kirchhofer et al. 2023
Y403 <sup>6.51</sup>	M <sub>2</sub>	Y403A Y403F	Barchad-Avitzur et al. 2016; Dekel et al. 2012; Navarro-Polanco et al. 2011
Y507 <sup>6.51</sup>	M <sub>3</sub>	Y507F	Hoppe et al. 2018
H297 <sup>6.52</sup>	μ-opioid	H297A H297F	Kirchhofer et al. 2023
N382 <sup>6.52</sup>	M <sub>1</sub>	N382Q	Birk et al. 2015
N508 <sup>6.52</sup>	M <sub>3</sub>	N508Q	
N459 <sup>6.52</sup>	M <sub>5</sub>	N459Q	
V300 <sup>6.55</sup>	μ-opioid	V300A V300N V300F V300L	Kirchhofer et al. 2023
W422 <sup>7.35</sup>	M <sub>2</sub>	W422A	Dekel et al. 2012
W526 <sup>7.35</sup>	M <sub>3</sub>	W526A	Hoppe et al. 2018
H319 <sup>7.36</sup>	μ-opioid	H319Y	Kirchhofer et al. 2023
Y426 <sup>7.39</sup>	M <sub>2</sub>	Y426A Y426F	Barchad-Avitzur et al. 2016; Dekel et al. 2012
Y530 <sup>7.39</sup>	M <sub>3</sub>	Y530F	Hoppe et al. 2018
R295 <sup>7.40</sup>	TP	R295A R295K	Kurz et al. 2020
Y326 <sup>7.43</sup>	μ-opioid	Y326F	Kirchhofer et al. 2023
W299 <sup>7.44</sup>	TP	W299L	Kurz et al. 2020
Y177 <sup>45.51</sup>	M <sub>2</sub>	Y177A	Hazan et al. 2024

List of site directed-mutagenesis on class A GPCR where functional role of the mutation was evaluated. The point mutations are abolishing (red), reducing (pink), increasing (green) or reversing (blue) the depolarization-induced effect on GPCR activation. The mutation affecting only gating charges is displayed in orange. The mutations ineffective to change the  $V_m$  effect are displayed in gray. Note the redundancy of the Ballesteros-Weinstein numbering of mutated amino acid on different receptors. Since the  $V_m$  sensitivity is highly agonist-dependent, the table preferentially display the endogenous agonist sensitivity (except for μ-opioid, and TP receptors, using synthetic or exogenous ligands). For the H297<sup>6.52</sup> AA, the H297A mutation increase (fentanyl) or reverse (methadone) the depolarization-induced effect.

redundancies are evident, such as Y<sup>3,33</sup>, mutations of which disrupt V<sub>m</sub> sensitivity in  $\mu$ -opioid, M<sub>1</sub>, M<sub>2</sub>, and M<sub>3</sub> receptors. Notably, as illustrated in Fig. 2, mutations affecting amino acids crucial for ligand binding generally severely impair V<sub>m</sub> sensing. Hence, a strong correlation between GPCR functionality and V<sub>m</sub> sensitivity is likely, as numerous studies have reported a significant reduction in agonist potency on mutated V<sub>m</sub>-insensitive GPCRs, with only a few remaining functionally unaffected by such mutations (e.g. (Rinne et al., 2015; Sahlholm et al., 2011)). Ultimately, it appears that the V<sub>m</sub> sensor is integral to the process of receptor activation via ligand binding or at least it is connected to alterations in the distribution of receptor conformations that are stabilized by agonists. The fact the steepness and the range of the voltage that affects receptor function is very similar for all voltage sensitive GPCRs studied so far, could be taken as strong argument for the existence of a conserved mechanism of voltage sensing for GPCRs.

This inherent proximity between the V<sub>m</sub> sensor and GPCR function has regrettably impeded research into GPCR V<sub>m</sub> sensitivity, as isolating V<sub>m</sub> sensitivity from receptor function proves challenging. This is particularly true concerning the physiological role of V<sub>m</sub> sensing. Nonetheless, recent exciting advancements have emerged, and a comprehensive overview of the latest research on this topic will follow.

### 3. The role of GPCR voltage sensitivity

The challenge of identifying the molecular determinants of a V<sub>m</sub>-sensitive GPCR and/or mutating them without affecting receptor functionality hampers the understanding of the functional role of V<sub>m</sub> sensitivity. Nevertheless, relevant functional studies have recently emerged, utilizing both non-excitatory heterologous and excitatory native expression systems. These studies offer correlations or even causal links to formulate hypotheses and reassess the role played by a GPCR in light of the cellular context and V<sub>m</sub>.

This issue is of particular interest in neurons, where membrane V<sub>m</sub> exhibits high dynamics and GPCRs are expressed at axonal terminals and dendrites (Huang & Thathiah, 2015), playing pivotal roles in various functions. Notably, they finely modulate neuronal transmission by regulating the release of NT from the presynapse and modulating the conductance and membrane expression of channels on the postsynapse (Huang & Thathiah, 2015). The V<sub>m</sub> variations to which GPCRs are exposed range from 20 to 25 mV for excitatory postsynaptic potentials (EPSPs, (Acker et al., 2016; Cornejo et al., 2022; Jayant et al., 2017; Popovic et al., 2015)) to 45–100 mV for retrograde action potentials (APs, (Nevian et al., 2007; Palmer & Stuart, 2009; Waters et al., 2005)) and 70 mV–110 mV for anterograde APs (Kandel et al., 2021). Moreover, GPCRs are expressed in numerous other cell types where V<sub>m</sub> varies during certain processes, such as the cell cycle or muscle contraction (Abdul Kadir et al., 2018).

#### 3.1. Role of voltage in GPCRs functions – impact in neuronal context

The impact of GPCRs' sensitivity to V<sub>m</sub> on their ability to modulate effectors was initially observed in non-excitatory cells, in heterologous expression systems like HEK 293 T cells, HeLa cells, or *Xenopus* oocytes. These heterologous expression systems offer the advantage of isolating the expressed receptor for focused study and also circumvent spontaneous variations in V<sub>m</sub>, thereby allowing better control. Typically, V<sub>m</sub> is experimentally controlled by tonic depolarizations of the order of 100 mV. For instance, the recruitment of G-proteins, measured using biosensors on live cells, has been demonstrated to be V<sub>m</sub>-sensitive (Birk et al., 2015; Boutonnet et al., 2024; Hoppe et al., 2018; Sahlholm et al., 2011; Tateyama & Kubo, 2013), suggesting that downstream signaling of G-proteins is also influenced by V<sub>m</sub>. Consistently, Ca<sup>2+</sup> signaling induced through the G<sub>q</sub> protein by mGlu5 or P2Y<sub>1</sub> receptor activation is affected by V<sub>m</sub> (Boutonnet et al., 2024; Martínez-Pinna et al., 2005). Additionally, the recruitment of GPCR Kinases (GRK) and

