Exploring the Regulation of Kv1.2 Homomeric and Heteromeric Channels by Redox, LMAN2, and Kvβ

by

Anson Wong

A thesis submitted in partial fulfillment of the requirements for the degree of

Master of Science

Department of Pharmacology

University of Alberta

ABSTRACT

Voltage-gated potassium channels generate diverse current properties influenced by various signaling mechanisms that are still not well understood. Among these channels, Kv1.2 in particular demonstrates highly variable activation properties based on their ability to shift between a fast permissive and slow resistant gating mode. This feature is coined "slow gating regulation" and is sensitive to modulation by extrinsic factors. Reducing agents can promote the resistant gating mode resulting in a profound depolarizing shift of voltage-dependence and use-dependent activation. Similarly, overexpression of Kv1.2 channels with transmembrane lectin LMAN2 recapitulates these functional outcomes. It remains uncertain whether Kv1.2 redox and LMAN2 sensitivity is present in Kv1 heteromeric channels and also whether these mechanisms are interconnected.

In my thesis, I report that redox and LMAN2 sensitivity are exclusive to Kv1.2 among Kv1 homomeric channels and can persist in Kv1.2-containing heteromeric channels. These findings demonstrate that Kv1.2 can act as an adaptor subunit capable of recruiting sensitivity to redox and LMAN2 to other Kv1 channels. Additionally, it highlights the overlapping subunit dependence (i.e. Kv1.2 α-subunits) of redox and LMAN2, supporting LMAN2 as a redox-sensitive auxiliary subunit that regulates Kv1.2 slow gating. Furthermore, my research reveals that multiple signaling pathways can simultaneously regulate ion channels, leading to even greater current diversity. Redox and LMAN2-mediated slow gating strongly suppress Kvβ and Kv1.4-mediated inactivation by decelerating channel opening. This inhibitory effect can then be rescued through depolarizing prepulses or mutagenesis targeting redox/LMAN2 sensitivity.

Overall, this thesis expands our understanding of Kv1.2 channel modulation, highlights the profound impact of extrinsic regulatory mechanisms, and provides a solid foundation for future investigations into ion channel regulation.

PREFACE

This thesis is an original work by Anson Wong. No part of this thesis has been previously published. Harley Kurata and Anson Wong conceived all the projects in this thesis. The hippocampal neuron recordings in Chapter 4 were conducted and analyzed by Victoria Baronas, while the accompanying Figure 4.1 was constructed by Harley Kurata. Anson Wong created all other figures and conducted all experiments and data analysis. Chapters 3 and 4 are currently being prepared as a series of manuscripts on channel modulation by redox and LMAN2.

ACKNOWLEDGEMENT

I am deeply grateful for the invaluable help and support I have received from my laboratory, friends, and family throughout the completion of my thesis. First and foremost, I would like to extend my heartfelt appreciation to Harley Kurata for his guidance and for providing me with the opportunity to work in his lab. It has been an absolute pleasure collaborating with him and to be able to listen to his various views on science, sports, financial investments, family, and life philosophies. If I am fortunate enough to establish my own lab one day, I aspire to embody the same admirable qualities that I have witnessed in him. I would also like to thank Patrick MacDonald and Xing-Zhen Chen for their invaluable contributions as members of my committee. Their guidance and insightful suggestions have undoubtedly played a significant role in shaping me into a better researcher and have greatly contributed to the quality of my thesis. I am immensely grateful to all my lab mates, including Shawn Lamothe, Damayantee Das, Richard Kanyo, Yubin Hao, Cynthia Liang, Chloe Koens, Ethan Smith, Tom Hammond, Haoyun Chen, and Nicholas Duta. The time we have spent together has created unforgettable memories, and I hope that our paths will cross again in the future. I want to express special appreciation to Shawn for his early instructions and constant support whenever I needed it. I wish you all the best of luck in your future endeavors. To all my friends and family, I want to convey my deepest gratitude. Your unwavering support has been the cornerstone of my journey, and I cannot thank you enough for being there for me throughout this chapter of my life.

To everyone who has contributed to my thesis and to those who have supported me along the way, thank you from the bottom of my heart.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

4-AP: 4-Aminopyridine

AIS: Axon initial segment

cDNA: Complementary DNA

CHO: Chinese Hamster Ovary cell line

DNA: Deoxyribonucleic acid

DRG: Dorsal root ganglion

DTT: Dithiothreitol

ER: Endoplasmic Reticulum

GABA: Gamma (γ)-aminobutyric acid

GFP: Green fluorescent protein

HEK: Human embryonic kidney cell line

Hz: Hertz

K^+: Potassium ion

K2P: Tandem pore channel

KCa: Calcium-activated channel

Kir: Inward rectifier potassium channel

Kv: Voltage-gated potassium ion channel

Kvα: Voltage-gated potassium ion channel alpha (α)-subunit

Kvß: Voltage-gated potassium ion channel beta (ß)-subunit

LM: Mouse fibroblast cell line

LMAN2 (VIP): Lectin Mannose Binding 2 (vesicular integral-membrane protein)

LSO: Lateral superior olive

MAM: Mitocohondrion-associated ER membrane

mM: Millimolar

MNTB: Medial nucleus of the trapezoid body

mRNA: Messenger RNA

ms: Millisecond

MSO: Medial superior olive

mV: Millivolt

MΩ: Megaohm

nA: Nanoamp

NADPH: Nicotinamide adenine dinucleotide phosphate

NADH: Nicotinamide adenine dinucleotide

nM: Nanomolar

pA: Picoamp

PCR: polymerase chain reaction

PIP2: Phosphatidylinositol 4,5-bisphosphate

Slc (LAT): Solute carrier (L-type amino acid transporter)

TCEP: Tris(2-carboxyethyl)phosphine

UDA: Use-dependent activation

VSD: voltage sensing domain

WT: Wild type

σ-1: Sigma-1

μM: Micromolar

Amino acid	Three letter abbreviation	One letter abbreviation
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartate	Asp	D
Cysteine	Cys	C
Glutamine	Gln	Q
Glutamate	Glu	E
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V

CHAPTER 1: INTRODUCTION

1.1 OVERVIEW OF VOLTAGE GATED POTASSIUM CHANNELS

Potassium channels are a diverse class of transmembrane proteins found in nearly all species (Kuo et al., 2005; Yu et al., 2005) and are involved in a variety of cellular and physiological processes including cellular excitability (Kim and Nimigean, 2016), apoptosis (Pal et al., 2006), cellular growth (Deutsch and Chen, 1993), cognitive processing (Baculis et al., 2020; Ghelardini et al., 1998), hormone secretion (MacDonald et al., 2002), and cardiac functioning (Wang et al., 1996). These proteins can be divided into four major classes: the inward rectifying channels (Kir), calcium-activated channels (K_{Ca}), tandem pore channels (K2P), and voltage-gated (Kv) channels (Grizel et al., 2014; Guéguinou et al., 2014). This thesis will focus on Kv channels which facilitate efflux of potassium ions (K^+) down their electrochemical gradient in response to membrane depolarization (Catterall, 2013). This outward movement of K^+ ions is responsible for the repolarization and hyperpolarization of the membrane potential (Kim and Nimigean, 2016) and as such, Kv channels play important roles in regulating action potential generation and termination (Robbins and Tempel, 2012; Wulff et al., 2009). While voltage is the primary determinant of Kv channel opening (Yellen, 1998), it is crucial to acknowledge the influence of various additional regulatory mechanisms that contribute to the remarkable functional diversity observed in these channels.

Kv channel assembly

Kv channels are the largest ion channel family in humans with over 40 voltage gated potassium genes divided among 12 families (Kv1-12) (Ranjan et al., 2019). Each gene encodes a single unique Kv α-subunit that can assemble to form functional channels containing four subunits (Wulff et al., 2009). These channels can either be homotetrameric (i.e. containing all four of the same subunits) or heterotetrameric (i.e. containing combinations of different subunits). A single Kv α-subunit has six

transmembrane helices (S1-S6) with cytosolic N and C termini (Figure 1.1) (Noda et al., 1984; Ranjan et al., 2019; Wulff et al., 2009). The first four transmembrane segments (S1-S4) form the voltage sensor domains (VSD) which sit at the periphery of the channel (Chen et al., 2010; Jiang et al., 2003a). The S4 domain is considered the main voltage sensing element and contains a series of positive arginine and lysine residues which are regularly spaced at intervals of three (in *Shaker* R362, R365, R368, K374, R377, and R380) (Gandhi and Isacoff, 2002). These S4 charges displace through the membrane in response to changes in membrane potential and this is the fundamental mechanism underlying how voltage gated ion channels respond to voltage (Bezanilla, 2002; Gandhi and Isacoff, 2002). The S5, transmembrane loop, and S6 helices assemble to form a central ion-conducting pore (Chen et al., 2010; Heginbotham et al., 1994; Jiang et al., 2003a).

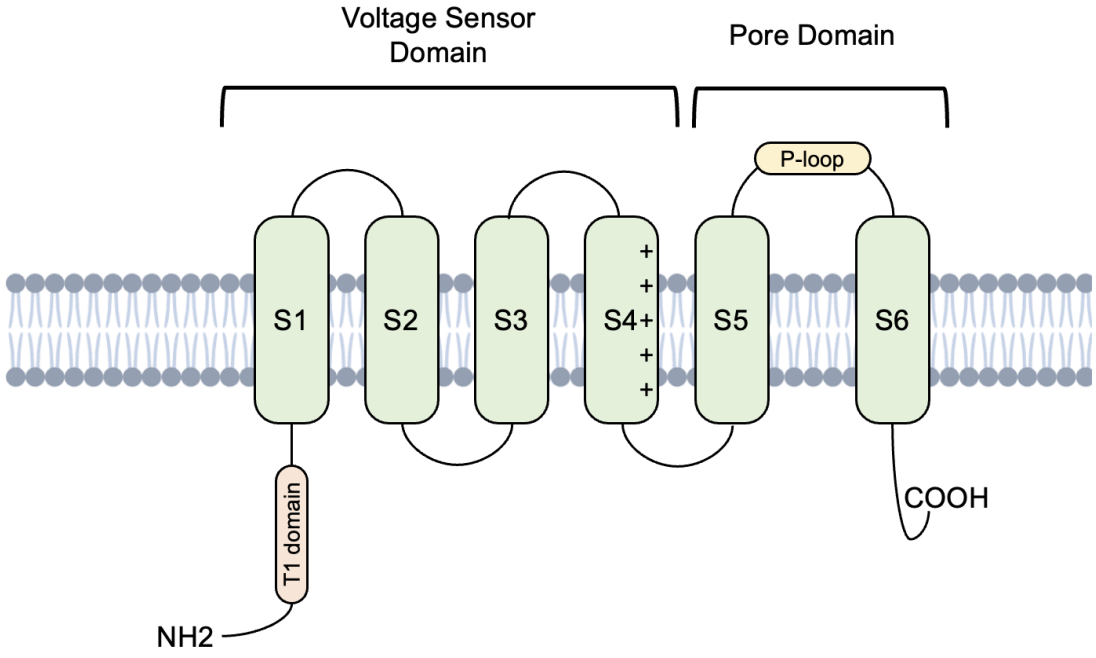


Figure 1.1. Structural schematic diagram of Kv1 α-subunit. Kv1 α-subunits are composed of six transmembrane helices (S1-S6) with cytoplasmic N- and C-termini. Four Kv1 α-subunits assemble to form functional channels, with the S1-S4 segments forming the voltage sensing domain and the S5-S6 segments forming the pore domain. The voltage sending domain detects changes in membrane potential and induces the necessary conformational changes for channel opening and closing. The pore domain allows for K^+ efflux. The P-loop, consisting of the TXGYG sequence, forms the selectivity filter which is responsible for K^+ selectivity. While the highly conserved T1 domain governs channel assembly, the NH_2

and COOH regions, which are relatively more variable, play crucial roles in inactivation and recruiting auxiliary proteins.

Located on the N-terminus is the highly conserved T1 domain which dictates proper Kv channel assembly (Bixby et al., 1999; Cushman et al., 2000). Kv channels only co-assemble with members of the same subfamily (Covarrubias et al., 1991; Li et al., 1992; Shen et al., 1993; Shen and Pfaffinger, 1995; Xu et al., 1995). For example, Kv1(KCNA gene family) α-subunits will only assemble with other Kv1 α-subunits, but not Kv2(KCNB), Kv3(KCNC), etc. (Li et al., 1992). Deletion of the T1 region hence allows Kv α-subunits to assemble promiscuously. For example, elimination of the T1 domain in Kv2.1 enabled it to assemble with Kv1.4 subunits (Lee et al., 1994).

Kv channel pore, selectivity filter, and S6 bundle crossing

As mentioned previously, the S5 and S6 transmembrane segments connected by the P loop constitute the Kv channel central pore, which is crucial for the passage of ions across the cell membrane (Chen et al., 2010; Heginbotham et al., 1994; Jiang et al., 2003a). This structure is often depicted as an inverted teepee with a wider diameter towards the extracellular side that narrows as it approaches the cytoplasmic side (Figure 1.2) (Doyle et al., 1998). The Kv channel pore can be characterized as having three distinct regions: the S6 bundle crossing at the base of the teepee, the selectivity filter at the top, and the central cavity connecting the two (Figure 1.2) (Kim and Nimigean, 2016; Kuang et al., 2015).

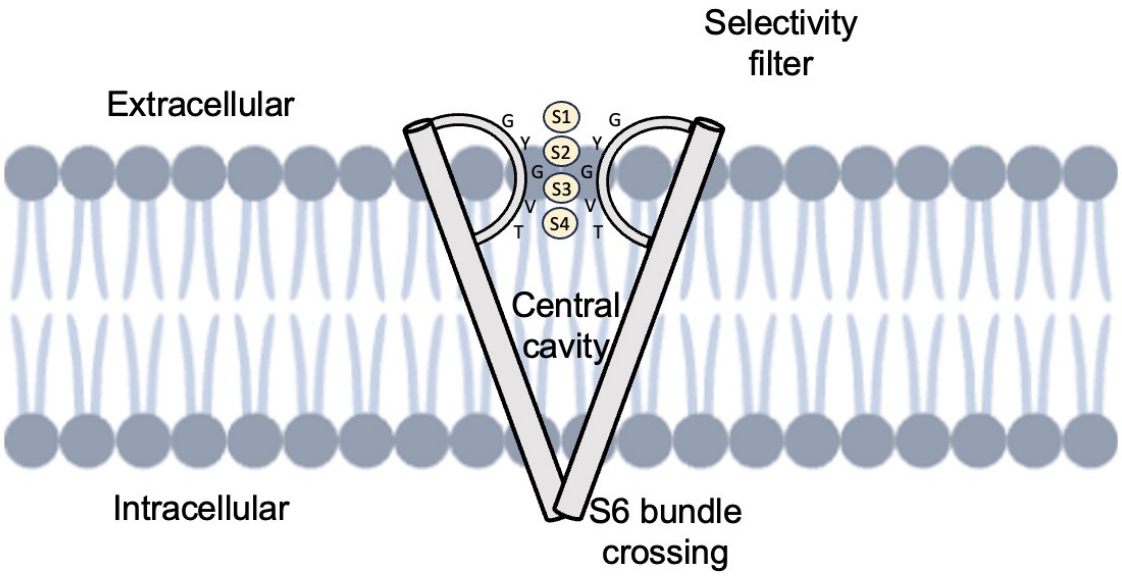


Figure 1.2. Inverted teepee structure and selectivity filter. The following shows two Kv1.2 α-subunits forming the inverted teepee structure with an S6 bundle crossing and central cavity. This representation excludes two other subunits for visibility. The P-loops form the selectivity filter with four K^+ binding sites (S1-S4). Upon channel activation, the S6 bundle crossing will open up allowing K^+ to enter the hydrophobic central cavity. K^+ ions can then enter the selectivity filter in a single-file manner wherein they will be pseudo-hydrated by the backbone carbonyl groups of the TVGYG sequence and the hydroxyl group of threonine. Charge-charge repulsion then rapidly pushes K^+ out the cell.

The S6 segments converge to form the S6 bundle crossing acting as a gate to regulate ion flow into the pore (Kuang et al., 2015; Labro and Snyders, 2012). At hyperpolarized voltages, the S6 bundle crossing constricts, preventing K^+ entry (Kim and Nimigean, 2016; Labro and Snyders, 2012). At depolarized voltages, the S6 bundle will bend and open up, thereby allowing K^+ flow. This mechanism is facilitated by the presence of the conserved PXP motif within the S6 segments, where X represents any amino acid (Labro and Snyders, 2012; Labro et al., 2003; Long et al., 2007). This PXP motif acts as a flexible "hinge" that bends when the channel is closed and unbends when the channel is open.

The selectivity filter is responsible for the high selectivity of K^+ and the near diffusion-limited rate of ion conductance (Doyle et al., 1998; Kim and Nimigean, 2016; Kuang et al., 2015). Unlike the central cavity which is predominantly composed of hydrophobic amino acids, the selectivity filter

contains hydrophilic residues, particularly the conserved TXGYG (TVGYG in Kv1.2) sequence located on the P-loop (Doyle et al., 1998; Kim and Nimigean, 2016). The P-loops form the narrowest region of the pore (~3Å) and the carbonyls of the TVGYG and hydroxyl group of the threonine form four K^+ binding sites (S1-S4) (Kim and Nimigean, 2016). K^+ ions enter the filter in a single file manner to occupy one of these binding sites during which they will be surrounded by eight oxygens (Doyle et al., 1998; Kim and Nimigean, 2016; Kuang et al., 2015). This configuration mimics K^+ hydration which lowers the energy required for entry to the selectivity filter. Due to the smaller size of Na^+ ions, the distance between the oxygens and Na^+ would be too great for effective hydration making the process energetically unfavorable (Doyle et al., 1998; Kim and Nimigean, 2016; Kuang et al., 2015). With regards to the fast diffusion rates, two K^+ ions will bind to the selectivity filter at either positions S1 and S3 or S2 and S4. The charge-charge repulsion results in rapid conduction of K^+ (Doyle et al., 1998; Kim and Nimigean, 2016).

Kv voltage sensing domain

The interaction between the S4 segment and electric field forms the basis of voltage sensing in voltage gated ion channels. In response to changes in the membrane voltage, the charged side chains in the VSD will move across the membrane electric field and produce a small amount of transient electric current known as “gating currents” (Bezanilla, 2018; Hodgkin and Huxley, 1952). These gating currents reflect the conformational change in the membrane protein which is coupled to opening of the channel pore to produce the ionic current. Various experimental approaches have confirmed this principle. Mutagenesis experiments combined with electrophysiology approaches have shown that mutating the conserved positive charges in the S4 segments can result in significant changes to voltage-dependence, thereby confirming the importance of the S4 region in voltage dependent gating (Chen et al., 1996; Kontis and Goldin, 1997; Stühmer et al., 1989). Site-directed fluorimetry, a technique in which fluorescent reporter dyes were introduced into the S4 region of *Shaker* potassium channels, further demonstrated that

the occurrence of gating currents was correlated with conformational changes in that region (Cha and Bezanilla, 1997; Mannuzzu et al., 1996).

How the VSD moves in response to changes in membrane potential has remained a source of contention with multiple models proposed over the years. The three most endorsed models include the helical-screw, the transporter, and the paddle model (Kuang et al., 2015). In the helical screw model, a depolarization triggers a 13.5 Å outward displacement of S4 along with a 180˚ rotation (Börjesson and Elinder, 2008; Durell and Guy, 1992; Keynes and Elinder, 1999). In doing so, positive residues in the S4 segment form a series of ion pairs with negative counter-charges in the S1-S3 segments. In contrast, the transporter model proposes that the VSD movement is due primarily to the 180˚ rotation with only minimal outward movement (Bezanilla, 2000; Chanda et al., 2005; Durell and Guy, 1992). This conformational change is proposed to shift the orientation of the basic S4 residues from an internal aqueous crevice to another crevice that is connected to the external environment. Finally, the paddle model suggests that the S3 and S4 segments form a "paddle-like structure" that undergoes a large translation across the membrane (15-20 Å) (Jiang et al., 2003b). With the development of new atomic resolution structures, a consensus model is emerging that incorporates aspects from all three previous models. This new model, coined the "focused electric field model", proposes a hydrophobic region that the positive charges pass through (Chen et al., 2010). When excited, S4 moves outward and each positive residue moves past the hydrophobic region and interacts with S1-S3 negative residues (Catterall, 2010). During this process, parts of the S4 within the hydrophobic region will adopt a 3:10-helical conformation while parts outside this region will maintain their alpha helical conformation (Durell and Guy, 1992). Doing so, the S4 segment can be extended which will allow S4 residues to maintain their electrostatic interactions (Yarov-Yarovoy et al., 2012).

N and C-type channel inactivation

Many Kv channels undergo a stepwise transition from closed (C) to open (O) to inactivated (I) states (Loots and Isacoff, 1998; Olcese et al., 1997). Different Kv1 subtypes exhibit variable rates of entry

into non-conducting inactivated states and this results in a time-dependent current decay during prolonged depolarizing stimuli. The two major categories of inactivation include fast N-type inactivation and (usually) slower C-type inactivation (Kuang et al., 2015).

N-type inactivation, also known as ball-and-chain inactivation, occurs very rapidly (milliseconds to tens of milliseconds time scale) and is mediated by a mobile peptide located on the N-terminus of channels that can occlude the channel pore (Hoshi et al., 1991; Kuang et al., 2015). Certain channels such as Kv1.4 possess a blocking peptide that enables N-type inactivation and confers these properties when co-assembled as heteromers with other Kv channels (Hoshi et al., 1991; Kim and Nimigean, 2016). Furthermore, assembly of Kv1 channels with certain Kvβ subunits can also result in N-type inactivation (Rettig et al., 1994). Most importantly, channels must be open for N-type inactivation to take place. Intracellular blockers such as TEA^+ can inhibit N-type inactivation. This is because internal blockers can compete with the N-terminal mobile peptide for occupancy of the open pore which then decelerates the rate of N-type inactivation (Choi et al., 1991). In contrast, extracellular blockers do not affect N-type inactivation.

C-type inactivation is typically slower and involves a distinct mechanism of conformational changes in the selectivity filter (Hoshi et al., 1991). Recent structural studies suggest more specifically that C-type inactivation occurs via the dilation of the external selectivity filter which disrupts two of their ion binding sites and this prevents ion permeation (Reddi et al., 2022; Tan et al., 2022). Unlike N-type inactivation, removal of the N-terminus does not abolish C-type inactivation (Choi et al., 1991). Instead, residues in the pore, particularly Thr449 and Ala463 in *Shaker*, dictate the rate of C-type inactivation (Hoshi et al., 1991). Furthermore, C-type inactivation was found to be coupled to channel opening but is usually not voltage-dependent through -25 to +50 mV (Hoshi et al., 1991). While internally applied TEA^+ can prevent N-type inactivation, intracellular blockers and N-type inactivation can promote the rate of C-type inactivation (Baukrowitz and Yellen, 1996; Kurata and Fedida, 2006). This is because by preventing potassium from entering the pore, this increases the possibility for an empty selectivity filter, which has a destabilizing effect and promotes entry into a C-type inactivated conformation (Baukrowitz and Yellen,

1996). Conversely, externally applied TEA^+ or high concentrations of extracellular potassium can inhibit the rate of C-type inactivation (Choi et al., 1991; Hoshi et al., 1990; Rasmusson et al., 1998). This occurs because the likelihood of either K^+ or TEA^+ to enter and occupy ion binding sites in the selectivity filter increases, which stabilizes the filter and prevents the conformational change needed for C-type inactivation (Rasmusson et al., 1998).

Given the importance of the extracellular pore in C-type inactivation, mutations in this region can have dramatic impacts. In particular, replacement of residue T449 in *Shaker* with a polar residue such as arginine, lysine, glutamate, or alanine drastically accelerates C-type inactivation (López-Barneo et al., 1993). Replacement with a hydrophobic residue such as tyrosine or valine greatly inhibits C-type inactivation. Other mutations including *Shaker* W434F and D447N mutants accelerate C-type inactivation to such a degree that channel conduction is abolished (Hurst et al., 1996; Pless et al., 2013; Yang et al., 1997). For Kv1.2, the V381T mutant (equivalent/homologous to Shaker T449) accelerates C-type inactivation and when combined with W366F mutant, results in a nonconducting channel similar to *Shaker* 434F and D447N (López-Barneo et al., 1993; Suárez-Delgado et al., 2020). Collectively, these studies highlight the significant impact that channel mutations can have on inactivation. These effects are similarly investigated in Chapter 4, specifically focusing on the influence of mutations on N-type inactivation.

1.2 LOCALIZATION AND FUNCTION OF KV1.2 CHANNELS

Axon initial segment

Neurons receive synaptic inputs that converge onto the axon initial segment (AIS). This area represents the site of action potential initiation and contains a high density of voltage-gated sodium and potassium channels (Huang and Rasband, 2018). Kv1.2 is commonly co-assembled with Kv1.1 channels and found throughout the AIS of various cell types where they perform multiple functions. These Kv1.1/1.2 clusters can control the action potential waveform of layer V pyramidal neurons in the neocortex and inhibit the excitability of fast-spiking neocortical GABAergic interneurons (Goldberg et al., 2008; Kole et al., 2007). Kv1.2 also regulates rodent lumbar motoneurons. Specifically Kv1.2 inactivation contributes to the delayed spike-frequency acceleration of spinal motoneurons such that pharmacological inhibition of Kv1.2 abolished this hallmark feature (Bos et al., 2018). These findings suggest that Kv1.2 channels may play an important role in regulating locomotion. Moreover, in the context of this thesis, this result demonstrates the potential significance of Kv1.2 inactivation as a powerful regulator of various physiological processes.

The mechanism by which Kv1.2 clusters in the AIS remains unclear. While Kv1 channels have been found to be colocalized with Caspr2 in the AIS of human cortical pyramidal neurons and Cortactin2 in motor neurons, neither of these cell adhesion proteins were required for Kv1 channel recruitment in the AIS (Duflocq et al., 2011; Ogawa et al., 2008). Rather, a membrane-associated guanylate kinase postsynaptic density-93 (PSD-93), localizes and mediates Kv1 clustering at the AIS in hippocampal neurons (Ogawa et al., 2008). More recently, the acetyltransferase ZDHHC14 was shown to control AIS clustering of both PSD-93 and Kv1 potassium channels (Sanders et al., 2020).

Juxtaparanode

The juxtaparanode represents the part of the axon underneath the myelin sheath and between the paranode and internode of the nodes of Ranvier (Arancibia-Carcamo and Attwell, 2014). While it has been well-established that Kv1 channels are highly expressed in this region with Kv1.1 and Kv1.2 being

the most abundant, the specific role of these channels remains elusive (Arancibia-Carcamo and Attwell, 2014; Rasband et al., 1998, 1999; H. Wang et al., 1993). Attempts to answer these questions have resulted in inconclusive and contradictory findings. In optic nerves, Devaux et al. reported that application of Kv1 channel blocker 4-aminopyridine (4-AP) resulted in profound changes to the amplitude, duration, and refractory periods of action potentials but Foster et al. found that 4-AP has only minor effects (Devaux et al., 2002; Foster et al., 1982). In central myelinated axons, potassium conductance was undetectable altogether and application of Kv1 channel blocker 4-AP only slightly reduced late outward currents (Kocsis and Waxman, 1980). These findings may reflect that depending on the type of axon, the contribution of potassium channels to the action potential may differ (Arancibia-Carcamo and Attwell, 2014). A possible explanation for the minor effects of 4-AP in spite of the high density of Kv1 channels is that the small voltage changes at the juxtaparanode may not be sufficient to activate Kv channels. It has been suggested that Kv channels offer a protective mechanism by maintaining the resting membrane potential and preventing improper action potential firing (Gittelman and Tempel, 2006; Kopp-Scheinpflug et al., 2003; Vabnick et al., 1999).

Over the years, various cell adhesion proteins have been identified to regulate Kv1 clustering to the juxtaparanode. Caspr2 and Cortactin2/TAG-1 form a complex that mediates the localization of Kv channels (Rasband, 2010). Caspr2 KO mice resulted in a significant but incomplete reduction in Kv1 channels at the juxtaparanode of the sciatic nerve, optic nerve, and spinal cord (Poliak et al., 2003; Saifetiarova et al., 2017). Likewise, Cortactin2 KO mice also exhibited a reduction in Kv1 channels at the juxtaparanode of optic and sciatic nerves (Traka et al., 2003). The scaffolding protein 4.1B also plays a role in Kv1.1/1.2 clustering. In myelinated DRG neurons, it was found that Kv1.1/1.2 channels were co-localized with Caspr2 at the distal juxtaparanodes and that knock-out of 4.1B disrupted this clustering while not altering the assembly of the paranode itself (Hivert et al., 2016). The incomplete attenuation of Kv1 channels in these knockout models suggest that compensatory mechanisms exist to maintain a lesser degree of Kv1 assembly or that the interaction between Caspr2, Cortactin2, and 4.1B, is not solely responsible for this effect. The known Kv1 juxtaparanodal complex is therefore likely incomplete with

future experiments needed to fully elucidate the various binding partners and the mechanism behind their effects.

Together, these findings parallel the core themes of this thesis, which proposes that Kv1.2 channels are regulated by various auxiliary binding partners which can then have important physiological relevance. The incompletely understood relationship between Kv1 channels, auxiliary subunits, and site of localization highlights the complexity of ion channels and the need for continual research and investigation.

Hippocampus

Toxin data and anti-Kv1 specific antibody studies show that both Kv1.1 and Kv1.2 channels are expressed throughout the hippocampus, often as heteromeric channels (Koschak et al., 1997; Sheng et al., 1994, 1993; Willis et al., 2018). Specifically, Kv1.2 was found to be most highly localized to the molecular layer of the dentate gyrus, CA3 stratum lucidum and stratum oriens, and CA1/2 stratum radiatum (Willis et al., 2018). Kv1.2 haploinsufficiency in CA3 pyramidal cells resulted in decreased threshold for long term potentiation. Kv1.2 +/- mice showed impaired ability to recognize context-threats during a contextual fear discrimination task (Eom et al., 2020). These findings suggest that Kv1.2 may play a role in memory and pattern recognition. In hippocampal neurons, Kv1.2 has been found to localize to the dendrites and AIS where it contributes to the dendrotoxin-sensitive current (D-current) which is responsible for regulating the threshold for action potential generation (Sánchez-Ponce et al., 2012; Sheng et al., 1994; Wu and Barish, 1992). Blockade of Kv1.2 with 4-AP increased action potential duration, repetitive firing, and neuronal hyperstimulation (Wu and Barish, 1992).

Auditory Cortex

The superior olivary complex represents a region of the brainstem that plays important roles in sound localization and is primarily composed of three nuclei: the medial superior olive (MSO), the lateral superior olive (LSO), and the medial nucleus of the trapezoid body (MNTB) (Fischl et al., 2016; Moore,

2000). Kv1.1 and Kv1.2 are found throughout the LSO and MNTB. Immunochemistry and Kv1 specific toxins confirmed that Kv1.2 homomeric and Kv1.2/1.1 heteromeric channels localized to the AIS of MNTB neurons where they contribute the majority of the low voltage activated current (Dodson et al., 2002). This current is important for ensuring that a sustained depolarization only generates a single action potential and thus, Kv1.2 regulates MNTB neuronal firing. Kv1.2 channels are also found between the axon and synaptic terminal of MNTB neurons where they regulate action potential duration to suppress terminal hyperexcitability (Dodson et al., 2003). Kv1.2 in LSO neurons are similarly responsible for maintaining proper generation and transmission of action potentials (Barnes-Davies et al., 2004; Gittelman and Tempel, 2006).

Cerebellum

Kv1.1 and Kv1.2 channels are expressed in the cerebellum, specifically in the axon terminals of basket cells and the dendrites and AIS of Purkinje cells (Feria Pliego and Pedroarena, 2020; Sheng et al., 1994). As basket cells are inhibitory neurons that integrate Purkinje cells at the pinceau synapse, inhibition of basket cells by dendrotoxin was able to increase Purkinje cell spontaneous inhibitory postsynaptic potential (IPSP) current frequency and amplitude (Southan and Robertson, 1998; Zhou et al., 2020). In various rodent and human models, KCNA1 and KCNA2 mutations are associated with ataxia and seizures (Browne et al., 1994; Helbig et al., 2016; Herson et al., 2003; Pena and Coimbra, 2015). Furthermore, KCNA1 mutations in mice showed increased IPSP current in Purkinje cells similar to the effects seen with Kv1 blockers (Herson et al., 2003). This has led to the postulation that the synapse where basket cells innervate onto Purkinje cells is important for the development of ataxia (Jan and Jan, 2012).

Cerebral Cortex

In the cerebral cortex, Kv1.2 is distributed throughout different regions which include the neuropil, the dendrites and AIS of pyramid V neurons, the AIS of pyramidal II/III neurons, and the

dendrites of pyramidal I neurons (Lorincz and Nusser, 2008; Sheng et al., 1994). Given the crucial role of the AIS for action potential initiation, it has been suggested that Kv1.2 channels play a significant role in modulating the activity of various pyramidal neurons. Application of Kv1 channel blockers to layer V pyramidal neurons would increase excitability, thereby increasing the firing rate of these neurons (Bekkers and Delaney, 2001; Guan et al., 2018; Kole et al., 2007). Similarly, pharmacological inhibition of Kv1 channels in layer II/III pyramidal neurons reduced the action potential threshold, decreased the rheobase, increased action potential width, decelerated deactivation kinetics and overall increased neuronal firing (Guan et al., 2007). These findings highlight the importance of Kv1.2 in regulating the excitability of various pyramidal neurons in the cerebral cortex.

Peripheral Nervous System

Dorsal root ganglia (DRG) neurons are sensory neurons that innervate both the periphery and spinal cord, and are responsible for transmitting sensory information to the CNS (Nascimento et al., 2018). This process is mediated by a variety of ion channels including Na^+, K^+, acid-gated ion channels, and ATP-gated channels (Krames, 2014). Kv1.2 and Kv1.1 channels are the predominant Kv channels expressed in large DRG neurons where they contribute to repolarization of the membrane, set the resting membrane potential, and protect the neuron from hyperexcitability by contributing to the delayed-rectifier current (I_{KDR}) and transient inactivating A current (I_{KA}) (Du and Gamper, 2013; Rasband et al., 2001; Vydyanathan et al., 2005; Yang et al., 2004). Nerve injury leads to consistent decreased expression of Kv1.2 and Kv1.1 which can then lead to over-sensitization and the development of neuropathic pain (Lee et al., 1999; Rasband et al., 2001; Vydyanathan et al., 2005; Yang et al., 2004).

Over the years, the decreased expression of Kv channels has been attributed to various regulatory factors. Liang et al., showed that euchromatic histone-lysine N-methyltransferase 2 (G9a) co-localizes with KCNA2 mRNA and suppresses KCNA2 gene transcription (Liang et al., 2016). Similarly, Zhao et al., showed that post peripheral nerve injury, DNA methyltransferase DNMT3a expression increases leading to methylation of KCNA2 and attenuated Kv1.2 expression (Zhao et al., 2017). More recently,

microRNA-137 was shown to downregulate Kv1.2 expression leading to neuropathic pain in rats with suppression of microRNA-137 leading to rescue of Kv1.2 and decreased pain (Zhang et al., 2021). Ultimately, these variable regulators demonstrate the importance of Kv1.2 in controlling the emergence of neuropathic pain.

Cardiovascular System

While Kv1.2 is primarily expressed in the CNS, Kv1.2 is also found at varying levels in the right and left coronary arteries, atrium, and ventricles of various mammalian models (Barry et al., 1995; Bertaso et al., 2002; Gautier et al., 2007; Kerr et al., 2001; Lee et al., 1999). Through the use of Kv channel blockers like dendrotoxin and 4-AP, it has been shown that Kv1.2 homomeric and Kv1.2-1.5 heteromeric channels contribute to the delayed rectifier K^+ current to repolarize the membrane potential in vascular smooth muscle (Barry et al., 1995; Kerr et al., 2001; Yuan et al., 1998). Kv1.2, along with Kv1.5, Kv2.1, and Kv3.1, also plays a minor role in regulating the ultrarapid outward IKur current in cardiomyocytes (Nattel et al., 1999).

1.3 KV1 FAMILY OVERVIEW

Kv1.1

Kv1.1 channels are expressed throughout the body and serve diverse functions (D'Adamo et al., 2020). In the CNS and PNS, Kv1.1 channels are prominently localized to the AIS, synaptic terminals, and juxtaparanode of various cells found throughout the hippocampus, auditory cortex, cerebellum, cerebral cortex, and periphery (Dodson et al., 2002; Feria Pliego and Pedroarena, 2020; Fischl et al., 2016; Huang and Rasband, 2018; Rasband et al., 2001; Sheng et al., 1993; H. Wang et al., 1993; Willis et al., 2018). Upon depolarization, Kv1.1 channels can activate to enable the efflux of potassium which serves to set the threshold potential, repolarize the membrane potential, and suppress cellular excitability (D'Adamo et al., 2020). Kv1.1 channel mutations or genetic knock out will therefore increase cellular excitability by lowering the threshold potential, broadening the action potential, and increasing the firing rate (Brunetti et

al., 2012; D'Adamo et al., 2020; Dodson and Forsythe, 2004; Herson et al., 2003). Mutations have been associated with various diseases and symptoms including episodic ataxia, eyelid myokymia (continuous contractions of the eyelid or fingers), slurred speech, blurred vision, and developmental delay (Demos et al., 2009; Graves et al., 2010; Jen et al., 2007). Kv1.1 is also found in pancreatic β-cells where they stimulate insulin secretion and in atrial myocytes to contribute to cellular repolarization and proper heart function (Glasscock et al., 2015, 2010; Ma et al., 2011). Kv1.1 can either assemble alone as homomeric channels or co-assemble with Kv1.2 and Kv1.4 α-subunits to form heteromeric channels (Dodson et al., 2002; Imbrici et al., 2007; Trimmer, 2015). Functional Kv1.1 channels can also form complexes with Kvβ1.2 and be influenced by its modulation (Rettig et al., 1994)). This interaction with Kvβ1.2 enables the channels to exhibit rapid N-type inactivation, which is induced by a large peptide region present on the Kvβ1.2 accessory protein (Rettig et al., 1994). When expressed alone, Kv1.1 expresses slow C-type inactivation that is believed to be regulated by conformational changes in the channel pore (Kuang et al., 2015). Other proteins that modulate Kv1.1 include LGI1, ADAM23, PSD-95, and CASPR (Lancaster et al., 2019; Poliak et al., 2003; Schulte et al., 2006)

Kv1.3

Kv1.3 channels are unique in that they regulate the immune response and are found in T and B lymphocytes, macrophages, microglia, fibroblasts, and platelets (Gutman et al., 2005; Wang et al., 2020). These channels are involved in regulating the membrane potential and Ca^{2+} homeostasis, which is critical for T-cell activation (Cahalan et al., 1985; Wulff et al., 2009). Importantly, upon T-cell activation, Kv1.3 channels can be upregulated (Wulff et al., 2009). Hence, dysregulation of Kv1.3 channels has been associated with a range of immune disorders, including multiple sclerosis, rheumatoid arthritis, diabetes, and asthma (Huang et al., 2017; Tanner et al., 2017; Toldi et al., 2010; Zhou et al., 2018). It has been proposed that blocking Kv1.3 channels could serve as a viable strategy for treating these diseases (Wang et al., 2020; Wulff et al., 2009).