$\beta$ -arrestin proteins has been reported to be V<sub>m</sub>-dependent (Birk et al., 2015; Rinne et al., 2013; Ruland et al., 2020). Notably,  $\beta$ -arrestin binding is known to facilitate GPCR internalization via a clathrin-dependent mechanism (Weis & Kobilka, 2018). While the effect of GPCR activation on internalization is extensively documented, the specific impact of V<sub>m</sub> on physiological GPCR expression at the cell membrane remains largely unexplored. This knowledge gap may prompt several inquiries, including whether GPCR internalization is modulated by synaptic activity. Furthermore, GPCRs can regulate in a V<sub>m</sub>-dependent manner, the opening of channels which themselves are not sensitive to V<sub>m</sub>, such as GIRK channels, Ca<sup>2+</sup>-activated Cl<sup>-</sup> currents, or transient receptor potential canonical 6 (TRPC6) channels (Ben-Chaim et al., 2003; Boutonnet et al., 2024; Ohana et al., 2006). These experiments suggest that neuronal excitability, modulated by the dialogue between GPCRs and channels, could be influenced by V<sub>m</sub> in physiological tissues. Collectively, these observations indicate that V<sub>m</sub> could impact a multitude of signaling pathways of GPCRs in their native environment.

The lingering question remains: Can the regulation of GPCR signaling be observed in a physiological context of V<sub>m</sub> variations? A first aspect of this query pertains to the kinetics of these V<sub>m</sub> variations: the gating charge movements measured in GPCRs exhibit kinetics rapid enough to be influenced by fast V<sub>m</sub> variations such as neuronal APs (< 1 ms, (Ben-Chaim et al., 2006)), suggesting that pre- and postsynaptic GPCRs sensitive to V<sub>m</sub> could indeed be affected by APs (anterograde or retrograde). Another aspect concerns the intensity of physiological V<sub>m</sub> variations in neurons. These neuronal depolarizations can range from approximately 20 mV for EPSPs (Acker et al., 2016; Cornejo et al., 2022; Jayant et al., 2017; Popovic et al., 2015) to up to 110 mV for APs (Nevian et al., 2007; Palmer & Stuart, 2009; Waters et al., 2005). However, typically, depolarizations between 75 mV and 150 mV are experimentally employed to demonstrate the V<sub>m</sub> sensitivity of GPCRs, and in the rare instances where variations of lower amplitudes are tested, they prove ineffective. This range of depolarization intensities allows for the examination of the effects of APs but not EPSPs. Only rare studies demonstrate the sensitivity of a GPCR to the range of physiological V<sub>m</sub> variations, such as the mGlu5 receptor (on the order of 20 mV, Boutonnet et al., 2024) or the small V<sub>m</sub> deviations affecting GIRK currents during small depolarizing pulses (Moreno-Galindo et al., 2011). Hence, apart from this study, the extrapolation of V<sub>m</sub> effects on postsynaptic receptors in neurons remains uncertain, particularly for those located in distal dendrites minimally influenced by retrograde APs. Last consideration, these V<sub>m</sub> variations are often applied tonically when studying the effect of V<sub>m</sub> on GPCRs. While it aptly depicts the variation in V<sub>m</sub> for GPCRs expressed in non-excitatory cells, wherein V<sub>m</sub> changes gradually, as observed during the cell cycle (Abdul Kadir et al., 2018), the neuronal V<sub>m</sub> variation bears no resemblance and manifests as rapid bursts of depolarization/repolarization. However, recent studies indicate that the effect of V<sub>m</sub> remains similar when variations are administered tonically or as trains of brief depolarizations at frequencies mimicking neuronal APs (Hoppe et al., 2018; Zhang et al., 2020). Collectively, these observations suggest that V<sub>m</sub> variations, particularly within the amplitude range of APs, likely directly influence GPCRs function within neurons.

#### 3.2. Correlative studies between voltage variation and GPCR function in excitable cells

Interestingly, V<sub>m</sub>-sensitive receptors were sometimes studied within a physiological context. It thus becomes apparent that V<sub>m</sub> may directly affect GPCRs. For example, the gating of many channels by GPCRs has been observed to be V<sub>m</sub>-dependent in neurons. This includes phenomena such as  $\mu$ -opioid induction of inward rectifying K<sup>+</sup> (Kir3.X) currents in neurons from the Locus Coeruleus (Ruland et al., 2020). Similarly, IK<sub>ACh</sub> currents in atrial myocytes, essential for controlling heart rate variability and susceptibility to atrial arrhythmias

(Kovoor et al., 2001; Wickman et al., 1998) appear to be  $V_m$ -dependent via intrinsic  $V_m$  dependence of muscarinic receptors (Moreno-Galindo et al., 2011; Navarro-Polanco et al., 2011). Another interesting example is the fact that mGlu5-mediated *N*-methyl-D-aspartate (NMDA) receptor facilitation as well as mGlu5-induced  $Ca^{2+}$  release from intracellular stores are both optimal at resting  $V_m$  of hippocampal neurons (Boutonnet et al., 2024).

The  $V_m$  sensitivity of GPCRs appears to contribute to physiological processes as fundamental as NT release mechanisms, as well as control of receptors triggering synaptic plasticity induction. At presynaptic terminals, Parnas and colleagues propose that a tonic block of ACh release relies on  $M_2$  receptor  $V_m$  sensitivity in frog neuromuscular junctions (H. Parnas et al., 2005). Using protocols involving two extremely close electrical pulses ( $< 1$  ms) to frog nerve fibers, triggering successive ACh releases, the first pulse appears to remove a block, allowing increased release with the second pulse. This receptor is directly inhibited by membrane depolarization when isolated in a heterologous expression system (Ben-Chaim et al., 2003). The rapidity of the protocols used (100  $\mu$ s pulse) suggests that the  $V_m$  sensor of the  $M_2$  receptor best explains these observations, enabling the modulation of ACh release kinetics via its sensitivity to  $V_m$  (Kupchik et al., 2011). The  $M_2$  receptor would constitutively restrict pre-synaptic neuron activity when the membrane is in the range of hyperpolarized potentials, and would be rapidly inhibited by an AP (H. Parnas & Parnas, 2007; I. Parnas & Parnas, 2010). At the postsynaptic level, our own work highlights a facilitation of NMDA receptors by mGlu5 receptors and an increase in  $Ca^{2+}$  transients, which is optimal at the resting potential of neurons (Boutonnet et al., 2024). Indeed, under depolarized conditions, inhibition of  $V_m$ -sensitive mGlu5 receptors prevents the facilitation of NMDA receptors by mGlu5. In addition to unveiling the  $V_m$  sensitivity of mGlu5 receptors, this discovery challenges the established dogma that NMDA receptors remain inactive at the cell's resting potential and solely function as detectors of coincident activation of pre- and post-synaptic elements. Furthermore, given that mGlu5 and NMDA receptors are main triggers in plasticity induction, these results open up perspectives for reconsideration of synaptic plasticity depending on the activity state of the two types of receptors, which exhibit clearly distinct or even opposite sensitivity to  $V_m$ . One could readily hypothesize that at the resting potential, the optimal activity of mGlu5 receptors would enable their significant involvement in inducing plasticity. The priming of NMDA receptors activity by mGlu5 receptors would allow co-involvement of both receptor types at  $V_m$  close to resting potential and/or slightly depolarized. In contrast, sustained depolarizations block the activity of mGlu5 receptors while promoting, through magnesium block relief, the activation of NMDA receptors, which under these conditions would thus become preferential actors for inducing plasticity.