Kv1.4

Kv1.4 is unique in that it contains an N-terminal inactivating particle which can enable rapid N-type inactivation when expressed either as a homomers or heteromers to produce A-type current (Lee et al., 1996). Kv1.4 also performs numerous functions and is found in the brain, heart, and pancreas. In the brain, Kv1.4 regularly coexpresses alongside Kv1.1 and Kv1.2 in various regions including the axon/nerve terminals, axon initial segments, layer V pyramidal neurons, substantia nigra, dentate gyrus, Schaffer collateral axons, and mossy fiber axons (Kole et al., 2007; Monaghan et al., 2001; Sheng et al., 1992; Shu et al., 2007; Trimmer and Rhodes, 2004; Vacher et al., 2008). In the heart, Kv1.4 is found in the myocardium alongside Kv4.3/Kv4.2 to generate the transient outward (I_{to}) current (Niwa and Nerbonne, 2010). Upon depolarization, this current activates and inactivates rapidly and is partially responsible for repolarizing the membrane - specifically in shaping the phase 1 initial rapid repolarization and setting the phase 2 initial plateau potential. Kv1.4 is also found in pancreatic β-cells where they inhibit glucose-stimulated insulin release such that suppression of Kv1.4 current either by GIP or truncating the C-terminus enhanced insulin secretion (Kim et al., 2005; MacDonald et al., 2001)

Kv1.5

In the cardiovascular system, Kv1.5 channels are chiefly expressed in the atria and pulmonary artery smooth muscles (Fedida et al., 1993; Wang et al., 1997). In the atria, Kv1.5 channels heavily distribute themselves at the intercalated disks and underlie the outward IKur current (Jeevaratnam et al., 2018; Snyders, 1999). This IKur current activates very rapidly within 10 milliseconds and plays a role in atrial repolarization (Jeevaratnam et al., 2018). Further demonstrating the importance of Kv1.5 is that various Kv1.5 mutations are associated with cardiac arrest, action potential prolongation, and atrial fibrillation (Nielsen et al., 2007; Olson et al., 2006). Given that Kv1.5 channels are not present in the ventricle, selective inhibition of Kv1.5 has long been considered as a viable strategy for treating atrial arrhythmias (Wulff et al., 2009). Despite this initial optimism, experimental results using 4-AP demonstrate both prolonged and shortened action potential duration that seems to be dependent on the

degree of electrical remodeling done in response to disease (Escande et al., 1985; Z. Wang et al., 1993; Wettwer et al., 2004).

In pulmonary artery smooth muscles, Kv1.5 contributes to the regulation of resting membrane potential and pulmonary vasomotor tone (Archer et al., 1998; Wang et al., 1997). Under hypoxic conditions, Kv1.5 expression decreases which results in increased depolarization of the pulmonary artery smooth muscles leading to increased pulmonary vasoconstriction (Archer et al., 1998; Wang et al., 1997). Using gene therapy to reintroduce Kv1.5 channels can therefore reverse pulmonary vasoconstriction further underscoring the importance of Kv1.5 channels in cardiovascular function (Pozeg et al., 2003).

Kv1.6

Kv1.6 is most found in the medulla-pons, inferior colliculus, and visceral sensory neurons likely either as homomeric or heteromeric channels along with Kv1.1 and Kv1.2 (Glazebrook et al., 2002; Grupe et al., 1990). More recently, four mutations of Kv1.6 have been identified resulting in varying degrees of epilepsy and neurodevelopmental defects (Salpietro et al., 2023).

Kv1.7

First reported in 1998, Kv1.7 is preferentially localized to the skeletal muscle, heart, kidney and pancreatic islet cells. It is also found in the liver to a lesser degree (Kalman et al., 1998; Kashuba et al., 2001). While Kv1.7 function has yet to be fully explored, it has been shown that Kv1.7 channels are involved in pancreatic insulin secretion such that selective inhibition of Kv1.7 led to increased insulin release in response to glucose (Finol-Urdaneta et al., 2012).

Kv1.8

Lang et al. cloned the Kv1.8 channel in 2000 and described its channel kinetics and sensitivities to various pharmacological agents (Lang et al., 2000). Kv1.8 is localized to the renal blood vessels, heart,

and inner ear where it is suspected to regulate renal vascular smooth muscle tone, cardiocyte firing, vestibular function, and hearing (Lang et al., 2000; Lee et al., 2013).

1.4 REGULATION OF KV1.2 CHANNELS

Aside from the intrinsic properties of Kv1.2 underlying voltage-dependent gating, there are numerous extrinsic regulatory mechanisms that comparatively have not received as much attention but have impacts on function and expression. This section aims to provide an overview of some of these regulatory mechanisms.

Redox-dependent slow gating

Across various mammalian cell lines including LM fibroblast, HEK, and CHO cells, Kv1.2 exhibits variable activation kinetics with reported $V_{1/2}$ ranging from -40 mV to +40 mV (Baronas et al., 2017, 2015; Grissmer et al., 1994; Lamothe and Kurata, 2020; Rezazadeh et al., 2007). This 80 mV variation in voltage-dependence has been attributed to Kv1.2 occupying distinct gating modes: a "fast" permissive gating mode and a "slow" resistant gating mode (Rezazadeh et al., 2007). In their fast gating mode, Kv1.2 activates quickly while conversely, in their slow gating mode, Kv1.2 activates slowly and requires strong depolarizations to open. Under ambient redox conditions (unbufferred redox), cells transfected with Kv1.2 exhibit considerable variability likely due to variable occupancy of channels between these two extreme gating modes (Baronas et al., 2017, 2015; Rezazadeh et al., 2007).

Due to the potential to operate in different gating modes, Kv1.2 channels exhibit use-dependent activation (UDA), whereby brief repetitive depolarizations can gradually increase channel current. UDA occurs because repetitive depolarizing stimuli cause Kv1.2 channels to progressively shift from the slow to fast gating modes, allowing them to activate more readily.

Past work has identified experimental manipulations that can promote channel gating in either the fast or slow modes. For example, strong depolarizing prepulses reduce variable behavior of Kv1.2 by shifting channels to a fast gating mode, characterized by accelerated activation, $V_{1/2}$ = ~-20 mV, and decreased % UDA (Baronas et al., 2015; Rezazadeh et al., 2007). Alternatively, extracellular reducing conditions induced by various reducing agents including DTT, reduced glutathione, and TCEP, strongly promote the slow gating mode of Kv1.2 resulting in slower activation, $V_{1/2}$ = +40 mV, and increased %

UDA (Baronas et al., 2017). Although this process is clearly redox sensitive, Kv1.2 transmembrane cysteine mutations do not abolish redox sensitivity, suggesting that these effects are modulated by an unknown extrinsic regulator rather than disulfides within the channel itself (Baronas et al., 2017).

Rezazdeh et al., identified the S2-S3 linker region as the key regulator of the activation gating switch in Kv1.2 channels. Replacement of the Kv1.2 S2-S3 linker with Kv1.5 resulted in cells that exclusively exhibited the fast gating phenotype. Notably, Thr252, Phe251, and Phe250 residues were found to be especially important such that single point mutations could attenuate Kv1.2 variable current properties (Baronas et al., 2016; Rezazadeh et al., 2007). It was further demonstrated that Thr252 mutations could greatly reduce Kv1.2 sensitivity to redox and LMAN2 (Lamothe and Kurata, 2023, 2020). Taken together, these findings suggest that redox and LMAN2 either directly or indirectly act on these residues to promote the slow gating behaviour. Interestingly, introducing Thr in the S2-S3 linker of Kv1.1, Kv1.3, and Kv1.4 was insufficient to produce UDA or redox sensitivity (Baronas et al., 2016). This suggests the presence of additional unidentified channel features or regulatory factors that are required for the slow gating phenomenon.

Lectin Protein LMAN2 (VIP36)

LMAN2 (VIP36) is a transmembrane mannose-binding lectin that cycles between the Golgi apparatus and transmembrane ER (Füllekrug et al., 1999). It interacts with glycosylated proteins and facilitates the transport of diverse cargo proteins, particularly GPRC5B and α1-antitrypsin, within the cell (Kwon et al., 2016; Reiterer et al., 2010). While LMAN2 has not been directly linked with any neurological diseases, mutations in its closely related homolog, LMAN2L, have been associated with intellectual disabilities and epilepsy (Alkhater et al., 2019; Rafiullah et al., 2016). We recently identified LMAN2 as a candidate protein modulator of Kv1.2 that promotes their slow gating modes (Lamothe and Kurata, 2023). Coexpression of Kv1.2 channels with LMAN2 yielded profound functional outcomes that mimicked effects of reducing agents, including a rightward shift ($V_{1/2}$ = +40 mV) in voltage-dependence and enhanced % UDA. Overexpression of LMAN2 also sensitizes Kv1.2 channels to reducing agents

such that sub-saturating concentrations of DTT (i.e. 30 μM DTT) generate maximal slow gating effects. The knockdown of LMAN2 significantly diminished the slow gating phenotypes and attenuated redox sensitivity, which were subsequently restored upon re-transfection with LMAN2. Taken together, these findings strongly support the role of LMAN2 as a regulator of the Kv1.2 slow gating mechanism and Kv1.2 sensitivity to extracellular redox.

Slc7a5 (LAT1)

Slc7a5 (LAT1) is a neutral amino acid transmembrane transporter that forms a heterodimeric complex with Slc3a2 (CD98) (El Ansari et al., 2018; Kanai et al., 1998; Scalise et al., 2018). Its primary function is antiport of neutral amino acids leucine, phenylalanine and tryptophan into the cell in exchange for glutamine out of the cell. Slc7a5 is believed to play an important role in proper CNS development because homozygous mutations result in autism, motor delay and seizures (Sokolov et al., 2020; Tărlungeanu et al., 2016). Furthermore, rodent knock down of Slc7a5 led to profound neural defects and death (Poncet et al., 2020, 2014). While this was suggested to be due to the role of Slc7a5 in maintaining proper amino acid concentrations, Slc7a5 has also been shown to have powerful effects on Kv1.2 channel expression and function (Baronas et al., 2018; Lamothe and Kurata, 2020).

Through mass spectrometry analysis, molecular biology techniques, and electrophysiology approaches, Slc7a5 was found to greatly inhibit Kv1.2 expression, suppress current density and induce a -40 mV hyperpolarizing shift in gating ($V_{1/2}$ = -58 mV) (Baronas et al., 2018). These effects could then be rescued by co-expression with the shortest isoform of Slc3a2. Slc7a5 was also shown to promote C-type inactivation and enable significant disinhibition of Kv1.2 by holding at supraphysiological negative potentials (i.e. -120 mV). Further demonstrating the neurological importance of this transporter, Slc7a5 mutations A246V and P375L, which have been linked with autism, prevented interaction with Kv1.2 channels thereby attenuating effects on Kv1.2 expression and gating (Baronas et al., 2018; Tărlungeanu et al., 2016). Additionally, Kv1.2 gain of function mutations R297Q and L289F caused hypersensitivity to Slc7a5 effects (Syrbe et al., 2015). This observation has been proposed as a possible explanation as to

why both gain and loss of function Kv1.2 mutants can induce epileptic seizures (Baronas et al., 2018; Syrbe et al., 2015).

Kvβ

The canonical auxiliary proteins of Kv1 channels are the Kvβ subunits. These proteins assemble with Kv1 α-subunits in a 1:1 stoichiometry by binding onto the cytoplasmic T1 domain to form heteromultimeric complexes (Gulbis et al., 2000, 1999; Kobertz et al., 2000). There are three Kvβ mammalian genes and their splice variants which include Kvβ1.1-1.3, Kvβ2.1-2.2, and Kvβ3.1-3.2. Each of these Kvβ subunits can modulate the function and properties of Kv1 channels in similar and different ways (Pongs and Schwarz, 2010).

When assembled with Kv1.2 channels, Kvβ1 can facilitate channel localization to the cell surface and confer rapid N-type inactivation via a N-terminal ball-and-chain peptide (Accili et al., 1997; Heinemann et al., 1996; Rettig et al., 1994). In contrast, Kvβ2, which is the most abundantly expressed β-subunit in the brain, does not induce N-type inactivation (Heinemann et al., 1996; Rhodes et al., 1995). This is due to the relatively shorter N-terminus of Kvβ2 (difference of ~70 amino acids) which lacks the inactivating ball domain. When the N-terminus of Kvβ2 is replaced with Kvβ1, these Kvβ2 chimeras can then induce fast inactivating currents to Kv1 channels. Instead, Kvβ2 interacts with Kv1 channels in the endoplasmic reticulum (ER) and promotes their cell surface expression and stability, and facilitates N-linked glycosylation (Shi et al., 1996). Unlike Kvβ1 and Kvβ2, Kvβ3 does not increase current size but is capable of inducing N-type inactivation depending on the expression system (Bähring et al., 2004; Heinemann et al., 1995; Morales et al., 1995). In oocytes, Kvβ3 exclusively affected the inactivation rate of Kv1.4 but in CHO cells, Kvβ3 modulated the currents of Kv1.1, 1.2, 1.4, 1.5, and 1.6 (Bähring et al., 2004; Heinemann et al., 1995; Morales et al., 1995). While the exact mechanism by which this occurs remains unknown, it does show that cell expression systems can result in different channel modulation. These effects are similar to how Kv1.2 slow gating varies depending on the expresion system (Baronas et al., 2015; Grissmer et al., 1994; Minor et al., 2000; Rezazadeh et al., 2007).

Additionally, Kvβ proteins are also oxidoreductases and belong to the aldo-keto reductase family (Pongs and Schwarz, 2010). While the Kvβ N-terminus is highly variable, the core domain and C-terminal domain is highly conserved with over 80% homology among the different subtypes. This core domain is characterized by a TIM barrel structure with eight alternating α-helices and β-strands, and an active site (Hyndman et al., 2003; McCormack and McCormack, 1994; Pongs and Schwarz, 2010). The active site is composed of a substrate binding site, a $NADP^+$ (NADPH) cofactor binding site, and a catalytic site. Kvβ can reduce aldehydes to alcohols via the oxidation of NADPH (Weng et al., 2006). The enzymatic activity of Kvβ is coupled to their inactivating properties. Mutations of key residues in the catalytic domain (D119A, D152A, and D124F) prevents Kvβ from conferring N-type inactivation to Kv1 channels (Bähring et al., 2001). More specifically, the oxidation of bound NADPH to NADP+ is responsible for disrupting Kvβ channel inactivation. Increasing the amount of Kvβ substrate to drive the consumption of NADPH will decrease the rate of inactivation and moderately increase current size (Weng et al., 2006). Alternatively, perfusing excess NADPH in the pipette can rescue these effects and restore N-type inactivation (Tipparaju et al., 2007; Weng et al., 2006). It has been proposed that upon NADPH oxidation to NADP+, positively charged residues on the N-terminus interact with negatively charged residues on the AKR core domain (Pan et al., 2011). When the core domain binds to the N-terminus, this then inhibits N-type inactivation. While further studies are required to elucidate the full mechanism, it is becoming increasingly clear that Kvβ proteins are redox sensitive and that their redox sensitivity can impart functional changes to Kv1.2.

These findings on Kvβ, along with previous studies examining Kv1.2 redox-dependent slow gating, demonstrate the existence of diverse mechanisms that have evolved to enable the modulation of Kv1 subtypes by redox. Despite these advances, our understanding of redox-dependent modulation of excitability remains limited. As such, a major objective of this thesis is to further elucidate this phenomenon and aim to gain insight into the intricate interplay between redox signaling, auxiliary proteins, and channel excitability.

Sigma-1 Receptor

The sigma-1 (σ-1) receptor is an intracellular chaperone protein and signaling modulator that directly and indirectly regulates a variety of proteins including ion channels, GABA and glutamate ionotropic receptors, dopamine D1 receptors, muscarinic and nicotinic acetylcholine receptors, tyrosine kinase receptor type 2, kinases and IP3 receptors (Kourrich et al., 2012b). While primarily localized to the mitocohondrion-associated ER membrane (MAM), upon stimulation, σ-1 receptors can translocate to various cellular compartments, including the ER and plasma membrane, to serve diverse functions (Hayashi and Su, 2007). In the context of Kv1.2 channels, activation of σ-1 receptors by agonists such as cocaine can induce a direct interaction with Kv1.2, leading to increased translocation of both proteins to the plasma membrane of Nucleus Accumbens shell (NAcs) medium spiny neurons (MSNs) (Delint-Ramirez et al., 2020; Kourrich et al., 2013, p. 201). The NAcs is critically involved in addiction, and dopamine release in this area is necessary for establishing appropriate responses to reward-predictive cues (Nicola et al., 2005). The increased presence of Kv1.2 channels at the membrane leads to augmented outward K^+ current and consequently induces neuronal hypoactivity. The reduced firing rate of NACs MSNs then results in drug-seeking behaviour (Kourrich et al., 2012a; Taha and Fields, 2006). Conversely, knockdown of σ-1 receptors abolishes the cocaine-induced increase in K^+ current and reduced firing of MSNs (Kourrich et al., 2013). Intriguingly, previous studies have suggested that σ-1 receptors may influence the gating state and activation kinetics of Kv1.2 channels, favoring a predominantly slow gating mode (Abraham et al., 2019). However, the reported effects of σ-1 receptor co-expression are modest in comparison to the pronounced functional changes induced by DTT, LMAN2 (see Chapter 3), or Slc7a5 (Baronas et al., 2018, 2017; Lamothe and Kurata, 2023).

PIP2

Phosphatidylinositol-(4,5)-bisphosphate (PIP2) is a phospholipid primarily located in the cytoplasmic leaflet of the cell membrane (Suh and Hille, 2008). Despite making up less than 1% of

membrane lipids, PIP2 plays diverse functions in regulating actin dynamics, membrane structure, vesicle trafficking, and ion channels (Mandal, 2020). Down regulation of PIP2 results in a 20-30% reduction in current amplitude, slower channel closure, and a hyperpolarizing shift in voltage dependence (Kruse and Hille, 2013; Rodriguez-Menchaca et al., 2012). Conversely, reintroduction of PIP2 can reverse these effects. The underlying mechanism for these effects involves the interaction between PIP2 and the S4-S5 linker - a region that couples the VSD and pore domain. In contrast, Kv1.1, 1.3, 1.4, and 1.5 are all insensitive to PIP2, highlighting the diverse and different signaling pathways of each Kv channel subtype (Kruse et al., 2012).

Phosphorylation

Kv1.2 channels have various phosphorylation sites that can affect channel gating, expression, and current amplitude (Park et al., 2008). Activation of protein kinase A promotes phosphorylation of the T46 residue in the N-terminal region, resulting in enhanced Kv1.2 cell surface localization and increased current (Huang et al., 1994). On the other hand, activation of tyrosine kinase exerts suppressive effects on Kv1.2 current through two distinct mechanisms and by acting on different sites. Phosphorylation of the Y132 residue in the N-terminus enhances Kv1.2 endocytosis, reducing the number of channels on the cell surface and leading to decreased current amplitude (Huang et al., 1993; Nesti et al., 2004). Additionally, tyrosine kinase-mediated phosphorylation of the C-terminus disrupts the interaction between Kv1.2 channels and cortactin, an actin cytoskeleton-associated protein, further reducing current amplitude (Hattan et al., 2002). Moreover, phosphorylation of specific phosphoserine sites (pS434, pS440, and pS441) on the C-terminal region can enhance Kv1.2 cell surface expression (Yang et al., 2007). Remarkably, Kv1.2 channels were capable of imparting the same phosphorylation-dependent effects to Kv1.4 α-subunits when assembled as Kv1.4-1.2 dimeric channels, underscoring the diverse functional properties exhibited by heteromeric Kv1 channels (Yang et al., 2007).

Despite the initial findings, more recent structural studies have raised doubts about the plausibility of phosphorylation at sites T46 and Y132 (Chen et al., 2010; Long et al., 2005). Additionally,

attempts to replicate these findings by other researchers have been unsuccessful (Rezazadeh et al., 2007).

Glycosylation

All Kv1 channels, except for Kv1.6, possess a conserved N-linked glycosylation consensus site located in the extracellular S1-S2 linker (Shi and Trimmer, 1999). In the case of Kv1.2 channels, this site corresponds to asparagine 207 (N207). N-linked glycosylation exerts significant effects on Kv1.2 channel function. Increasing the number of glycosylation sites through mutations in Kv1.2 leads to a hyperpolarizing shift in $V_{1/2}$, faster activation kinetics, and slower C-type inactivation (Watanabe et al., 2007). Conversely, the Kv1.2[N207Q] mutant, lacking glycosylation sites, exhibited a depolarizing shift in $V_{1/2}$, slower activation, and faster C-type inactivation. Furthermore, N-linked glycosylation plays crucial roles in the forward trafficking of Kv1.2 channels to the cell surface and their degradation. The Kv1.2[N207Q] mutant displays reduced cell surface expression compared to WT Kv1.2 (Thayer et al., 2016; Watanabe et al., 2007). Moreover, internal Kv1.2[N207Q] channels underwent more rapid degradation compared to the wild-type counterpart (Thayer et al., 2016). Treatment of wild-type Kv1.2 channels with glycosidase, an enzyme that cleaves sialic acid residues from glycoproteins, resulted in accelerated degradation rates similar to those observed in the N207Q mutant. Together, these findings highlight the importance of glycosylation in the function, localization, and turnover of Kv1.2 channels.

Summary

This section sheds light on several extrinsic regulatory mechanisms that can influence Kv1.2 channel function and expression. However, there remain limitations in the field of Kv1 regulation. Firstly, the impact of these diverse regulatory mechanisms on Kv1 heteromeric channels is still not well understood. Secondly, the interactions between these mechanisms in combination are even less comprehended. My thesis aims to tackle both of these limitations.

1.5 SCOPE OF THESIS INVESTIGATION

This thesis is dedicated to investigating the regulation of Kv1.2 potassium channels by novel signaling pathways and accessory proteins. Chapter 3 describes the influence of redox and LMAN2 on Kv1 homomeric and heteromeric channels. Building on these findings, Chapter 4 further explores the impact of redox and LMAN2 on N-type inactivation induced by Kvβ and Kv1.4 α-subunits in Kv1.2-containing channels. The comprehensive findings presented in this research offer valuable insights into the diverse signaling pathways of Kv1.2 channels and the intricate interplay among these mechanisms, ultimately contributing to a better understanding of the distinctive current properties observed in vivo.

CHAPTER 2: MATERIALS AND METHODS

Molecular biology and ion channel constructs

In the current thesis, I utilized previously generated rat Kv1 channel constructs (Baronas et al., 2015). These constructs encompassed a range of Kv1 homomeric (Kv1.1-1.5) and heteromeric (Kv1.2-1.1, ΔN1.4-1.2, 1.4-1.2, and 1.5-1.2) channels to investigate use-dependent activation in channels with diverse subtype arrangements (Baronas et al., 2015). Building upon this foundation, I reused these Kv1 constructs to examine redox, LMAN2 sensitivity, and N-type inactivation in homomeric and heteromeric complexes.

All channels were generated by expressing their corresponding cDNAs in the pcDNA3.1 vector (Invitrogen). For the generation of heteromeric Kv1 channels, the leading subunit promoter was subcloned into the vector at the NheI and XhoI sites, while the trailing subunit promoter was subcloned in the EcoRI and HindIII sites. This strategy allowed the formation of tandem linked dimers, which could subsequently co-assemble to produce functional channels with a 1:1 subunit stoichiometry (refer to Figure 2.1 for visualization). In Chapter 3, the Kv1.1, Kv1.4, and Kv1.4-1.2 were chimeras with the N-terminus to the T1 domain being replaced by the Kv1.5 N-terminus. The S2-S3 linker region that is responsible for redox and LMAN2 sensitivity remained unaltered. This modification was necessary because WT Kv1.1 did not express well in mouse ltk-fibroblast (LM) cell lines and the inactivating properties of WT Kv1.4 and WT Kv1.4-1.2 interfered with the slow gating phenotype induced by reducing agents and LMAN2. In Chapter 4, the WT Kv1.4 and WT Kv1.4-1.2 channels were used. The 1.2[S2-S3L]1.5 mutant was previously generated by a PhD student, Victoria Baronas, by performing two-step overlapping PCR on WT Kv1.2. To create the Kv1.4-1.2[S2-S3L]1.5, standard PCR was performed on Kv1.2[S2-S3L]1.5 using 5' EcoRI forward and 3' HindIII reverse primers. The PCR product, which contains the Kv1.2 S2-S3 linker region, was then subcloned into WT Kv1.4-1.2 at the EcoRI and HindIII sites. To confirm the accuracy and identity of the constructs, Sanger sequencing (Genewiz) and Western blots were performed as previously described (Baronas et al. 2015).

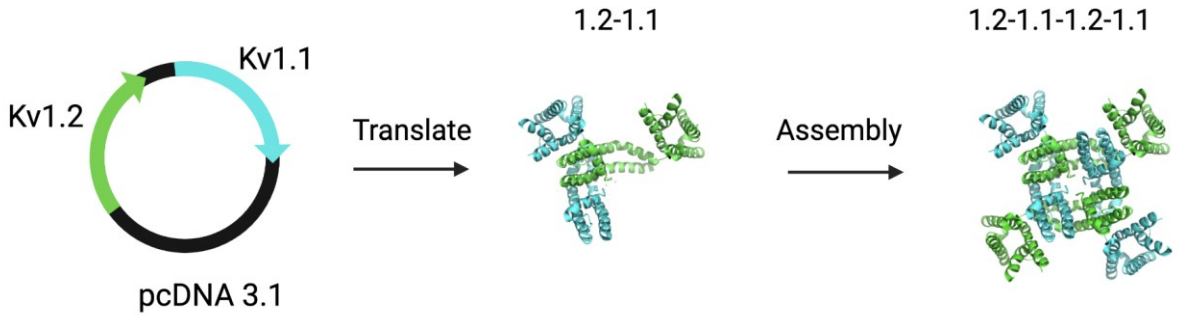


Figure 2.1. Molecular cloning and assembly of Kv heteromeric channels. A sample workflow of how Kv1.2-1.1 heteromeric channels were generated. The same approach was used to generate other Kv heteromeric channel variants. In accordance with the methods described, the leading protomer (i.e. Kv1.2) was subcloned into the pcDNA3.1 vector using the NheI and XhoI sites, while the trailing protomer (i.e. Kv1.1) was subcloned using the EcoRI and HindIII sites. Upon translation, the resulting assembly consists of one Kv1.2 α-subunit linked to one Kv1.1α construct. These dimers then coassemble with other dimers to form functional channels exhibiting a Kv1.2-1.1-1.2-1.1 subunit arrangement, consisting of two Kv1.2 α-subunits and two Kv1.1 α-subunits.

Cell culture

Mouse LM (ltk-) fibroblast cells (ATCC CCL-1.3, referred to as LM cells throughout the thesis) were cultured in a 5% CO_2 incubator set to 37˚C. Cells were maintained in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% FBS and 1% penicillin/streptomycin. We have chosen this specific cell model for two compelling reasons. First, LM cells are devoid of endogenous currents, a characteristic that enables us to specifically isolate Kv1.2 currents in our experimental setup. Additionally, existing evidence strongly suggests that LM cells prominently express a significant degree of slow gating, making them exceptionally suited for a comprehensive exploration of this mechanism. (Baronas et al., 2015; Rezazadeh et al., 2007). LM cells were seeded in 12 or 24 well plates and allowed to grow for 24-48 hours prior to transfection. Transfection was then carried out using jetPRIME transfection reagent (Polyplus). In Chapter 3, cells were transfected with 200 ng of various voltage-gated potassium channel plasmids and either 400 ng of green fluorescent protein plasmid (GFP) or LMAN2 plasmid N-terminally tagged with GFP. In Chapter 4, for experiments involving Kvβ, we transfected cells

with 100 ng of channel plasmid, 1200 ng of Kvβ plasmid, and 400 ng GFP or EGFP-LMAN2 plasmid. For experiments involving Kv1.4-1.2 heterodimers, we transfected cells with 100 ng of channel plasmid, 100 ng of TMEM33 plasmid, and 400 ng fluorescent protein plasmid. TMEM33 disrupts Kv1 channel localization to the cell surface and was used to control current amplitudes (Unpublished data). Following a 6 hour transfection period, cells were plated on 22x22-1.5 coverslips (Fisher Scientific 12541B) in 6 well plates. Electrophysiology recordings were performed the subsequent day, roughly 24 hours post-transfection.

Whole-cell patch clamp recordings

Patch pipettes were prepared from soda lime capillary glass (Fisher) using a Sutter P-97 puller (Sutter Instrument). Pipettes had a tip resistance of 1-3 MΩ when filled with standard internal recording solution containing: 135 mM KCl, 5 mM K-EGTA, 10 mM HEPES and was adjusted to pH 7.2 using KOH. External (bath) solution contained: 135 mM NaCl, 5 mM KCl, 1 mM CaCl2, 1 mM MgCl2, 10 mM HEPES, and was adjusted to pH 7.4 with NaOH. Recordings were filtered at 5 kHz, sampled at 10kHz, with manual capacitance compensation and 80% series resistance compensation, and stored directly on a computer hard drive using Clampex 10 software (Molecular Devices). Dithiothreitol (DTT) was purchased from Fisher Scientific (BP172-25). It was prepared as a 1 M stock solution in ddH2O, stored at -20˚C, and diluted to 200 μM using the external bath solution before being used in experiments. Patch clamp experiments were done at room temperature (22 ± 1°C).

Hippocampal neuron extraction and electrophysiology setup

Embryonic day 18 male and female Sprague-Dawley rats were used to extract hippocampi, following a previously established protocol (Xie et al., 2000). Neurons were cultured for 7-16 days in vitro before electrophysiology experiments were conducted. To isolate for tityustoxin-sensitive currents (also known as Kv1.2-containing currents in this study) neurons were first exposed to 100-300 nM tityustoxin and then, the toxin-insensitive current was subtracted from the total current. The recording of

hippocampal neurons and subsequent data analysis were conducted by Victoria Baronas following established protocols. Harley Kurata then created the accompanying figure (Figure 4.1).

Pipette tips made from soda lime glass were prepared with a resistance of 1.5-2.5 MΩ and filled with the internal recording solution mentioned above. The external recording solution consisted of 135 mM N-methyl-D-glucamine, 5 mM KCl, 1 mM CaCl2, 1 mM MgCl2, 10 mM HEPES, and the pH was adjusted to 7.4 using NaOH. A filtering frequency of 5 kHz was applied, and the sampling rate was set at 10 kHz. Manual capacitance compensation and 80% series resistance compensation were performed. The recorded data was directly stored on a computer hard drive using Clampex 10 software by Molecular Devices.

Electrophysiology Analysis

Conductance-voltage (G-V) relationships were fitted with a Boltzmann equation.

$$G = \frac{1}{1 + e^{-(V - V_{1/2})/k}}$$

G is the normalized conductance, V is the voltage applied, $V_{1/2}$ is the half-maximal activation voltage, and k is the respective slope factor. G–V relationships were fitted for each individual cell using a least squares minimization approach in Microsoft Excel (Solver tool). The time constant of activation and deactivation was calculated by fitting current traces with the following single exponential equation:

$$A(t) = A(1 - e^{-t/\tau}) + C$$

A(t) is the current at time (t), A is the maximum current, and C is the constant.

To calculate the percent inactivation, the steady-state current (typically measured ~250 ms after initial depolarization) was subtracted from the peak current, and the result was divided by the peak

current for the given test pulse. Box plots were used to visually represent the data. These plots display the median, 25th, and 75th percentiles (which define the edges of the box), as well as the 10th and 90th percentiles (shown as whiskers). The statistical tests performed to determine significance are described throughout the text and in figure legends when applicable. Tests were typically performed to compare fitted gating parameters such as $V_{1/2}$.

CHAPTER 3: KV1.2 CONFERS REDOX AND LECTIN SENSITIVITY TO HETEROMERIC VOLTAGE GATED POTASSIUM CHANNELS

3.1 INTRODUCTION

Kv1.2 is a prominent voltage gated potassium channel in the central nervous system where it regulates action potential thresholds and neuronal firing (Bean, 2007; Rama et al., 2017). Among the Kv1 family, Kv1.2 appears to have an especially important function in the central nervous system, as mutations in Kv1.2 cause uniquely severe neurological phenotypes including intractable epilepsy and developmental delay. Moreover, the phenotype of Kv1.2 knockout mice is especially severe, including 100% mortality within three weeks of birth due to generalized seizures (Coetzee et al., 1999; Masnada et al., 2017; Robbins and Tempel, 2012; Syrbe et al., 2015; Wang et al., 1994).

Kv1.2 channels are unique in that they display variable kinetics of activation and voltage-dependence that are absent in other Kv1 members (Baronas et al., 2015; Grissmer et al., 1994; Lamothe and Kurata, 2020; Rezazadeh et al., 2007). Depending on the type of expression system, the extent of this variability can change. For instance, while this functional phenotype has been reported in HEK, CHO, and LM cells, it is completely absent in oocytes (Rezazadeh et al., 2007). As such, it is believed that Kv1.2 channels are regulated by a variety of known and unknown extrinsic factors. Phosphorylation of Kv1.2 promotes both cell surface expression and internalization, and affects its interaction with cortactin, an actin binding protein (Hattan et al., 2002; Nesti et al., 2004; Yang et al., 2007). Phosphatidylinositol 4,5-bisphosphate (PIP2) depletion has been shown to induce a hyperpolarizing shift in the half-activation voltage ($V_{1/2}$) and reduce total current amplitude (Rodriguez-Menchaca et al., 2012). Slc7a5, a neutral amino acid transporter, influences numerous Kv1.2 and Kv1.1 properties including surface expression, and voltage-dependence of activation $V_{1/2}$ (Baronas et al., 2018). Apart from these regulators that may have a specific influence on Kv1.2, the most studied accessory proteins with general effects on Kv1 channels are the Kvβ subunits. The Kvβ subunits associate with Kv1 α-subunits in a 1:1 stoichiometry with functional channels containing up to four Kvβ subunits (Xu et al., 1998). Kvβ subunits can influence

Kv1 cell surface expression, channel assembly, and certain subtypes confer rapid N-type inactivation via a NH2-terminal “ball and chain” mechanism (Pongs and Schwarz, 2010; Rettig et al., 1994).

We have reported an additional mode of Kv1.2 regulation by extracellular redox that does not involve intrinsic redox sensitivity of the channel (Baronas et al., 2015). Thus, it is likely that an extrinsic regulatory molecule influences channel activity in response to altered extracellular redox conditions. With this mechanism of modulation, exposure to modest extracellular reducing conditions promotes a slow ‘resistant’ gating mode of Kv1.2 characterized by a +60 mV depolarizing shift of the voltage-dependence of activation, and prominent use-dependent activation (Baronas et al., 2015). In this system, use-dependent activation arises because activation of channels temporarily relieves the redox-mediated inhibition, allowing channels to progressively accumulate in a rapidly-activating mode in response to trains of repetitive depolarizations. More recently, we also reported that LMAN2 strongly influences redox modulation of Kv1.2, suggesting that LMAN2 is a contributor to the molecular mechanism underlying this unique gating behavior. Co-expression of Kv1.2 with LMAN2 mimics many of the slow gating features observed in reducing conditions, including a depolarizing shift of voltage-dependent activation and use-dependent activation, and enhanced sensitivity to reducing agents (Lamothe and Kurata, 2023). Kv1.2 can also assemble to form a wide variety of heterotetrameric channels in combination with other Kv1 α-subunits (Plane et al., 2005; Po et al., 1993; Ruppersberg et al., 1990; Shamotienko et al., 1997; Sheng et al., 1993), and this heteromerization can recruit distinct gating properties and sensitivities to regulatory pathways from different subunits (Coleman et al., 1999; Ranjan et al., 2019). For instance, Kv1.4 subunits transfer their rapid N-type inactivation properties when expressed as tandem constructs with Kv1.5 non-inactivating subunits (Lee et al., 1996). Alternatively, Kv1.5 can confer Src induced current suppression when coexpressed with Kv1.4 (Nitabach et al., 2001).

In this study, we have explored the subunit specificity of LMAN2 and redox sensitivity, and investigated whether Kv1.2 can generate redox and LMAN2 sensitivity in heteromeric channels. We found that sensitivity to DTT and LMAN2 has overlapping subunit specificity, as only Kv1.2 subunits exhibit sensitivity among Kv1 subtypes. When coexpressed in heteromeric channels with various redox

and LMAN2 insensitive subunits (Kv1.1, 1.4, and 1.5), the presence of Kv1.2 subunits endows heteromeric channels with prominent redox and LMAN2 modulation of gating. Furthermore, we demonstrated that these changes in gating were due to decelerated activation kinetics, but not deactivation. Overall, our findings reinforce that LMAN2 is a likely contributor to redox sensitive gating in Kv1.2, and that Kv1.2 subunits can recruit this modulatory mechanism into heteromeric Kv1 channels.