However, these observations, suggesting fundamental roles for GPCRs  $V_m$  sensitivity in a physiological context, remain correlative in nature. To ascertain a causal effect of  $V_m$  on a given GPCR, it is imperative to ensure the use of a modified receptor where  $V_m$ -sensing is altered, yet still functional. Only recently have causal demonstrations emerged using this type of GPCR mutants.

### 3.3. Causal links between voltage sensitivity and GPCR activity

Despite the challenge of identifying  $V_m$ -sensing domains within GPCRs and mutating them without drastically affecting receptor function, recent groundbreaking studies enable the establishment of a causal link between the property of  $V_m$  sensitivity and certain receptor functions. For example, catecholamine release from sympathetic adrenal chromaffin cells, classified as excitable (Holman et al., 1994), is inhibited by adenosine triphosphate (ATP) via P2Y<sub>12</sub> metabotropic receptors, a tonic block recently elucidated to be alleviated by membrane depolarization as demonstrated by Zhang and colleagues (Zhang et al., 2020). Conversely, they screened for mutated P2Y<sub>12</sub>

receptor variants with reduced voltage sensitivity and came up with two amino acid substitutions (D76<sup>2,50</sup> to A and D127<sup>3,49</sup> to A), that showed attenuated responses to  $V_m$  fluctuations while retaining functional activity. In chromaffin cells, membrane depolarization fails to alleviate the tonic inhibition of NT release mediated by the mutant P2Y<sub>12</sub> receptor, marking the first direct evidence of a causal relationship between GPCR  $V_m$  sensitivity and a physiological process. Similar conclusions regarding NT release were observed in adrenal gland slices, indicating that circulating hormone levels could be directly modulated by the  $V_m$  of chromaffin cells, underscoring the physiological relevance of this mechanism (Zhang et al., 2020). Nonetheless, the relevance of this GPCR property *in vivo* remains ambiguous. Given the pivotal role of GPCRs in synaptic transmission modulation, elucidating this aspect is of paramount importance (Huang & Thathiah, 2015). More recently, a study has unveiled the functional significance of GPCR  $V_m$  sensitivity *in vivo* through causal observations linking GPCR  $V_m$  sensitivity to the physiology and behavior of *Drosophila melanogaster* (Rozenfeld et al., 2021). This investigation showcases that the  $V_m$ -sensitive mAChR-A receptor, homologous to the mammalian  $M_1$  receptor, loses its  $V_m$  sensitivity when the KDLPN motif within the intracellular loop 3, analogous to the  $M_2$  receptor's KKDKK motif known to be crucial for  $V_m$  sensitivity, is substituted with KKLKN. Notably, the receptor remains functionally intact in a highly active state. For the first time, it demonstrates the necessity of GPCR  $V_m$  sensitivity in synaptic potentiation within cholinergic olfactory neurons located in the antennal lobes of live *Drosophila* expressing this mutated receptor. Furthermore, it illustrates the crucial role of mAChR-A receptor  $V_m$  sensitivity in mitigating bursts of APs induced by odor exposure and in the odor habituation process reliant on this neural circuitry, as evidenced by olfactory preference behavioral tests conducted on *Drosophila*. Significantly, the divergence of the *Drosophila* mAChR-A receptor from the mammalian muscarinic receptor approximately 700 million years ago suggests that GPCR  $V_m$  sensitivity may have emerged millions of years ago and could potentially be widely shared across phyla (Ren et al., 2015).

### 3.4. Pharmacological opportunities in the context of voltage sensitivity of GPCRs

Given the significance of the physiological roles of GPCRs, the multitude of pathologies arising from their dysfunction, and their importance as pharmacological targets in the treatment of diseases, it is imperative to investigate the impact of their  $V_m$  sensitivity in these conditions. A key feature of the voltage sensitivity of GPCRs is its ligand dependency (Kirchhofer et al., 2023; Moreno-Galindo et al., 2016; Rinne et al., 2015; Ruland et al., 2020; Sahlholm et al., 2011). Even in cases where the voltage sensitivity of a GPCR is absent or neglectable for the natural agonist, it might be possible to find or develop agonists that exhibit a voltage dependence for pharmacological purposes. Two examples for receptors that are clinically highly relevant are muscarinic receptors and  $\mu$ -opioid receptors. In the first case  $M_2$  muscarinic receptors exhibit voltage sensitivity upon stimulation with the natural transmitter acetylcholine (Ben-Chaim et al., 2003). A depolarization leads to a decrease of the potency of ACh to activate receptors. In contrast the clinically used drug pilocarpine is more active upon depolarization highlighting the possibility for developing agonists with distinct profiles of voltage sensitivity (Moreno-Galindo et al., 2016; Rinne et al., 2015). A second example is the  $\mu$ -opioid receptor which does not show a pronounced voltage sensitivity if activated via natural agonist metenkephalin (Ruland et al., 2020). However, the efficacy of the analgesic morphine is robustly enhanced upon depolarization, whereas fentanyl and methadone are more active in hyperpolarized cells (Kirchhofer et al., 2023; Ruland et al., 2020). As revealed by the latter study, the contact sites of the different ligands with different helices are critical for the determination of the directionality of the voltage effect. Pharmacologically, an increase in efficacy with depolarization as seen with morphine will

lead to an enhanced effect of morphine in cells that are more frequently depolarized (such as nociceptors sensing acute pain) compared to more inactive hyperpolarized cells. Even though the clinically relevant pharmacological differences of the different opioid analgesics are not fully understood, the differential voltage effects on the efficacy of the different opioids adds another pharmacological dimension which might be of potential use also for future drug development.

Another illustration of the possible importance of GPCR sensitivity in clinics, is the 5HT<sub>1A</sub> polymorphism associated with major depression (R220<sup>S63L</sup>, (Haenisch et al., 2009)) that has recently been demonstrated to be insensitive to  $V_m$  variations (Tauber & Ben-Chaim, 2023). While the link between  $V_m$  sensitivity and the development of pathological conditions has not been assessed, it raises intriguing inquiries. Given the results demonstrating an effect of this property on neuronal physiology, mutations related to the  $V_m$ -dependent role of the receptor may be decisive in a pathological phenotype, and related pharmacological drugs development and testing.