3.2 RESULTS

Kv1 subtype specificity of LMAN2 and redox sensitivity

Previous reports described redox-dependent shifts of Kv1.2 to a 'slow' gating mode that exhibits a prominent shift of the voltage-dependence of activation to depolarized potentials (Baronas et al., 2017). This slow gating behavior is strongly favored by extracellular reducing conditions, and potentiating prepulses can temporarily relieve this inhibitory gating effect and allow channels to open more rapidly. Recent work in our group has identified LMAN2 as a candidate regulator of Kv1.2 as co-expression with LMAN2 biases channels towards this slow gating behavior (Lamothe and Kurata, 2023). To confirm the subunit-dependence of redox sensitivity, and compare this with the LMAN2 subunit sensitivity, we measured the effects of DTT and LMAN2 on the voltage-dependence of activation of most members of the Kv1 subfamily (Figure 3.1A-E). Reducing agents (200 μM DTT) or co-transfection with LMAN2 does not significantly alter the activation $V_{1/2}$ for Kv1.1, 1.3, 1.4, and 1.5 (Figure 3.1F, H-J). This insensitivity is also apparent in exemplar traces in Figure 3.1A and Figure 3.1 C-E where the highlighted +40 mV pulse is unchanged in all three conditions. In contrast, DTT and LMAN2 induced a prominent +60 mV depolarizing shift of activation for Kv1.2 (Figure 3.1G; Table 3.1). This shift is also exemplified by the slow activation kinetics and minimal channel activation observed at +40 mV (green/blue sweeps) in DTT and LMAN2 conditions relative to control (Figure 3.1B). Together, these data demonstrate that Kv1.2 subunits are biased to a slow gating mode by reducing conditions and LMAN2. Also, redox sensitivity and LMAN2 sensitivity have the same subtype dependence among the Kv1 channels.

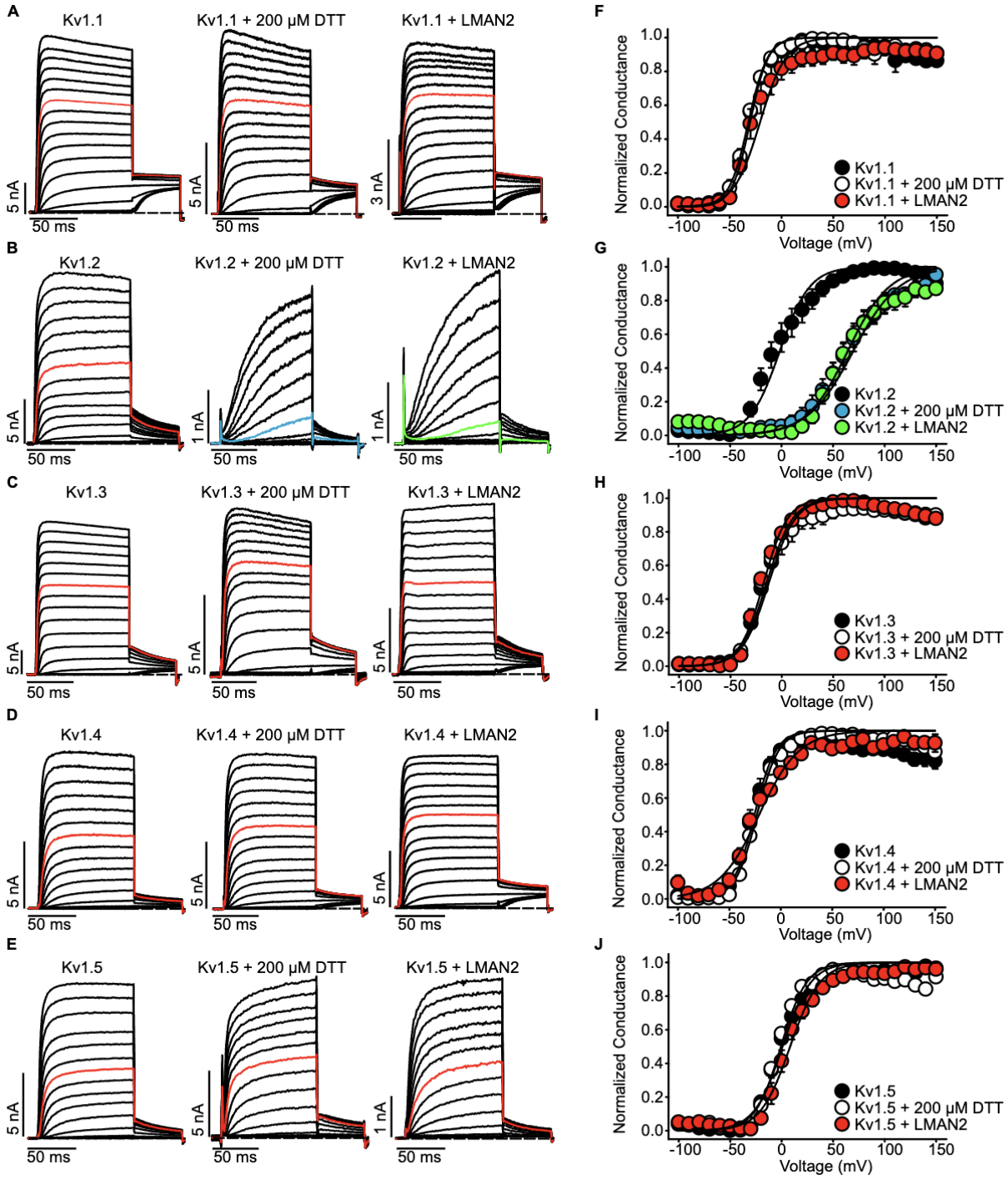


Figure 3.1. Redox and LMAN2 induce depolarizing shifts of activation exclusively to Kv1.2 among Kv1 homomeric channels. (A-E) Representative current traces of LM fibroblast cells transfected with various Kv1 homomeric channels (Kv1.1-1.5) and LMAN2 recorded in ambient redox or 200 µM DTT conditions. Cells were held at -100 mV and then stepped to voltages between -100 to +150 mV for 100 ms (10 mV steps) followed by a -30 mV depolarization for tail currents. The highlighted trace represents

a +40 mV pulse. (F-J) Conductance-voltage plots were generated by normalizing tail currents to peak tail current and fitting with a Boltzmann function.

Redox and LMAN2 sensitivity of Kv1 heteromeric channels

Previous work has highlighted that features of slow gating could be conferred to heteromeric Kv1 channels containing one or more Kv1.2 subunits (Baronas et al., 2015). We tested whether similar subunit dependence would extend to LMAN2 sensitivity in heteromeric channels. We used patch clamp recording to test the DTT and LMAN2 sensitivity of linked dimeric constructs (Kv1.2-1.1, Kv1.4-1.2, Kv1.5-1.2), leading to channels with forced 1:1 stoichiometry of Kv1.2 and a different Kv1 subtype. In all cases, these heteromeric channels exhibited prominent depolarizing shifts of the voltage-dependence of activation with either DTT or LMAN2 (Figure 3.2). The Kv1.3-1.2 dimers we constructed are problematic in terms of reproducibility of currents and expression, and so they were not examined further.

Kv1.4-1.2 and Kv1.5-1.2 heteromeric channel constructs both exhibited prominent depolarizing shifts of activation in either DTT or LMAN2 conditions, in the range of ~75-85 mV (Figure 3.2 and Table 3.1). The large magnitude of this effect is likely due to both the large Kv1.2-mediated sensitivity to the gating modulators tested, but also because both channels have fairly hyperpolarized $V_{1/2}$s of activation under control conditions (ambient redox, no co-transfection with LMAN2). Responses in Kv1.2-1.1 channels differed slightly in the sense that properties of Kv1.2 were quite apparent even in ambient redox conditions, leading to a fairly depolarized $V_{1/2}$ of activation (19.3 ± 8.3 mV) which is further shifted by LMAN2 or DTT (Figure 3.2 and Table 3.1). The varying $V_{1/2}$ in ambient conditions and degree of shifts induced by DTT and LMAN2 could indicate that different Kv1 subtypes influence the magnitude of the impact of Kv1.2 on LMAN2 and/or redox sensitivity.

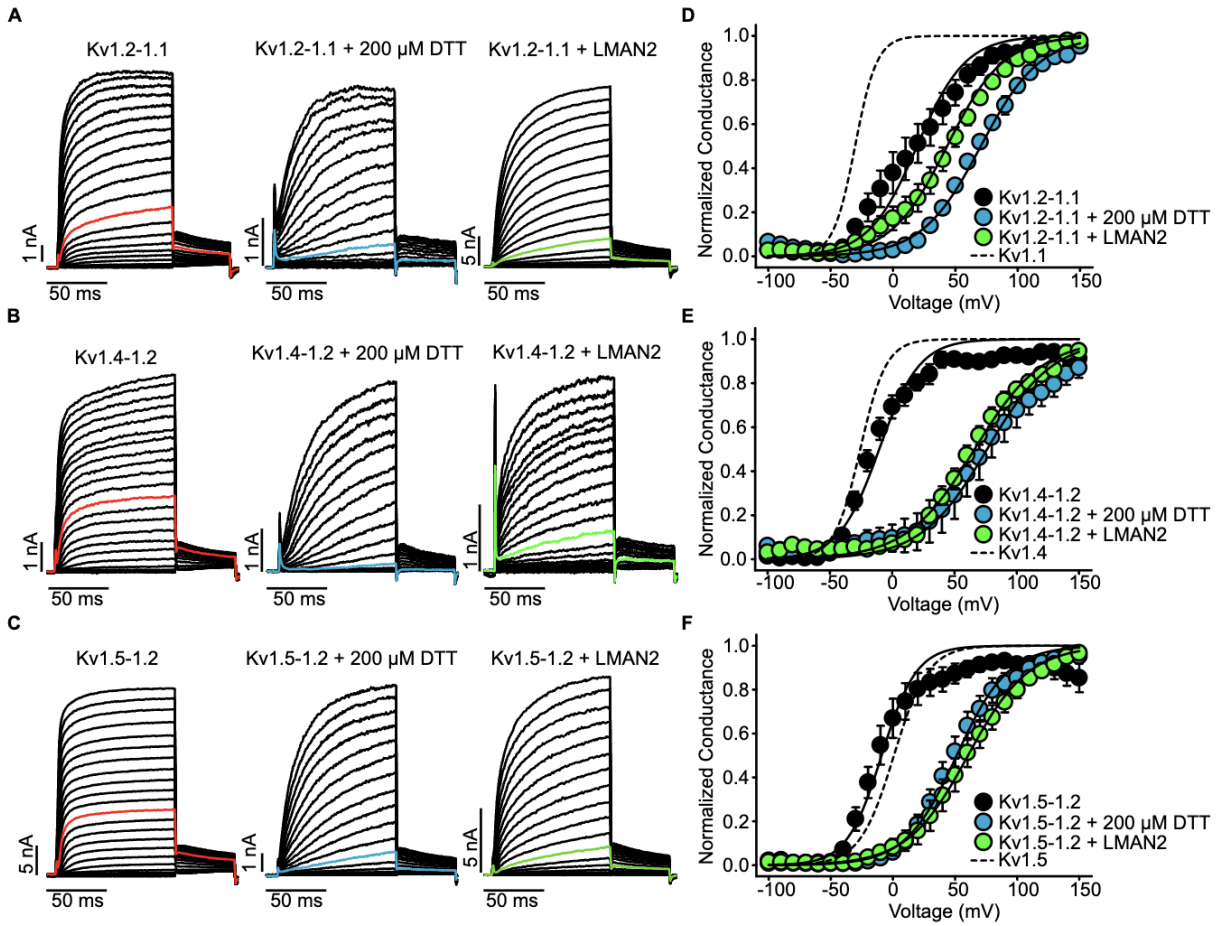


Figure 3.2. Kv1.2 transfers redox and LMAN2 induced sensitivity to Kv1 heteromeric channels. (A-C) Sample current traces of LM fibroblast cells transfected with various Kv1 heterodimeric channels +/- LMAN2 in ambient and redox conditions. Highlighted sweeps represent a +40 mV pulse. (D-F) GV relationships were obtained from tail currents as described in Figure 3.1. Dashed lines represent homomeric Kv1 channel data from Figure 3.1, included for comparison.

	$V_{1/2}$ [mean ± SEM]	k [mean ± SEM]	n
Kv1.1	-30.4 ± 1.6	2.9 ± 0.30	6
Kv1.1 + 200 μM DTT	-32.0 ± 1.9	2.8 ± 0.19	9
Kv1.1 + LMAN2	-20.8 ± 6.7	2.0 ± 0.53	5
Kv1.2	-1.7 ± 6.8	1.8 ± 0.22	7
Kv1.2 + 200 μM DTT	64.2 ± 7.8*	1.3 ± 0.22	4
Kv1.2 + LMAN2	69.5 ± 8.1*	1.2 ± 0.20	5
Kv1.3	-14.5 ± 2.2	2.0 ± 0.28	6
Kv1.3 + 200 μM DTT	-12.5 ± 5.8	2.0 ± 0.46	5
Kv1.3 + LMAN2	-17.5 ± 2.0	2.2 ± 0.15	8
Kv1.4	-25.6 ± 4.2	2.5 ± 0.28	7
Kv1.4 + 200 μM DTT	-25.6 ± 1.7	2.3 ± 0.081	8
Kv1.4 + LMAN2	-25.3 ± 2.7	1.6 ± 0.16	9
Kv1.5	0.79 ± 2.8	1.9 ± 0.19	5
Kv1.5 + 200 μM DTT	-0.69 ± 2.8	2.1 ± 0.23	6
Kv1.5 + LMAN2	8.2 ± 3.9	1.8 ± 0.19	6
Kv1.2-1.1	19.3 ± 8.3	1.3 ± 0.19	8
Kv1.2-1.1 + 200 μM DTT	70.4 ± 3.3*	1.1 ± 0.039	6
Kv1.2-1.1 + LMAN2	43.4 ± 6.0$^{\#}$	1.1 ± 0.089	11
Kv1.4-1.2	-10.4 ± 4.1	1.6 ± 0.22	6
Kv1.4-1.2 + 200 μM DTT	76.2 ± 15.3$^{\delta}$	0.97 ± 0.076	6
Kv1.4-1.2 + LMAN2	66.2 ± 5.5$^{\delta}$	0.93 ± 0.023	6
Kv1.5-1.2	-5.3 ± 7.0	1.9 ± 0.39	6
Kv1.5-1.2 + 200 μM DTT	51.3 ± 6.8*	1.2 ± 0.091	6
Kv1.5-1.2 + LMAN2	60.4 ± 8.6*	1.05 ± 0.031	5

Table 3.1. Summary data of $V_{1/2}$ [mean ± SEM], k [mean ± SEM], and number of cells recorded for various Kv1 channels in control, DTT, and LMAN2 conditions. A one-way ANOVA followed by Bonferroni post hoc test was performed on the same ion channel in different conditions (e.g. Kv1.1,

Kv1.1 + 200 μM DTT, and Kv1.1 + LMAN2). Significant differences relative to the control group are indicated with * $p<0.001$ and # $p<0.05$. A non-parametric Kruskal-Wallis ANOVA on Ranks test followed by Dunn's method was performed on the Kv1.4-1.2 channel as the data was not normally distributed. Significant differences relative to the control group are indicated with δ $p<0.05$.

Kv1.2 transfers use-dependent activation properties to heteromeric channels

The redox-dependent slow gating mode of Kv1.2 results in use-dependent activation (UDA), which arises because channels temporarily shift to a potentiated or facilitated gating mode as they are activated. Thus, if channels are pulsed rapidly enough to prevent significant recovery of the slow gating mode, repetitive trains of depolarizations will cause a gradual increase in current amplitude as channels that have been activated accumulate in the potentiated mode (Baronas et al., 2015). As it appears that LMAN2 is causing a similar gating mode shift as extracellular reducing conditions, we compared the effects of LMAN2 and DTT on use-dependent activation properties. Previous studies have shown that LMAN2 significantly enhances the degree of use-dependent activation in cells expressing Kv1.2 channels, similar to the effects of extracellular reducing conditions (Baronas et al., 2017; Lamothe and Kurata, 2023). Here, we demonstrate that cells transfected with Kv1.1, 1.4, and Kv1.5 did not exhibit use-dependence and were unaffected by reducing agents and LMAN2 (Figure 3.3). However, consistent with the effects of DTT and LMAN2 in Kv1.2 homomers (Baronas et al., 2017; Lamothe and Kurata, 2023), Kv1.2-1.1, Kv1.4-1.2, and Kv1.5-1.2 all exhibited moderate and variable UDA under control conditions (Figure 3.3), which was markedly enhanced by DTT and LMAN2. This further confirms that Kv1.2 can transfer its slow gating, redox sensitivity, and LMAN2 sensitivity when expressed in heteromeric channels.

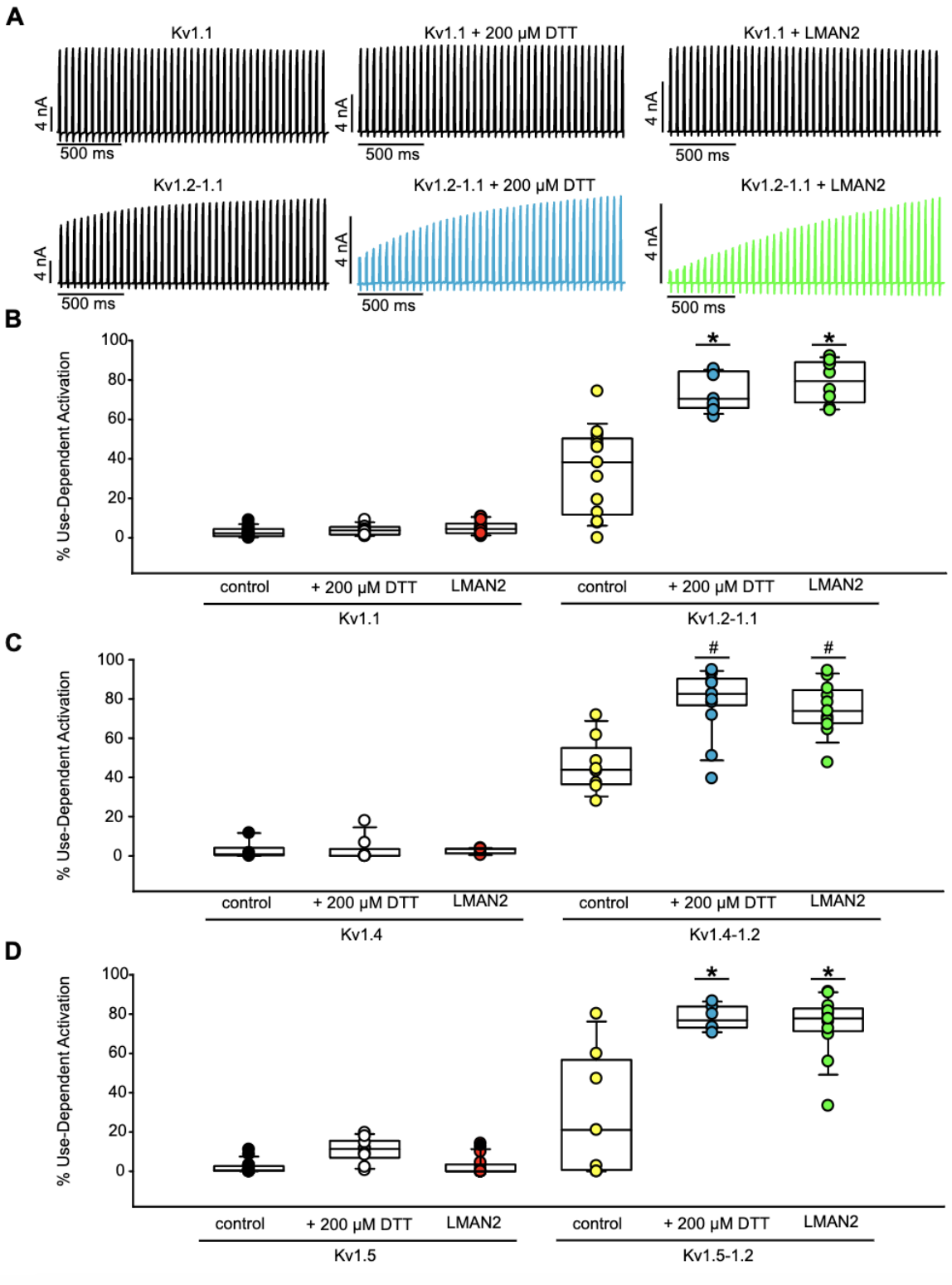


Figure 3.3. Redox and LMAN2 enhance use-dependent activation in Kv1 heterodimeric channels. (A) Representative traces of LM fibroblast cells expressing Kv1.1 and Kv1.2-1.1 ± 200 µM DTT/LMAN2 being subjected to 20 Hz trains of +60 mV 10 ms depolarizations from a holding potential

of -80 mV. (B-D) Box plots illustrate % use-dependent activation (UDA). UDA was calculated by subtracting the first pulse from the peak pulse and then dividing by the peak pulse (n = 5-20). Kruskal-Wallis ANOVA and Dunn's post hoc test were performed on the same ion channel in different conditions. Significance relative to control group (i.e. channel in ambient conditions) was indicated with * <0.05. One-way ANOVA and Bonferroni tests were performed on Kv1.4-1.2 channels as normality and equal variance were assumed. Significance was indicated with # <0.05.

LMAN2 mirrors DTT effects on activation and deactivation of Kv1 channels

To further confirm the parallels between LMAN2 and DTT in terms of functional outcomes and subtype specificity, we compared the effects of DTT and LMAN2 on activation and deactivation rates of Kv1 homomeric and Kv1.2-containing heteromeric channels. Time constants of activation were measured with single exponential fits of activation kinetics between +100 and 180 mV (10 mV steps, 100 ms pulses), from a holding potential of -80 mV. Consistent with the previously described effects of DTT and LMAN2 on voltage-dependent activation, only Kv1.2 exhibited a slower activation time constant in DTT and LMAN2 (Figure 3.4) (Baronas et al., 2017; Lamothe and Kurata, 2023). Also, all Kv1.2-containing heteromeric channels exhibited DTT or LMAN2-sensitive activation kinetics (Figure 3.5A-C). Notably, Kv1.2-1.1 had the slowest activation time constant in control conditions, which was further shifted by DTT and LMAN2 (Figure 3.5A). Kv1.4-1.2 and Kv1.5-1.2 both exhibited relatively fast activation kinetics in ambient conditions, but markedly slower kinetics in the treatment groups (Figure 3.5B-C and E-F).

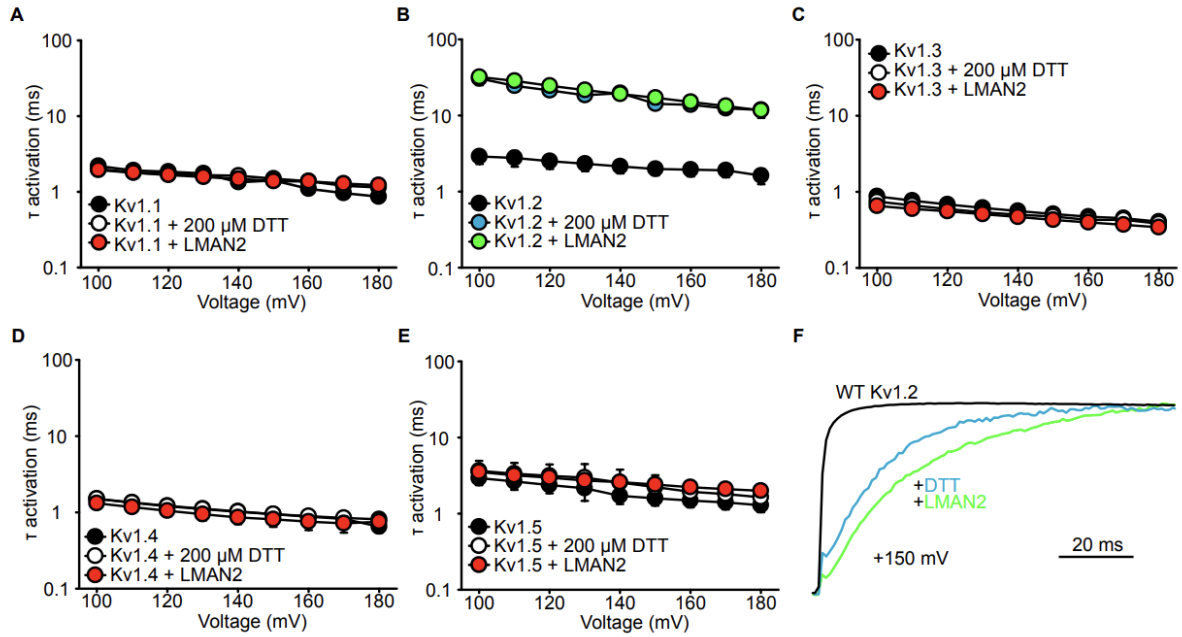


Figure 3.4. Redox and LMAN2 decelerate activation kinetics of Kv1.2 homomeric channels. (A-E) Time constants of activation (τ activation) were fitted with single exponential curves as described in the methods and plotted against voltage (mV) (mean ± SEM). LM fibroblast cells were initially held at -100 mV and then underwent step depolarizations ranging from +100 to 180 mV for 100 ms in 10 mV increments. A Kruskal-Wallis ANOVA and Dunn's post hoc test were performed on τ activation at +150 mV. Only Kv1.2 + 200 μM DTT and Kv1.2 + LMAN2 groups showed significant differences relative to control ($p<0.05$). n = 3-7 individual cells recorded for each group. (F) Sample current trace showing activation of WT Kv1.2 ± DTT/LMAN2 being depolarized to +150 mV.

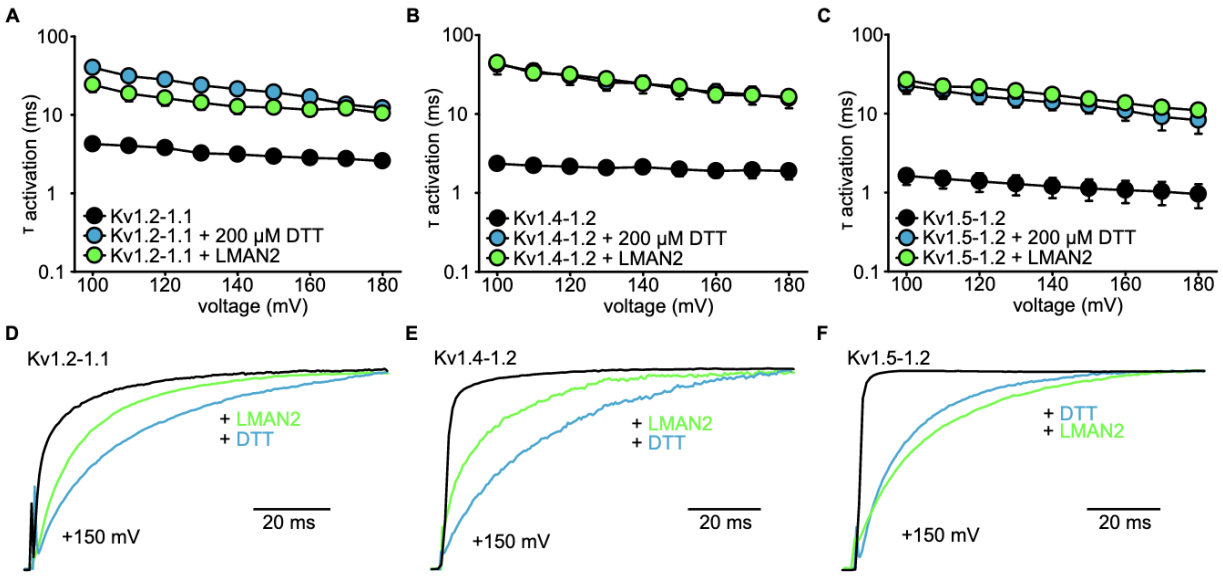


Figure 3.5. Redox and LMAN2 decelerate activation kinetics of heteromeric channels containing Kv1.2 α-subunits. (A-C) Time constants of activation (τ activation) for Kv1.2-1.1, Kv1.4-1.2, and Kv1.5-1.2 in control, DTT, and LMAN2 conditions were obtained using the protocol described in Figure 3.4. Recordings were obtained using LM fibroblast cells. A one-way ANOVA and Bonferroni t-test were performed on τ activation at +150 mV. Significant differences relative to control groups were found for all +DTT and +LMAN2 groups ($p<0.05$). n = 3-7 individual cells recorded for each group. (D-F) Representative current traces of cells expressing Kv1.2-1.1, Kv1.4-1.2, and Kv1.5-1.2 channels ± DTT/LMAN2 being depolarized to +150 mV for 100 ms.

Deactivation kinetics were measured over a range of negative voltages (-80 mV to -150 mV, 10 mV steps) after a depolarization to +60 mV (Figure 3.6). As shown by the exemplar traces, there were no consistent differences in deactivation between control, 200 μM DTT, and LMAN2 conditions in Kv1.1, 1.2, and 1.2-1.1 (Figure 3.6). Redox and LMAN2 also had no effect on deactivation for all other Kv1 homomeric or heteromeric channels (Supplementary Figures 1-2). These findings indicate that DTT and LMAN2 exert comparable effects on channel activation, while leaving deactivation unaltered. Importantly, their shared specificity for Kv1.2 α-subunits strongly suggests that they operate through a common or overlapping mechanism that targets the gating properties of Kv1.2 channels. Although the precise nature of this mechanism remains elusive and requires further investigation, we present a comprehensive working model in our discussion section that integrates all existing findings.

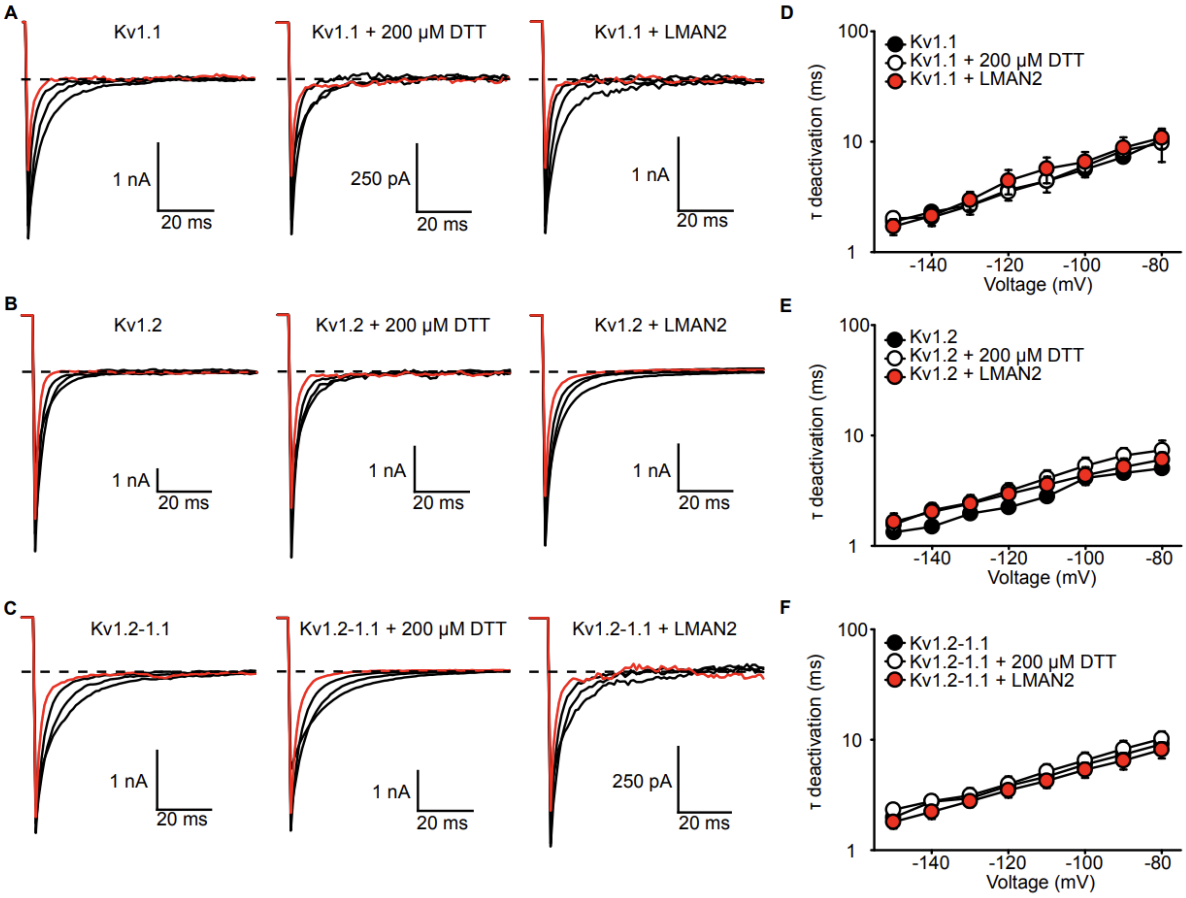


Figure 3.6. Redox and LMAN2 induce no changes in deactivation kinetics of Kv1 homomers and heteromers. (A-C) Sample traces of LM fibroblast cells expressing Kv1.1, Kv1.2, and Kv1.2-1.1 channels in control, 200 µM DTT, and LMAN2 conditions being depolarized to +60 mV for 600 ms from a -100 mV holding potential and then being repolarized to -80 to -150 mV (10 mV steps) for 500 ms. To ensure clarity, only sweeps at -90, -110, -130, and -150 mV repolarization are shown. The highlighted red traces depict the -150 mV depolarization. (D-F) Time constants of deactivation (τ deactivation) was measured by fitting deactivation curves with a single exponential function as mentioned in the methods and measured against voltage (mV). Either a one-way ANOVA or Kruskal-Wallis one-way ANOVA was run at -120 mV, depending on if normality and equal variance assumptions were fulfilled, to compare between groups. In all conditions, there were no statistically significant differences. n = 4-8 individual cells.

3.3 DISCUSSION

Relative to other Kv1 subtypes, Kv1.2 channels appear especially prone to modulation, and this likely underlies the wide variation in the reported kinetics and voltage-dependence of activation (Baronas et al., 2015; Grissmer et al., 1994; Rezazadeh et al., 2007). However, apart from the well-characterized

Kvβ subunits, extrinsic regulatory proteins that account for the pronounced plasticity of Kv1.2 gating properties are not well understood. Further identification and characterization of mechanisms of modulation of Kv1.2 may be of general importance because of their propensity for intra-family heteromerization, and thus the potential to recruit regulatory mechanisms by individual subunits in heteromeric channels in vivo (Lee et al., 1996; Nitabach et al., 2001; Po et al., 1993; Sheng et al., 1993).

We have reported that Kv1.2 channels expressed in mammalian cell lines are highly responsive to changes in extracellular redox potential. Reducing agents shift channels to a slow gating mode with a pronounced depolarized shift of activation $V_{1/2}$ (to ~+60 mV) and use-dependent activation (Baronas et al., 2015). There is strong evidence that this sensitivity is not governed by the channel itself: a) redox sensitivity persists when transmembrane or extracellular cysteines are mutated, b) redox sensitivity is variable between different expression systems (ie. oocytes vs. cell lines), and c) homologous redox-sensitive cysteines in the TM or extracellular regions are present in all Kv1 channels, but only Kv1.2 exhibits pronounced redox sensitivity. We have worked towards identifying potential extrinsic regulators of redox sensitivity, and recently found that co-expression with LMAN2 strongly sensitizes Kv1.2 to redox changes and biases channels towards a slow gating mode (Lamothe and Kurata, 2023). In this current study, we have extended these findings and bolstered evidence for a relationship between redox sensitivity and LMAN2 modulation of Kv1.2. We systematically characterized the redox sensitivity of the most prominently studied Kv1 subunits (Kv1.1-Kv1.5), and demonstrated overlapping subtype specificity for DTT and LMAN2 effects, suggesting they operate by the same mechanism. We also demonstrated that Kv1.2 confers redox and LMAN2 sensitivity to heteromeric potassium channels. Heteromeric Kv1.2-1.1, 1.4-1.2, and 1.5-1.2 channels all exhibited $V_{1/2}$ shifts to depolarized voltages, decelerated activation, and increased UDA in response to either DTT or LMAN2 (Figure 3.2-3.5). For both LMAN2 co-expression and DTT application, the underlying mechanism for gating changes exclusively affected the rate of activation, but not deactivation (Figure 3.4-3.6). Overall, all channels (Kv1.2 homomers or heteromers) with LMAN2 sensitivity also exhibited redox sensitivity. No channel demonstrated one sensitivity without the other. This suggests that the mechanism behind both sensitivities are related. In

this regard, previous work identified a highly localized site in the intracellular S2-S3 linker that strongly influences variability of Kv1.2 gating - this site (Kv1.2 Thr252, which is a positively charged Lys or Arg side chain in nearly all other Kv channels), is also a powerful determinant of redox and LMAN2 sensitivity (Baronas et al., 2016, 2015; Lamothe and Kurata, 2023; Rezazadeh et al., 2007). The convergence of these findings indicates that the unusual variability of Kv1.2 gating is caused by the same regulatory mechanisms that underlie redox and LMAN2 sensitivity.

Our hypothesis is that an extrinsic regulatory molecule interacts and influences Kv1.2 in a redox-sensitive manner, and that cell-to-cell differences in expression of this regulator (along with perhaps localized variations in redox potential) can lead to the remarkable variability of Kv1.2 gating that has been reported. In this general model, the association/effect of this regulator with Kv1.2 would be more prominent in reducing conditions, and cause stabilization of resting states of the channel. Our recent findings characterizing the effects of LMAN2 on Kv1.2 (Lamothe and Kurata, 2023), and evidence here presenting the overlapping subunit dependence of LMAN2 and redox sensitivity, indicate that LMAN2 is a contributor to this mechanism of redox modulation of Kv1.2-containing channels. However, a clear gap in our findings thus far is that we have not been able to demonstrate a direct physical interaction between LMAN2 and Kv1.2. Some possible mechanisms that may explain the functional outcomes of LMAN2 co-expression are that it could be directly involved in sensing extracellular redox changes but its interaction with the channel is difficult to detect or possibly mediated by an additional intermediary. Alternatively, LMAN2 may influence the expression or assembly of a different redox-sensitive protein that influences Kv1.2. Based on our findings thus far, we view LMAN2 as a potential candidate for extracellular sensitivity as it contains numerous extracellular disulfide bonds, however we have recognized that co-expression with LMAN2 is not sufficient to reconstitute redox-sensitivity of Kv1.2 in the oocyte expression system (data not shown). Thus, it seems fair to say that LMAN2 can strongly influence redox-dependent gating of Kv1.2 but is likely only one part of a more complex set of regulatory proteins underlying the variable redox-sensitive gating of Kv1.2.