Collectively, these findings support the notion that the diverse effects of pharmacological drugs targeting GPCRs may be explained by differences in the receptor's ligand-dependent sensitivity to  $V_m$ . Consequently, the effects of pharmacological drugs in clinical settings should be evaluated considering the ligand-specific GPCR-  $V_m$  sensitivity, particularly in the context of pathologies presenting aberrant variations in neuronal  $V_m$ , such as epilepsy.

### 3.5. Conclusion & future perspectives

Over the past two decades, approximately 30 GPCRs have been found to be sensitive to  $V_m$ . Considering the rarity of GPCRs shown to be insensitive to  $V_m$  for their activation by endogenous ligand (3 out of 30 published receptors) and the diversity of ligands detected by  $V_m$ -sensitive GPCRs (lipid, peptide, aminergic, or nucleotide),  $V_m$  sensitivity appears to be a major property shared by GPCRs, indicating a crucial role of  $V_m$  in GPCR function.  $V_m$  regulates both G-protein and  $\beta$ -arrestin pathways, crucial for synaptic transmission modulation. However, the molecular aspects of GPCR  $V_m$  sensitivity remain elusive. To date, although the  $V_m$  sensitivity of some GPCRs is functionally altered by specific site-directed mutations, no method has succeeded in completely abolishing the  $V_m$  sensor of GPCR. A large-scale screening of GPCR residues involved in  $V_m$  sensitivity, using, for instance, an automated patch-clamp recording pipeline for GPCR-induced currents (such as the GIRK currents), should elucidate the global structure and organization of the GPCR  $V_m$  sensor. Additionally, recent simulations have revealed effects on residues whose importance for  $V_m$ -sensing is experimentally unquestionable, illustrating the efficacy of these approaches for future investigations (López-Serrano et al., 2020). All these considerations lead us to conclude that the property of being sensitive to voltage is indeed fundamental to the receptor's function, hence the limited success in mutating these domains without compromising receptor function, and conversely, the failures to completely abolish sensitivity to  $V_m$  when attempting to preserve a functional receptor.

From a different perspective, it is worth noting that  $V_m$  also affects lipids, such as the dynamics of phospholipids at the plasma membrane (Zhou et al., 2015). Yet, these same phospholipids can allosterically regulate the activation of GPCRs, such as the  $\beta_2$ -Adrenergic receptor (Dawaliby et al., 2016), which has also been shown to be sensitive to  $V_m$  (Birk et al., 2015). It is thus conceivable that  $V_m$  may indirectly affect certain GPCRs in a  $V_m$ -dependent manner through the modulation of membrane lipids. While a link between lipids and  $V_m$  sensor has been established for membrane channels (Swartz, 2008), with, for example, the immobilization of a  $K^+$  channel  $V_m$  sensor by lipids lacking their phosphate group at their head (Xu et al., 2008), the association between GPCR-  $V_m$  sensitivity and lipid interactions remains to be experimentally explored, warranting further research in this domain.

Understanding the molecular mechanisms and physiological role of  $V_m$  sensitivity in GPCRs has garnered significant interest in recent years, and its suspected importance in physiology is starting to be demonstrated in various contexts. Notably, the role of GPCR  $V_m$  sensitivity remains elusive in mammalian physiology, and future endeavors are necessary to elucidate the role of this unique property in vivo.

### CRedit authorship contribution statement

**Marin Boutonnet:** Writing – review & editing. **Moritz Bünemann:** Writing – review & editing. **Julie Perroy:** Writing – review & editing, Supervision.

### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

### Acknowledgments

This work was supported by the Agence Nationale de la Recherche (JP, ANR-22-CE16-0013 LEARN), the Fondation pour la recherche médicale (FRM, JP, grant agreement No. EQU202303016311) and the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme (JP, grant agreement No. 646788 VERTICAL CITY).

### References

- Abdul Kadir, L., Stacey, M., & Barrett-Jolley, R. (2018). Emerging roles of the membrane potential : Action beyond the action potential. *Frontiers in Physiology* 9. <https://www.frontiersin.org/articles/10.3389/fphys.2018.01661>.
- Acker, C. D., Hoyos, E., & Loew, L. M. (2016). EPSPs measured in proximal dendritic spines of cortical pyramidal neurons. *eNeuro* 3(2). <https://doi.org/10.1523/ENEURO.0050-15.2016>.
- Ågren, R., & Sahlholm, K. (2020). Voltage-dependent dopamine potency at D1-like dopamine receptors. *Frontiers in Pharmacology* 11, Article 581151. <https://doi.org/10.3389/fphar.2020.581151>.
- Ågren, R., Sahlholm, K., Nilsson, J., & Århem, P. (2018). Point mutation of a conserved aspartate, D69, in the muscarinic M2 receptor does not modify voltage-sensitive agonist potency. *Biochemical and Biophysical Research Communications* 496(1), 101–104. <https://doi.org/10.1016/j.bbrc.2018.01.005>.
- Anis, Y., Nürnberg, B., Visoček, L., Reiss, N., Naor, Z., & Cohen-Armon, M. (1999). Activation of go-proteins by membrane depolarization traced by in situ Photoaffinity labeling of G $\alpha$ -proteins with [ $\alpha$ 32P]GTP-azidoanilide. *Journal of Biological Chemistry* 274(11), 7431–7440. <https://doi.org/10.1074/jbc.274.11.7431>.
- Barchad-Avitzur, O., Priest, M. F., Dekel, N., Bezanilla, F., Parnas, H., & Ben-Chaim, Y. (2016). A novel voltage sensor in the Orthosteric binding site of the M2 muscarinic receptor. *Biophysical Journal* 111(7), 1396–1408. <https://doi.org/10.1016/j.bpj.2016.08.035>.
- Ben Chaim, Y., Bochnik, S., Parnas, I., & Parnas, H. (2013). Voltage affects the dissociation rate constant of the m2 muscarinic receptor. *PLoS One* 8(9). <https://doi.org/10.1371/journal.pone.0074354> Article 9.
- Ben-Chaim, Y., Broide, C., & Parnas, H. (2019). The coupling of the M2 muscarinic receptor to its G protein is voltage dependent. *PLoS One* 14(10). <https://doi.org/10.1371/journal.pone.0224367> Article 10.
- Ben-Chaim, Y., Chanda, B., Dascal, N., Bezanilla, F., Parnas, I., & Parnas, H. (2006). Movement of 'gating charge' is coupled to ligand binding in a G-protein-coupled receptor. *Nature* 444(7115). <https://doi.org/10.1038/nature05259> Article 7115.
- Ben-Chaim, Y., Tour, O., Dascal, N., Parnas, I., & Parnas, H. (2003). The M2 muscarinic G-protein-coupled receptor is voltage-sensitive. *The Journal of Biological Chemistry* 278(25), 22482–22491. <https://doi.org/10.1074/jbc.M301146200>.
- Bezanilla, F. (2000). The voltage sensor in voltage-dependent ion channels. *Physiological Reviews* 80(2), 555–592. <https://doi.org/10.1152/physrev.2000.80.2.555>.
- Bezanilla, F. (2008). How membrane proteins sense voltage. *Nature Reviews Molecular Cell Biology* 9(4). <https://doi.org/10.1038/nrm2376> Article 4.
- Birk, A., Rinne, A., & Bünemann, M. (2015). Membrane potential controls the efficacy of catecholamine-induced  $\beta$ 1-adrenoceptor activity. *The Journal of Biological Chemistry* 290(45), 27311–27320. <https://doi.org/10.1074/jbc.M115.665000>.
- Bradley, S. J., Langmead, C. J., Watson, J. M., & Challiss, R. A. J. (2011). Quantitative analysis reveals multiple mechanisms of allosteric modulation of the mGlu5 receptor in rat astroglia. *Mol Pharmacol* 79. <https://doi.org/10.1124/mol.110.068882>.
- Boutonnet, M., Carpena, C., Bouquier, N., Chastagnier, Y., Font-Ingles, J., Moutin, E., Tricoire, L., Chemin, J., & Perroy, J. (2024). Voltage tunes mGlu5 receptor function,