The potential involvement of LMAN2 in the regulation of ion channel gating is unusual and a departure from the functions typically studied for this protein. LMAN2 is a transmembrane lectin that binds to glycoproteins and cycles through the Golgi apparatus and ER, while its localization at the plasma membrane has been debated in different publications and may vary depending on cell type and expression level (Kamiya et al., 2008, 2005). The function of LMAN2 continues to be investigated but it has been suggested to have roles in both anterograde and retrograde vesicle/cargo trafficking (Füllekrug et al., 1999; Kwon et al., 2016; Reiterer et al., 2010). Our previous characterization of LMAN2 demonstrated that mutations introducing strong ER retention signals prevent the reported modulatory effects on Kv1.2 (Lamothe and Kurata, 2023). Additionally, the location of EGFP fusion to LMAN2 strongly influences its ability to modulate Kv1.2. N-terminal EGFP tagged LMAN2 can reach the plasma membrane and influence Kv1.2 gating, whereas C-terminal EGFP tagged LMAN2 is retained intracellularly and has no effect on Kv1.2. Evidence linking LMAN2 to in vivo ion channel function or neurological diseases is largely unexplored. A recent paper reported downregulation of LMAN2 in multiple systems atrophy, although the underlying mechanism was undetermined (Bettencourt et al., 2020). Interestingly, LMAN2L (a member of the LMAN family and close homolog of LMAN2) mutations have resulted in intellectual disabilities, schizophrenia, bipolar disorder, and epilepsy, but potential links to Kv1.2 or perhaps other cargo remain undetermined (Alkhater et al., 2019; Lim et al., 2014; Rafiullah et al., 2016).

We previously reported features of redox-sensitive gating of Tityustoxin-isolated (likely Kv1.2-containing) channel currents in primary hippocampal and dorsal root ganglion neurons (Baronas et al., 2017; Lamothe and Kurata, 2023). Much like our findings in cell lines, these currents exhibit highly variable rates and voltage-dependence of activation and inactivation. This likely involves the assembly of Kv1.2 with different Kv1 α-subunits and potentially β-subunits that confer inactivation. Keeping in mind this wide potential for variation in vivo, we were interested by our observation that different Kv1.2 heteromeric channel assemblies can vary significantly in terms of their gating properties in ambient redox or the saturating effects of LMAN2 and DTT. For example, Kv1.2-1.1 heteromers exhibited differences in the maximal gating shift generated by LMAN2 vs DTT, which is consistent with our suggestion that

LMAN2 is not the sole protein responsible for redox-sensitivity (Figure 3.2, Table 3.1). Perhaps more importantly however, Kv1.2-1.1 was more sensitive to Kv1.2 mediated effects under ambient redox conditions, compared to Kv1.4-1.2 and Kv1.5-1.2. Kv1.2-1.1 channels exhibited a strongly depolarized $V_{1/2}$ in ambient redox (19.3 ± 8.3 mV) (Table 3.1), diverging significantly from Kv1.1 homomers, indicating that Kv1.1 subunits may be more permissive for Kv1.2 modulation of heteromeric channel gating, relative to Kv1.4 or Kv1.5.

3.4 CONCLUSION

In short, we confirm that Kv1.2 channels can confer redox and LMAN2 sensitivity to other Kv1 channel members when assembled as heteromeric channels. This sensitivity is dependent on the presence of Kv1.2 α-subunits, indicating a connected mechanism in which LMAN2 is a candidate sensor for redox modulation. Additionally, our results demonstrate that redox and LMAN2 influence the channels by decelerating activation kinetics without affecting deactivation. These findings contribute to our overall comprehension of the modulation mechanisms of heteromeric voltage-gated potassium channels and specifically, contribute to the ongoing investigation of the Kv1.2 slow gating mechanism.

CHAPTER 4: REDOX AND LMAN2 SENSITIVE GATING ALTER N-TYPE INACTIVATION OF KV1.2 CHANNELS

4.1 INTRODUCTION

Kv1.2 was the first eukaryotic voltage-gated channel with a reported atomic resolution structure, and has thus served as a valuable model for investigating ion channel gating mechanisms and as a template for molecular dynamics studies of ion channel relatives (Han and Zhang, 2008; Jogini and Roux, 2007; Long et al., 2005; Treptow and Tarek, 2006). Although the intrinsic molecular mechanisms of voltage-dependent opening and closing of these channels have been studied in depth, the interplay of voltage-dependent gating with extrinsic regulatory mechanisms has received relatively less attention and is likely important to understanding Kv1.2 function in vivo. Kv1.2 is ubiquitously expressed in neurons throughout the CNS and PNS where it influences action potential properties and regulates neuronal excitability (Devaux et al., 2002; Dodson et al., 2003; Goldberg et al., 2008; Kole et al., 2007; Sheng et al., 1994). Relative to other Kv1 family members (Kv1.1-1.8), Kv1.2 stands out for its critical functions. For example, Kv1.2 knock-out mice exhibit 100% lethality within three weeks of birth (Brew et al., 2007) and Kv1.2/KCNA2 mutations are associated with especially severe neurological phenotypes (Allen et al., 2016; Gao et al., 2022; Nilsson et al., 2022; Pena and Coimbra, 2015; Syrbe et al., 2015).

Although it has not been directly linked to pathophysiological outcomes, Kv1.2 channels are reported to exhibit notable modulation of activation properties in primary neuronal cultures and when heterologously expressed in mammalian cell lines (Baronas et al., 2017, 2015; Grissmer et al., 1994; Ishida et al., 2015; Rezazadeh et al., 2007; Scholle et al., 2004). We have made some progress in identifying the molecular mechanisms and regulators underlying the highly variable activation properties of Kv1.2, including the recognition that extracellular reducing conditions strongly shift Kv1.2 gating, and that a candidate regulatory protein LMAN2 can influence this redox sensitivity (Baronas et al., 2017; Lamothe and Kurata, 2023). These modulators can prominently shift Kv1.2 gating properties, although other candidate regulators may also contribute. For example, Sigma-1 receptors are reported to physically

interact with Kv1.2 and influence current amplitude (Abraham et al., 2019) and cell surface expression (Kourrich et al., 2013). Additionally, RhoA associates with Kv1.2 and suppresses channel expression via multiple mechanisms (Cachero et al., 1998; Hattan et al., 2002; Stirling et al., 2009). Lastly, we have reported that the amino acid transporter Slc7a5 influences Kv1.2 gating and expression (Baronas et al., 2018).

Amongst regulators of Kv1 channels, the most widely investigated auxiliary proteins are the Kvβ subunits. Kvβ subunits assemble with Kv1 α-subunits in a 1:1 stoichiometry by direct association with the N-terminal T1 domain (Gulbis et al., 2000, 1999; Pongs and Schwarz, 2010). Their effects include enhancing the maturation, expression, and localization of Kv1 channels (Accili et al., 1997; Gu et al., 2003; Heinemann et al., 1995; Shi et al., 1996). Furthermore, Kvβ1 and Kvβ3 subunits possess a N-terminal peptide that generates N-type inactivation and rapid current decay (Rettig et al., 1994).

Since N-type inactivation of Kv channels has a state-dependence, and requires channels to be open for association with the N-terminal inactivation peptide, we considered whether the modulation of Kv1.2 channel activation properties could influence the apparent rate or extent of N-type inactivation (Hoshi et al., 1991). Moreover, since activation kinetics of Kv1.2 can be manipulated in an activity-dependent manner, could interaction between these regulatory mechanisms generate activity-dependent changes in the properties of Kv currents? With recent progress in our understanding of mechanisms that regulate Kv1.2 activation, we sought to investigate how manipulation of Kv1.2 activation properties could influence N-type inactivation properties. We report moment-to-moment and cell-to-cell variability of inactivation of Kv1.2-like currents in primary hippocampal neurons. Furthermore, we also describe how manipulation of Kv1.2 activation kinetics in mammalian cells can recapitulate several characteristics of these neuronal currents. These include the activity-dependent changes in the extent of N-type inactivation, and the appearance of slowly activating currents even in the presence of Kvβ subunits. Overall, our study contributes to the ongoing exploration of the various regulatory mechanisms governing Kv1.2 channels.

4.2 RESULTS

Diverse properties of Kv1.2-containing currents in primary hippocampal neurons.

We previously investigated and reported use-dependent or activity-dependent properties of tityustoxin-sensitive currents in primary hippocampal neurons (Baronas et al., 2015). In primary cell cultures, there is notable variability between cells, presumably because there are varying degrees and types of modulation of Kv1.2 in different cells. We took a similar approach and investigated the inactivating properties of tityustoxin-sensitive currents (attributed to Kv1.2-containing channels) in this cell model.

When these neurons were stimulated with a +40 mV pulse for 50 ms, we observed that the kinetics and degrees of activation and inactivation varied greatly across different neurons. Some neurons displayed slow activation and no inactivation (left), some neurons exhibited fast activation and high levels of inactivation (right), and some neurons exhibited moderate amounts of activation and inactivation (middle) (Figure 4.1A).

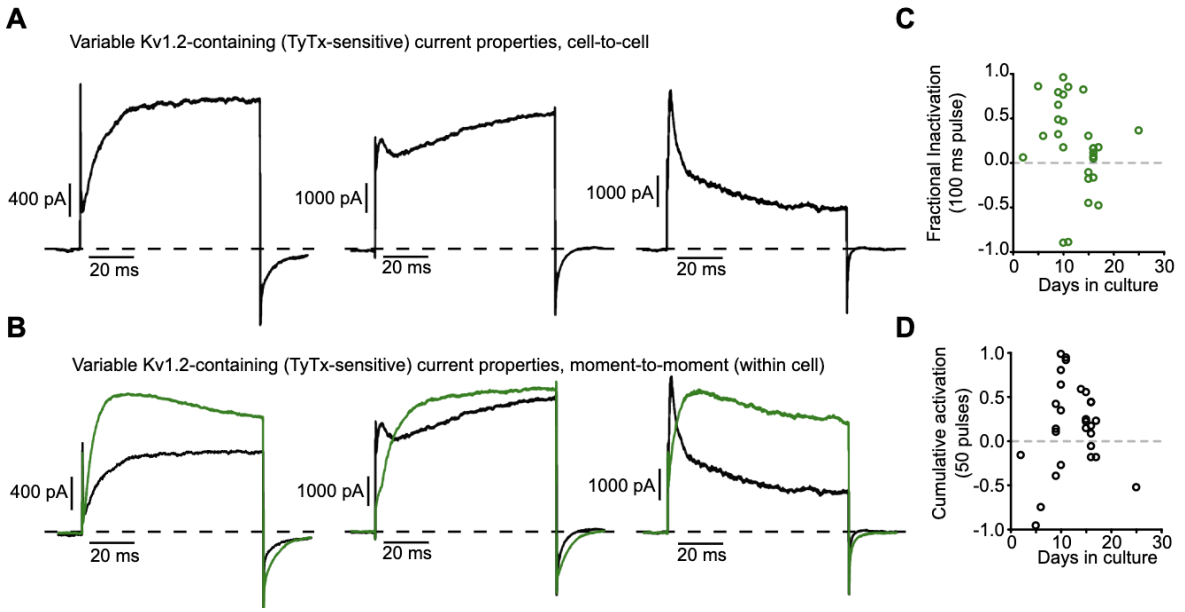


Figure 4.1. Variable Kv1.2-containing current properties in dissociated hippocampal neurons. (A) Sample traces of Kv1.2-containing currents isolated from three different hippocampal neurons using tityustoxin. These three traces demonstrate the wide range of Kv1.2 kinetic properties: slow activation and no inactivation (left), fast activation and high degrees of inactivation (right), and intermediate activation and inactivation effects (middle). (B) Sample traces of three neurons undergoing 50 pulses. Black trace represents the first pulse and green trace represents the 50th pulse. (C) Fractional inactivation was measured by calculating the difference between peak current within 10 ms and steady state current divided by total peak current of the first pulse. Each dot represents a single neuron recorded (n = 28). (D) Individual activation was measured from a single pulse by calculating the difference between final steady state current and peak current within 10 ms divided by total peak current. Cumulative activation was the sum of individual activation for all 50 pulses. Each dot represents a single neuron recorded (n = 28). These experiments were conducted and the data were analyzed by Victoria Baronas.

Notably, aside from the variability between cells (i.e. cell-to-cell), these neurons also demonstrated internal variability over time within the same cell (i.e. moment-to-moment). To explore this variability, neurons next underwent 50 repetitive +40 mV pulses. In Figure 4.1B, we observed that the first current sweep highlighted in green can be vastly different from the 50th current sweep highlighted in black. When we measure the fractional inactivation of a single sweep or the cumulative activation of 50 pulses, we again see high variability between cells that are not dependent on days in culture (Figure 4.1C-D). These findings reinforce that Kv1.2 can exhibit remarkable cell-to-cell and moment-to-moment

diversity in current properties. This intriguing phenomenon suggests the involvement of various unknown extrinsic regulators that are competing for and modulating Kv1.2 channel activity.

Kv1.2 and Kvβ generate variable levels of N-type inactivation.

Kv1.2 channels have been reported to exhibit widely variable kinetics and voltage-dependence of activation in mammalian cell lines. Kv1.2 can exhibit a slow "resistant" gating mode with slow activation kinetics, and can also be shifted to a fast "permissive" gating mode (with much faster activation kinetics) in response to conditioning prepulses (Baronas et al., 2015; Rezazadeh et al., 2007). We examined whether this activity-dependent plasticity of Kv1.2 activation rate would affect the appearance of Kvβ-mediated N-type inactivation because the onset of N-type inactivation requires channel activation to occur first (Hoshi et al., 1991).

We measured N-type inactivation in LM cells transfected with Kv1.2 and Kvβ1.2 in a 1:12 ratio (to promote association of Kv1.2 with the β-subunit). In response to a single depolarizing pulse (+150 mV, 300 ms), channels exhibited wide variability of inactivation between cells (Figure 4.2A). In some cases, there was little to no inactivation and even some delayed apparent slow activation (Figure 4.2A, left). Other cells exhibited intermediate (Figure 4.2A, middle) or high (Figure 4.2A, right) degrees of inactivation.

As previously described, conditioning prepulses to a strong depolarizing voltage will transiently relieve the slow gating, and thus lead to accelerated gating kinetics and activation of a second pulse at more negative voltages. In the case of Kvβ-mediated inactivation, this acceleration of activation could lead to more prominent inactivating behavior (Figure 4.2B). This was indeed the case even in very slow cells that initially exhibited 0% inactivation. The average % inactivation significantly increased from 28% to 47%, but more importantly, each cell exhibited enhanced % inactivation when potentiated with a conditioning pulse (Figure 4.2C). The important outcome of this experiment is that it demonstrates that variable gating kinetics of Kv1.2 can mask the apparent onset of N-type inactivation. Use-dependent potentiation can then reveal this hidden N-type inactivation.

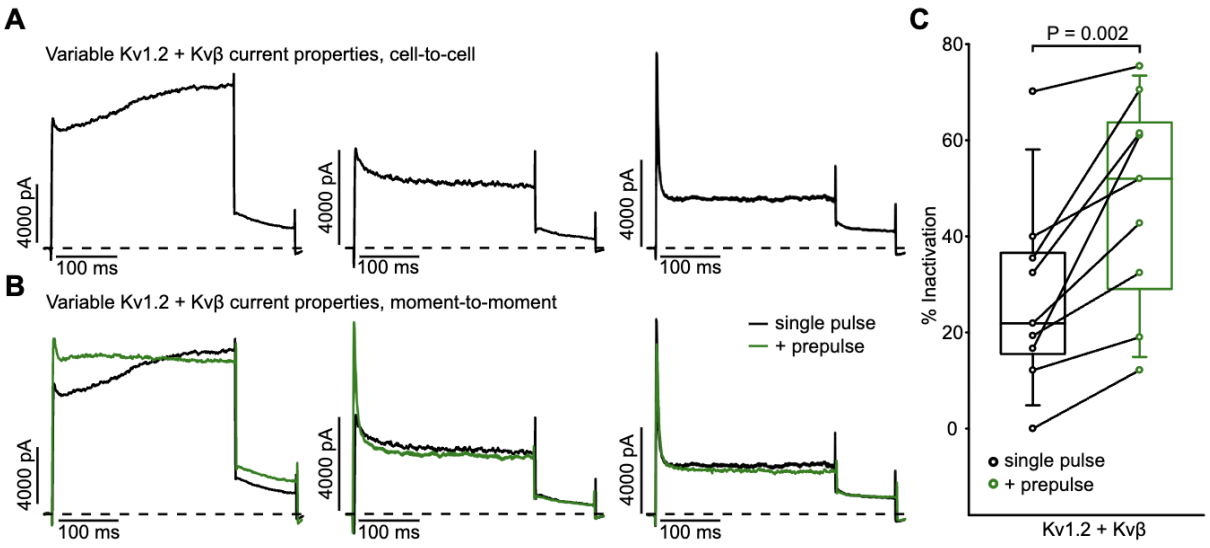


Figure 4.2. Variable Kvβ induced N-type inactivation in Kv1.2 channels in cell lines. (A) Representative traces recorded from three LM fibroblast cells transfected with Kv1.2 and Kvβ (1:12). Cells were held at -80 mV and then depolarized to +150 mV for 300 ms. The three traces illustrate the variable levels of percent inactivation (%) observed: low percent inactivation (left), medium percent inactivation (middle), high percent inactivation (right). (B) Sample current traces of LM fibroblast cells in the presence (green) and absence (black) of a 100 mV 300 ms prepulse. Recordings shown were again from cells that were held at -80 mV and then depolarized to +150 mV for 300 ms. (C) Box plots showing % inactivation. The % inactivation was measured by subtracting peak current by end current divided by peak current (n = 9). A paired t-test was performed to compare groups. P-values are indicated above box plots.

Redox and LMAN2 inhibit Kvβ inactivation in Kv1.2 channels

Experimental manipulations including extracellular reducing conditions and/or co-expression with the transmembrane lectin LMAN2 can strongly bias Kv1.2 towards the slow gating mode, with extreme deceleration of activation kinetics and a depolarizing shift of voltage-dependent activation (Baronas et al., 2017).

In terms of effects on Kvβ-mediated inactivation, application of DTT masks virtually all apparent inactivation, leading to 0% inactivation in the majority of cells tested (Figure 4.3B). This effect occurs because the DTT-mediated deceleration of activation masks the time course of onset of inactivation (Figure 4.3A). To demonstrate that the attenuation of % inactivation arises primarily from an indirect effect on the kinetics of activation, we again used a conditioning prepulse (double pulse protocol) to

transiently relieve the slow gating mode. Under these conditions, channels activate rapidly, and this reveals the fast time course of onset of N-type inactivation (Figure 4.3B). Use-dependent potentiation of cells in the extracellular reducing condition shifted average % inactivation from 2.8% to 33% (Figure 4.3B). This finding is helpful to demonstrate that redox-dependent mode shifting of Kv1.2 gating can markedly influence the appearance of N-type inactivation and can be modulated with prior depolarizing stimuli.