- impacting synaptic transmission. *British Journal of Pharmacology*. <https://doi.org/10.1111/bph.16317>.
- Cohen-Armon, M., & Sokolovsky, M. (1993). Evidence for involvement of the voltage-dependent Na<sup>+</sup> channel gating in depolarization-induced activation of G-proteins. *The Journal of Biological Chemistry* 268(13), 9824–9838.
- Cornejo, V. H., Ofer, N., & Yuste, R. (2022). Voltage compartmentalization in dendritic spines in vivo. *Science (New York, N.Y.)* 375(6576), 82–86. <https://doi.org/10.1126/science.abg0501>.
- David, D., Bentulila, Z., Tauber, M., & Ben-Chaim, Y. (2022). G protein-coupled receptors regulated by membrane potential. *International Journal of Molecular Sciences* 23(22). <https://doi.org/10.3390/ijms232213988> Article 22.
- Dawaliby, R., Trubbia, C., Delporte, C., Masureel, M., Van Antwerpen, P., Kobilka, B. K., & Govaerts, C. (2016). Allosteric regulation of G protein-coupled receptor activity by phospholipids. *Nature Chemical Biology* 12(1), 35–39. <https://doi.org/10.1038/nchembio.1960>.
- De Lean, A., Stadel, J. M., & Lefkowitz, R. J. (1980). A ternary complex model explains the agonist-specific binding properties of the adenylate cyclase-coupled beta-adrenergic receptor. *The Journal of Biological Chemistry* 255(15), 7108–7117.
- Dekel, N., Priest, M. F., Parnas, H., Parnas, I., & Bezanilla, F. (2012). Depolarization induces a conformational change in the binding site region of the M2 muscarinic receptor. *Proceedings of the National Academy of Sciences of the United States of America* 109(1), 285–290. <https://doi.org/10.1073/pnas.1119424109>.
- Foster, D. J., & Conn, P. J. (2017). Allosteric modulation of GPCRs: New insights and potential utility for treatment of schizophrenia and other CNS disorders. *Neuron* 94(3), 431–446. <https://doi.org/10.1016/j.neuron.2017.03.016>.
- Friedman, S., Tauber, M., & Ben-Chaim, Y. (2020). Sodium ions allosterically modulate the M2 muscarinic receptor. *Scientific Reports* 10(1). <https://doi.org/10.1038/s41598-020-68133-9> Article 1.
- Goldberger, E., Tauber, M., & Ben-Chaim, Y. (2022). Voltage dependence of the cannabinoid CB1 receptor. *Frontiers in Pharmacology* 13, Article 1022275. <https://doi.org/10.3389/fphar.2022.1022275>.
- Grishina, G., & Berlot, C. H. (2000). A surface-exposed region of G(salpa) in which substitutions decrease receptor-mediated activation and increase receptor affinity. *Molecular Pharmacology* 57(6), 1081–1092.
- Gurung, I. S., Martinez-Pinna, J., & Mahaut-Smith, M. P. (2008). Novel consequences of voltage-dependence to G-protein-coupled P2Y1 receptors. *British Journal of Pharmacology* 154(4), 882–889. <https://doi.org/10.1038/bjp.2008.97>.
- Haenisch, B., Linsel, K., Brüsch, M., Gilsbach, R., Propping, P., Nöthen, M. M., ... Bönisch, H. (2009). Association of major depression with rare functional variants in norepinephrine transporter and serotonin1A receptor genes. *American Journal of Medical Genetics. Part B, Neuropsychiatric Genetics: The Official Publication of the International Society of Psychiatric Genetics* 150B(7), 1013–1016. <https://doi.org/10.1002/ajmg.b.30912>.
- Hazan, S., Tauber, M., & Ben-Chaim, Y. (2024). Voltage dependence of M2 muscarinic receptor antagonists and allosteric modulators. *Biochemical Pharmacology* 227, Article 116421. <https://doi.org/10.1016/j.bcp.2024.116421>.
- Holman, M. E., Coleman, H. A., Tonta, M. A., & Parkinson, H. C. (1994). Synaptic transmission from splanchnic nerves to the adrenal medulla of guinea-pigs. *The Journal of Physiology* 478(Pt 1), 115–124.
- Hoppe, A., Marti-Solano, M., Drabek, M., Bünemann, M., Kolb, P., & Rinne, A. (2018). The allosteric site regulates the voltage sensitivity of muscarinic receptors. *Cellular Signalling* 42, 114–126. <https://doi.org/10.1016/j.cellsig.2017.10.011>.
- Huang, Y., & Thathiah, A. (2015). Regulation of neuronal communication by G protein-coupled receptors. *FEBS Letters* 589(14), 1607–1619. <https://doi.org/10.1016/j.febslet.2015.05.007>.
- Ilouz, N., Branski, L., Parnis, J., Parnas, H., & Linal, M. (1999). Depolarization affects the binding properties of muscarinic acetylcholine receptors and their interaction with proteins of the exocytic apparatus. *The Journal of Biological Chemistry* 274(41), 29519–29528. <https://doi.org/10.1074/jbc.274.41.29519>.
- Itoh, T., Seki, N., Suzuki, S., Ito, S., Kajikuri, J., & Kuriyama, H. (1992). Membrane hyperpolarization inhibits agonist-induced synthesis of inositol 1,4,5-trisphosphate in rabbit mesenteric artery. *The Journal of Physiology* 451(1), 307–328. <https://doi.org/10.1113/jphysiol.1992.sp019166>.
- Jayant, K., Hirtz, J. J., Plante, I. J., -L, Tsai, D. M., De Boer, W. D. A. M., Semonche, A., ... Yuste, R. (2017). Targeted intracellular voltage recordings from dendritic spines using quantum-dot-coated nanopipettes. *Nature Nanotechnology* 12(4), 335–342. <https://doi.org/10.1038/nnano.2016.268>.
- Kandel, E. R., Koester, J. D., Mack, S. H., & Siegelbaum, S. A. (2021). Nerve cells, neural circuitry and behavior. *Principles of neural science* (p. 67) (6th éd.).
- Katada, T. (2012). The inhibitory G protein G(i) identified as pertussis toxin-catalyzed ADP-ribosylation. *Biological & Pharmaceutical Bulletin* 35(12), 2103–2111. <https://doi.org/10.1248/bpb.121024>.
- Kirchofer, S. B., Lim, V. J. Y., Ernst, S., Karsai, N., Ruland, J. G., Canals, M., ... Bünemann, M. (2023). Differential interaction patterns of opioid analgesics with  $\mu$  opioid receptors correlate with ligand-specific voltage sensitivity. *eLife* 12, Article e91291. <https://doi.org/10.7554/eLife.91291>.
- Kovoor, P., Wickman, K., Maguire, C. T., Pu, W., Gehrman, J., Berul, C. L., & Clapham, D. E. (2001). Evaluation of the role of I(KACH) in atrial fibrillation using a mouse knockout model. *Journal of the American College of Cardiology* 37(8), 2136–2143. [https://doi.org/10.1016/s0735-1097\(01\)01304-3](https://doi.org/10.1016/s0735-1097(01)01304-3).
- Kruse, A. C., Ring, A. M., Manglik, A., Hu, J., Hu, K., Eitel, K., ... Kobilka, B. K. (2013). Activation and allosteric modulation of a muscarinic acetylcholine receptor. *Nature* 504(7478). <https://doi.org/10.1038/nature12735> Article 7478.
- Kupchik, Y. M., Barchad-Avitzur, O., Wess, J., Ben-Chaim, Y., Parnas, I., & Parnas, H. (2011). A novel fast mechanism for GPCR-mediated signal transduction—Control of neurotransmitter release. *The Journal of Cell Biology* 192(1), 137–151. <https://doi.org/10.1083/jcb.201007053>.
- Kurz, M., Krett, A. -L., & Bünemann, M. (2020). Voltage dependence of prostanoid receptors. *Molecular Pharmacology* 97(1), 267–277. <https://doi.org/10.1124/mol.119.118372>.
- López-Serrano, A. L., De Jesús-Pérez, J. J., Zamora-Cárdenas, R., Ferrer, T., Rodríguez-Menchaca, A. A., Tristani-Firouzi, M., ... Navarro-Polanco, R. A. (2020). Voltage-induced structural modifications on M2 muscarinic receptor and their functional implications when interacting with iperoxo. *Biochemical Pharmacology* 113961. <https://doi.org/10.1016/j.bcp.2020.113961>.
- Luqmani, Y. A., Bradford, H. F., Birdsall, N. J. M., & Hulme, E. C. (1979). Depolarisation-induced changes in muscarinic cholinergic receptors in synaptosomes. *Nature* 277(5696). <https://doi.org/10.1038/277481a0> Article 5696.
- Mahaut-Smith, M. P., Hussain, J. F., & Mason, M. J. (1999). Depolarization-evoked Ca<sup>2+</sup> release in a non-excitatory cell, the rat megakaryocyte. *The Journal of Physiology* 515(Pt 2), 385–390. <https://doi.org/10.1111/j.1469-7793.1999.385ac.x>.
- Mahaut-Smith, M. P., Martinez-Pinna, J., & Gurung, I. S. (2008). A role for membrane potential in regulating GPCRs? *Trends in Pharmacological Sciences* 29(8), 421–429. <https://doi.org/10.1016/j.tips.2008.05.007>.
- Martinez-Pinna, J., Gurung, I. S., Mahaut-Smith, M. P., & Morales, A. (2010). Direct voltage control of endogenous lysophosphatidic acid G-protein-coupled receptors in Xenopus oocytes. *The Journal of Physiology* 588(10), 1683–1693. <https://doi.org/10.1113/jphysiol.2009.183418>.
- Martinez-Pinna, J., Gurung, I. S., Vial, C., Leon, C., Gachet, C., Evans, R. J., & Mahaut-Smith, M. P. (2005). Direct voltage control of signaling via P2Y1 and other Gq-coupled receptors. *Journal of Biological Chemistry* 280(2), 1490–1498. <https://doi.org/10.1074/jbc.M407783200>.
- Martinez-Pinna, J., Tolhurst, G., Gurung, I. S., Vandenberg, J. I., & Mahaut-Smith, M. P. (2004). Sensitivity limits for voltage control of P2Y receptor-evoked Ca<sup>2+</sup> mobilization in the rat megakaryocyte. *The Journal of Physiology* 555(Pt 1), 61–70. <https://doi.org/10.1113/jphysiol.2003.056846>.
- Marty, A., & Tan, Y. P. (1989). The initiation of calcium release following muscarinic stimulation in rat lacrimal glands. *The Journal of Physiology* 419, 665–687. <https://doi.org/10.1113/jphysiol.1989.sp017892>.
- Mason, M. J., Hussain, J. F., & Mahaut-Smith, M. P. (2000). A novel role for membrane potential in the modulation of intracellular Ca<sup>2+</sup> oscillations in rat megakaryocytes. *The Journal of Physiology* 524(Pt 2), 437–446. <https://doi.org/10.1111/j.1469-7793.2000.00437.x>.
- Mason, M. J., & Mahaut-Smith, M. P. (2001). Voltage-dependent Ca<sup>2+</sup> release in rat megakaryocytes requires functional IP3 receptors. *The Journal of Physiology* 533(Pt 1), 175–183. <https://doi.org/10.1111/j.1469-7793.2001.0175b.x>.
- Moreno-Galindo, E. G., Alamilla, J., Sanchez-Chapula, J. A., Tristani-Firouzi, M., & Navarro-Polanco, R. A. (2016). The agonist-specific voltage dependence of M2 muscarinic receptors modulates the deactivation of the acetylcholine-gated K<sup>+</sup> current (IKACH). *Pflügers Archiv - European Journal of Physiology* 468(7), 1207–1214. <https://doi.org/10.1007/s00424-016-1812-y>.
- Moreno-Galindo, E. G., Sánchez-Chapula, J. A., Sachse, F. B., Rodríguez-Paredes, J. A., Tristani-Firouzi, M., & Navarro-Polanco, R. A. (2011). Relaxation gating of the acetylcholine-activated inward rectifier K<sup>+</sup> current is mediated by intrinsic voltage sensitivity of the muscarinic receptor. *The Journal of Physiology* 589(Pt 7), 1755–1767. <https://doi.org/10.1113/jphysiol.2010.204115>.
- Nasrallah, C., Cannone, G., Briot, J., Rottier, K., Berizzi, A. E., Huang, C. -Y., ... Pin, J. -P., et al. (2021). Agonists and allosteric modulators promote signaling from different metabotropic glutamate receptor 5 conformations. *Cell Reports* 36(9). <https://doi.org/10.1016/j.celrep.2021.109648>.
- Navarro-Polanco, R. A., Aréchiga-Figueroa, I. A., Salazar-Fajardo, P. D., Benavides-Haro, D. E., Rodríguez-Eliás, J. C., Sachse, F. B., ... Moreno-Galindo, E. G. (2013). Voltage sensitivity of M2 muscarinic receptors underlies the delayed rectifier-like activation of ACh-gated K<sup>+</sup> current by choline in feline atrial myocytes. *The Journal of Physiology* 591(17), 4273–4286. <https://doi.org/10.1113/jphysiol.2013.255166>.
- Navarro-Polanco, R. A., Galindo, E. G. M., Ferrer-Villada, T., Arias, M., Rigby, J. R., Sánchez-Chapula, J. A., & Tristani-Firouzi, M. (2011). Conformational changes in the M2 muscarinic receptor induced by membrane voltage and agonist binding. *The Journal of Physiology* 589(7), 1741–1753. <https://doi.org/10.1113/jphysiol.2010.204107>.
- Nevejan, T., Larkum, M. E., Polsky, A., & Schiller, J. (2007). Properties of basal dendrites of layer 5 pyramidal neurons: A direct patch-clamp recording study. *Nature Neuroscience* 10(2). <https://doi.org/10.1038/nn1826> Article 2.
- Ohana, L., Barchad, O., Parnas, I., & Parnas, H. (2006). The metabotropic glutamate G-protein-coupled receptors mGluR3 and mGluR1a are voltage-sensitive. *The Journal of Biological Chemistry* 281(34), 24204–24215. <https://doi.org/10.1074/jbc.M513447200>.
- Ong, B. H., Ohsaga, A., Sato, K., Oshiro, T., Shirato, K., & Maruyama, Y. (2001). G protein modulation of voltage-sensitive muscarinic receptor signalling in mouse pancreatic acinar cells. *Pflügers Archiv* 441(5), 604–610. <https://doi.org/10.1007/s004240000459>.
- Palmer, L. M., & Stuart, G. J. (2009). Membrane potential changes in dendritic spines during action potentials and synaptic input. *Journal of Neuroscience* 29(21), 6897–6903. <https://doi.org/10.1523/JNEUROSCI.5847-08.2009>.
- Parnas, H., & Parnas, I. (2007). The chemical synapse goes electric: Ca<sup>2+</sup> and voltage-sensitive GPCRs control neurotransmitter release. *Trends in Neurosciences* 30(2), 54–61. <https://doi.org/10.1016/j.tins.2006.12.001>.
- Parnas, H., Slutsky, I., Rashkovan, G., Silman, I., Wess, J., & Parnas, I. (2005). Depolarization initiates phasic acetylcholine release by relief of a tonic block imposed by presynaptic M2 muscarinic receptors. *Journal of Neurophysiology* 93(6), 3257–3269. <https://doi.org/10.1152/jn.01131.2004>.

- Parnas, I., & Parnas, H. (2010). Control of neurotransmitter release: From Ca<sup>2+</sup> to voltage dependent G-protein coupled receptors. *Pflügers Archiv / European Journal of Physiology* 460(6), 975–990. <https://doi.org/10.1007/s00424-010-0872-7>.
- Pin, J. -P., & Bettler, B. (2016). Organization and functions of mGlu and GABA B receptor complexes. *Nature* 540(7631). <https://doi.org/10.1038/nature20566> Article 7631.
- Popovic, M. A., Carnevale, N., Rozsa, B., & Zecevic, D. (2015). Electrical behaviour of dendritic spines as revealed by voltage imaging. *Nature Communications* 6, 8436. <https://doi.org/10.1038/ncomms9436>.
- Ren, G. R., Folke, J., Hauser, F., Li, S., & Grimmelikhuijzen, C. J. P. (2015). The A- and B-type muscarinic acetylcholine receptors from *Drosophila melanogaster* couple to different second messenger pathways. *Biochemical and Biophysical Research Communications* 462(4), 358–364. <https://doi.org/10.1016/j.bbrc.2015.04.141>.
- Rinne, A., Birk, A., & Bünemann, M. (2013). Voltage regulates adrenergic receptor function. *Proceedings of the National Academy of Sciences* 110(4), 1536–1541. <https://doi.org/10.1073/pnas.1212656110>.
- Rinne, A., Mobarec, J. C., Mahaut-Smith, M., Kolb, P., & Bünemann, M. (2015). The mode of agonist binding to a G protein-coupled receptor switches the effect that voltage changes have on signaling. *Science Signaling* 8(401), Article ra110. <https://doi.org/10.1126/scisignal.aac7419>.
- Rozenfeld, E., Tauber, M., Ben-Chaim, Y., & Parnas, M. (2021). GPCR voltage dependence controls neuronal plasticity and behavior. *Nature Communications* 12(1), 7252. <https://doi.org/10.1038/s41467-021-27593-x>.
- Ruland, J. G., Kirchofer, S. B., Klindert, S., Bailey, C. P., & Bünemann, M. (2020). Voltage modulates the effect of  $\mu$ -receptor activation in a ligand-dependent manner. *British Journal of Pharmacology* 177(15), 3489–3504. <https://doi.org/10.1111/bph.15070>.
- Sahlholm, K., Barchad-Avitzur, O., Marcellino, D., Gómez-Soler, M., Fuxe, K., Ciruela, F., & Arhem, P. (2011). Agonist-specific voltage sensitivity at the dopamine D2S receptor –Molecular determinants and relevance to therapeutic ligands. *Neuropharmacology* 61(5–6), 937–949. <https://doi.org/10.1016/j.neuropharm.2011.06.022>.
- Sahlholm, K., Marcellino, D., Nilsson, J., Fuxe, K., & Arhem, P. (2008). Differential voltage-sensitivity of D2-like dopamine receptors. *Biochemical and Biophysical Research Communications* 374(3), 496–501. <https://doi.org/10.1016/j.bbrc.2008.07.052>.
- Sahlholm, K., Marcellino, D., Nilsson, J., Fuxe, K., & Arhem, P. (2008). Voltage-sensitivity at the human dopamine D2S receptor is agonist-specific. *Biochemical and Biophysical Research Communications* 377(4), 1216–1221. <https://doi.org/10.1016/j.bbrc.2008.10.117>.
- Sahlholm, K., Nilsson, J., Marcellino, D., Fuxe, K., & Arhem, P. (2008). Voltage-dependence of the human dopamine D2 receptor. *Synapse (New York, N.Y.)* 62(6), 476–480. <https://doi.org/10.1002/syn.20509>.
- Shukla, A. K., Manglik, A., Kruse, A. C., Xiao, K., Reis, R. I., Tseng, W. -C., ... Lefkowitz, R. J. (2013). Structure of active  $\beta$ -arrestin-1 bound to a G-protein-coupled receptor phosphopeptide. *Nature* 497(7447), 137–141. <https://doi.org/10.1038/nature12120>.
- Slutsky, I., Parnas, H., & Parnas, I. (1999). Presynaptic effects of muscarine on ACh release at the frog neuromuscular junction. *The Journal of Physiology* 514(3), 769–782. <https://doi.org/10.1111/j.1469-7793.1999.769ad.x>.
- Slutsky, I., Rashkovan, G., Parnas, H., & Parnas, I. (2002). Ca<sup>2+</sup>-independent feedback inhibition of acetylcholine release in frog neuromuscular junction. *Journal of Neuroscience* 22(9), 3426–3433. <https://doi.org/10.1523/JNEUROSCI.22-09-03426.2002>.
- Stanfield, P. (2006). Voltage sparks a GPCR. *Nature Cell Biology* 8(12). <https://doi.org/10.1038/ncb1206-1323> Article 12.
- Sutkeviciute, I., & Vilardaga, J. -P. (2020). Structural insights into emergent signaling modes of G protein-coupled receptors. *Journal of Biological Chemistry* 295(33), 11626–11642. <https://doi.org/10.1074/jbc.REV120.009348>.
- Swartz, K. J. (2008). Sensing voltage across lipid membranes. *Nature* 456(7224). <https://doi.org/10.1038/nature07620> Article 7224.
- Tateyama, M., & Kubo, Y. (2013). Binding of Gq protein stabilizes the activated state of the muscarinic receptor type 1. *Neuropharmacology* 65, 173–181. <https://doi.org/10.1016/j.neuropharm.2012.10.006>.
- Tauber, M., & Ben-Chaim, Y. (2022). The activity of the serotonergic 5-HT1A receptor is modulated by voltage and sodium levels. *The Journal of Biological Chemistry* 298(6), Article 101978. <https://doi.org/10.1016/j.jbc.2022.101978>.
- Tauber, M., & Ben-Chaim, Y. (2023). Functional consequences of a rare human serotonergic 5-HT1A receptor variant. *Frontiers in Pharmacology* 14, Article 1270726. <https://doi.org/10.3389/fphar.2023.1270726>.
- Vickery, O. N., Carvalheda, C. A., Zaidi, S. A., Pislakov, A. V., Katritch, V., & Zachariae, U. (2018). Intracellular transfer of Na<sup>+</sup> in an active-state G-protein-coupled receptor. *Structure* 26(1), 171–180.e2. <https://doi.org/10.1016/j.str.2017.11.013>.
- Vickery, O. N., Machtens, J. -P., Tamburrino, G., Seeliger, D., & Zachariae, U. (2016). Structural mechanisms of voltage sensing in G protein-coupled receptors. *Structure (London, England: 1993)* 24(6), 997–1007. <https://doi.org/10.1016/j.str.2016.04.007>.
- Vickery, O. N., Machtens, J. -P., & Zachariae, U. (2016). Membrane potentials regulating GPCRs: Insights from experiments and molecular dynamics simulations. *Current Opinion in Pharmacology* 30, 44–50. <https://doi.org/10.1016/j.coph.2016.06.011>.
- Waters, J., Schaefer, A., & Sakmann, B. (2005). Backpropagating action potentials in neurones: Measurement, mechanisms and potential functions. *Progress in Biophysics and Molecular Biology* 87(1), 145–170. <https://doi.org/10.1016/j.pbiomolbio.2004.06.009>.
- Weis, W. I., & Kobilka, B. K. (2018). The molecular basis of G protein-coupled receptor activation. *Annual Review of Biochemistry* 87(1), 897–919. <https://doi.org/10.1146/annurev-biochem-060614-033910>.
- Wickman, K., Nemeč, J., Gendler, S. J., & Clapham, D. E. (1998). Abnormal heart rate regulation in GIRK4 knockout mice. *Neuron* 20(1), 103–114. [https://doi.org/10.1016/s0896-6273\(00\)80438-9](https://doi.org/10.1016/s0896-6273(00)80438-9).
- Wootten, D., Christopoulos, A., Marti-Solano, M., Babu, M. M., & Sexton, P. M. (2018). Mechanisms of signalling and biased agonism in G protein-coupled receptors. *Nature Reviews Molecular Cell Biology* 19(10). <https://doi.org/10.1038/s41580-018-0049-3> Article 10.
- Wootten, D., Christopoulos, A., & Sexton, P. M. (2013). Emerging paradigms in GPCR allostery: Implications for drug discovery. *Nature Reviews Drug Discovery* 12(8). <https://doi.org/10.1038/nrd4052> Article 8.
- Wu, H., Wang, C., Gregory, K. J., Han, G. W., Cho, H. P., Xia, Y., ... Stevens, R. C. (2014). Structure of a class C GPCR metabotropic glutamate receptor 1 bound to an allosteric modulator. *Science* 344(6179), 58–64. <https://doi.org/10.1126/science.1249489>.
- Xu, Y., Ramu, Y., & Lu, Z. (2008). Removal of phospho-head groups of membrane lipids immobilizes voltage sensors of K<sup>+</sup> channels. *Nature* 451(7180), 826–829. <https://doi.org/10.1038/nature06618>.
- Zarzycka, B., Zaidi, S. A., Roth, B. L., & Katritch, V. (2019). Harnessing ion-binding sites for GPCR pharmacology. *Pharmacological Reviews* 71(4), 571–595. <https://doi.org/10.1124/pr.119.017863>.
- Zhang, Q., Liu, B., Li, Y., Yin, L., Younus, M., Jiang, X., Lin, Z., Sun, X., Huang, R., Liu, B., Wu, Q., Zhu, F., & Zhou, Z. (2020). Regulating quantal size of neurotransmitter release through a GPCR voltage sensor. *Proceedings of the National Academy of Sciences of the United States of America*. <https://doi.org/10.1073/pnas.2005274117>.
- Zhou, Y., Wong, C. -O., Cho, K., van der Hoeven, D., Liang, H., Thakur, D. P., ... Hancock, J. F. (2015). Membrane potential modulates plasma membrane phospholipid dynamics and K-Ras signaling. *Science (New York, N.Y.)* 349(6250), 873–876. <https://doi.org/10.1126/science.aaa5619>.
- Zohar, A., Dekel, N., Rubinsky, B., & Parnas, H. (2010). New mechanism for voltage induced charge movement revealed in GPCRs—Theory and experiments. *PLoS One* 5(1), Article e8752. <https://doi.org/10.1371/journal.pone.0008752>.