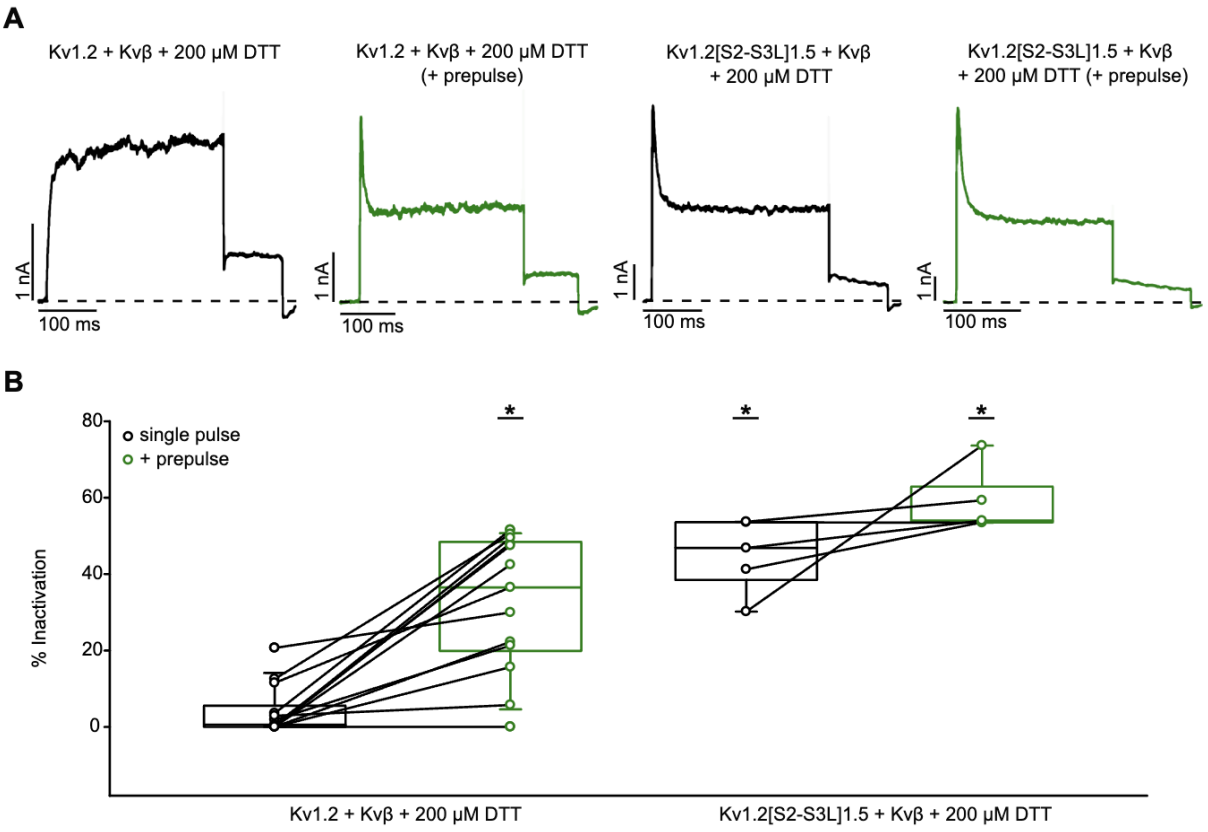


Figure 4.3. Prepulse potentiation and mutagenesis can rescue redox attenuated Kvβ induced N-type inactivation. (A) Representative traces recorded from LM cells expressing Kv1.2 or redox-insensitive mutant Kv1.2[S2-S3L]1.5 and Kvβ in 200 μM DTT conditions. Black traces were recorded from cells that underwent a single 300 ms +150 mV pulse. Green traces were recorded from cells that underwent an initial 300 ms +100 mV prepulse and then depolarized to +150 mV for 300 ms. (B) Box plots illustrating % inactivation of Kv1.2 + Kvβ + 200 μM DTT (n = 13) and Kv1.2[S2-S3L]1.5 + Kvβ + 200 μM DTT (n = 5). Measurements were taken using single pulse protocols (black) and double pulse protocols (green). A nonparametric Kruskal-Wallis test followed by pairwise multiple comparison Dunn's post hoc test was used to compare groups. Significant differences relative to Kv1.2 + Kvβ + 200 μM DTT single pulse are indicated with * $p < 0.05$.

It is also of interest that although Kvβ-subunits are closely-related to the redox-sensitive NADPH-oxidoreductase protein family, the redox-sensitivity of these effects on inactivation largely occur independently of the redox-responsiveness of Kvβ (Pan et al., 2011; Pongs and Schwarz, 2010; Weng et al., 2006). Rather, they are attributable to an alternative redox-sensitive mechanism that controls channel activation. To confirm this further, we used a chimeric Kv1.2-Kv1.5 construct that abolishes responsiveness to both redox and LMAN2 by replacing two residues in the intracellular linker of Kv1.2 with sequence from Kv1.5 (construct labeled Kv1.2[S2-S3L]1.5, which amounts to the double mutation F251S/T252R in Kv1.2). This loss of sensitivity causes Kv1.2[S2-S3L]1.5 channels to only exhibit their fast gating modes. The outcome of this loss of sensitivity is that Kv1.2[S2-S3L]1.5 exhibited fast activation kinetics and consequently significant inactivation in all conditions (Figure 4.3). This occurs because unlike Kv1.2 channels which can be shifted to slow gating modes in the presence of DTT or LMAN2, Kv1.2[S2-S3L]1.5 only has a fast gating mode and therefore does not exhibit pulse-to-pulse variation of activation and (indirectly) inactivation kinetics. In terms of overall redox modulation of Kv1.2 currents, this reaffirms that the modulation of inactivation is chiefly due to the effects redox has on Kv1.2 activation rates and not due to effects on Kvβ itself (Figure 4.3B).

We also tested the effects of combining LMAN2 co-expression with DTT application on N-type inactivation. The purpose of this approach was to determine whether further biasing the channels towards the slow gating mode would continue to suppress inactivation in repeated pulses. Our prior work has demonstrated that LMAN2 is involved in the mechanism underlying DTT sensitivity of Kv1.2, and the combination of these treatments further promotes the slow gating mode (Lamothe and Kurata, 2023). Similar to findings in Figure 4.3, combination of DTT and LMAN2 causes extremely low % inactivation, by decelerating activation and masking the onset of inactivation (Figure 4.4). Delivering a conditioning prepulse caused faster Kv1.2 activation unmasking a higher % inactivation (26%) (Figure 4.4). Consistent with our previous reports that the Kv1.2[S2-S3L]1.5 chimera is insensitive to both LMAN2 and redox, this conditioning pulse-dependence of inactivation was absent for the chimeric channel, and prominent

inactivation was apparent in all conditions (Figure 4.4) (Lamothe and Kurata, 2023). Overall, these findings demonstrate that reducing conditions and LMAN2 promote the slow gating mode of Kv1.2 channels which indirectly prevents it from undergoing inactivation by Kvβ.

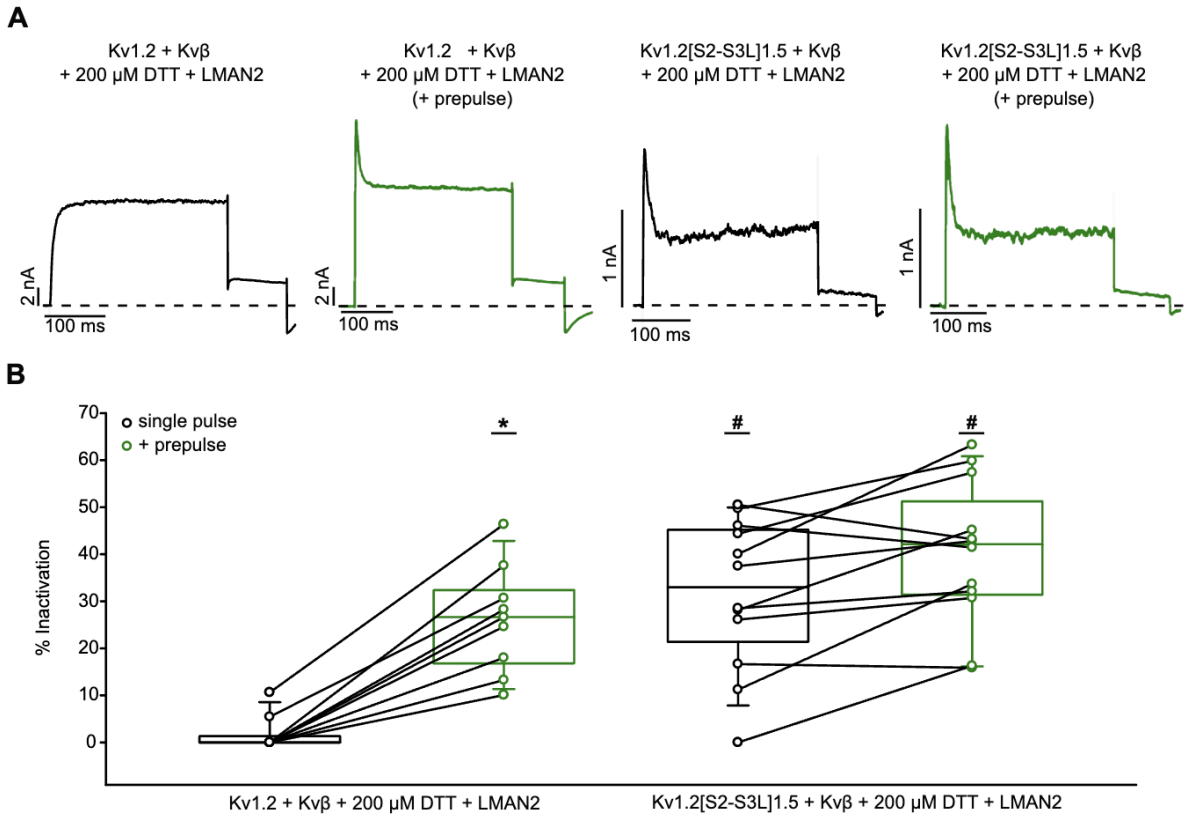


Figure 4.4. Prepulse potentiation and mutagenesis can rescue redox and LMAN2 attenuated Kvβ induced N-type inactivation. (A) Representative traces recorded from LM cells expressing Kv1.2, Kv1.2[S2-S3L]1.5, Kvβ, and LMAN2 in 200 μM DTT conditions. Black traces were recorded from cells that underwent a single 300 ms +150 mV pulse. Green traces were recorded from cells that underwent an initial 300 ms +100 mV prepulse and then depolarized to +150 mV for 300 ms. (B) Box plots illustrating % inactivation of Kv1.2 + Kvβ + LMAN2 + 200 μM DTT (n = 9) and Kv1.2[S2-S3L]1.5 + Kvβ + LMAN2 + 200 μM DTT (n = 12). Measurements were taken using single pulse protocols (black) and double pulse protocols (green). A one-way ANOVA followed by Bonferroni post hoc test was used to compare groups. Significant differences relative to Kv1.2 + Kvβ + 200 μM DTT + LMAN2 are indicated with * p=0.002 and # p <0.001.

Variable N-type inactivation of Kv1.4-1.2 heteromeric channels

Kv1 channel subtypes assemble as heteromeric channels in vivo which can recruit regulatory properties from distinct subunits. For example, Kv1.2 confers 'slow gating' sensitivity when assembled in heteromeric channels with Kv1.1, Kv1.4ΔN, or Kv1.5 (see Chapter 3). Since WT Kv1.4 contains a N-terminal peptide that generates N-type inactivation, we tested the interaction between the Kv1.2-dependent slow gating mode, and the Kv1.4-dependent N-type inactivation, when these subtypes combine to generate heteromeric channels. We used a concatenated dimer of full-length Kv1.4 (N-type inactivation intact and accessible at the N-terminus), and Kv1.2.

Similar to Kvβ-mediated N-type inactivation, WT Kv1.4-1.2 channels displayed a wide range of inactivation varying from 0% to 80% (Figure 4.5), likely due in part to the cell-to-cell variability of the slow gating mode of Kv1.2 (ie. when slow activation kinetics were prominent, then very little inactivation was apparent). We confirmed this interaction of slow gating and N-type inactivation by introducing the redox and LMAN2-insensitive form of Kv1.2 (Kv1.2[S2-3L] chimera) into these heterodimeric channels, leading to higher levels of % inactivation (Figure 4.5). This suggests that Kv1.2 induced slow gating inhibits Kv1.4 N-type inactivation. Interestingly, recovery from Kv1.4-mediated N-type inactivation progressed much slower than for Kvβ-mediated inactivation, and this prevented the development of peak current using the 'double pulse' protocols described in Figures 4.2, 4.3 or 4.4 (data not shown). This suggests that although Kv1.2 can strongly influence the kinetics of N-type inactivation, differences in pulse-to-pulse variability of N-type inactivation may emerge depending on the subunits used to confer inactivation.

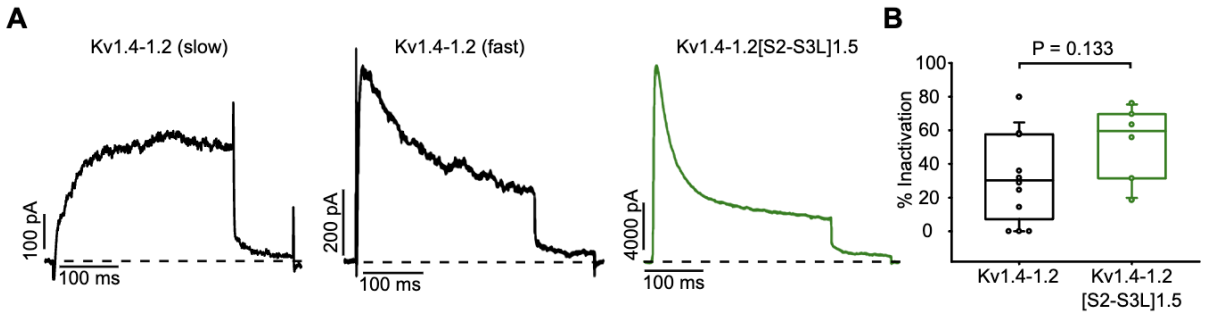


Figure 4.5. Variable levels of inactivation in Kv1.4-1.2 heteromers. (A) Exemplar recordings from LM fibroblast cells transfected with either Kv1.4-1.2 (black traces) or Kv1.4-1.2[S2-S3L]1.5 heterodimers (green traces) stimulated with a 300 ms pulse to +50 mV from a -80 mV resting potential. Left and middle sweeps demonstrate the extreme kinetics of Kv1.4-1.2 with a slow cell exhibiting no inactivation (left) and a fast cell exhibiting high % inactivation (middle). (B) Box plots depicting % inactivation of Kv1.4-1.2 heteromers in the absence (black box) and presence (green box) of prepulse (n = 6). A Mann-Whitney Rank Sum Test was performed to compare groups. P-values are indicated above box plots.

Attenuated redox/LMAN2 sensitivity enhances Kv1.4-mediated N-type inactivation

Similar to our experiments with Kv1.2 + Kvβ (Figures 4.3 and 4.4), we tested the effects of reducing agents and LMAN2 on WT Kv1.4-1.2 inactivation. When WT Kv1.4-1.2 channels were exposed to 200 μM DTT and LMAN2, N-type inactivation was nearly completely masked, as cells exhibited delayed channel activation and no current decay (Figure 4.6). Due to the previously mentioned redox/LMAN2 insensitivity of the Kv1.2[S2-3L] chimera, prominent inactivation persisted in most cells expressing Kv1.4-1.2[S2-S3L]1.5 channels, even in the presence of LMAN2 and DTT (Figure 4.6). These findings indicate that the slow gating mode markedly limits the appearance of N-type inactivation caused by both Kv1.4 and Kvβ.

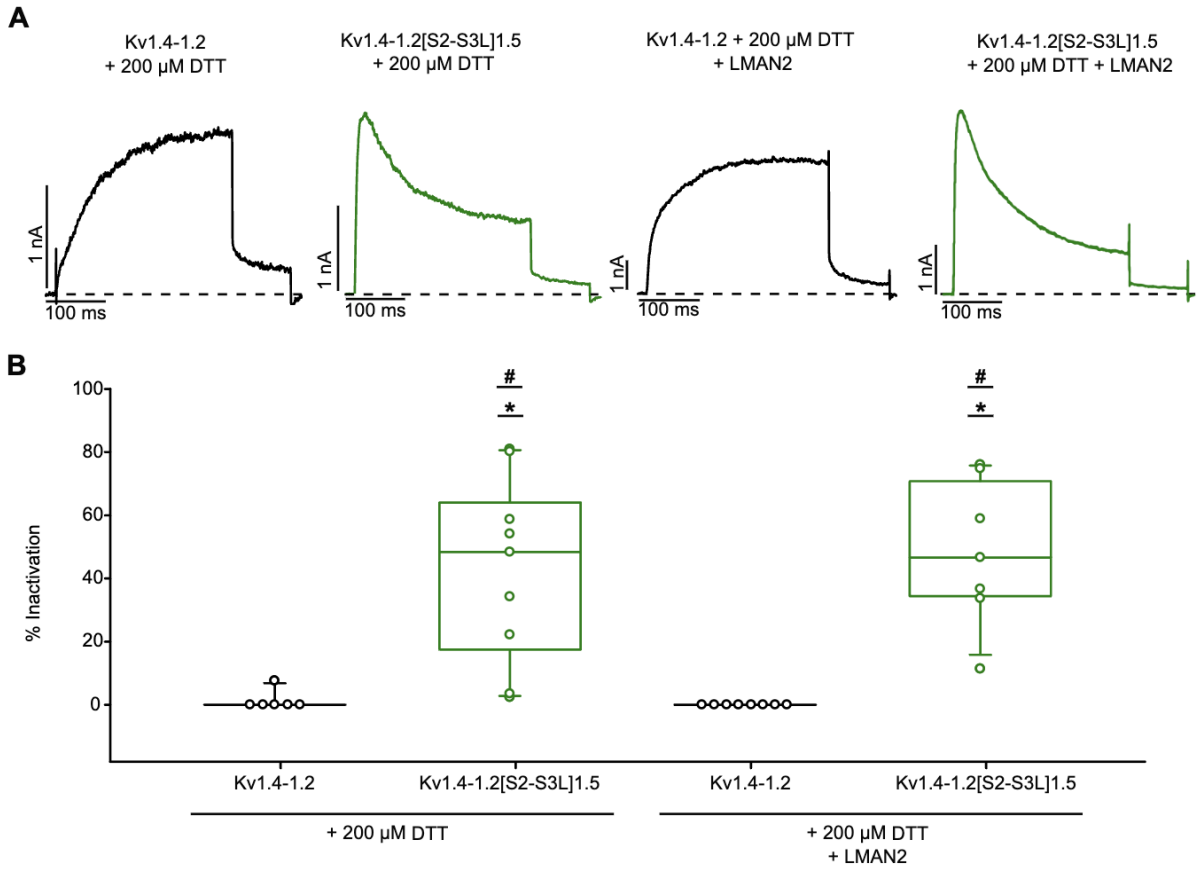


Figure 4.6. Inhibition of inactivation is absent in redox and LMAN2 resistant Kv1.4-1.2[S2-S3L]1.5 mutant. (A) Exemplar recordings of LM cells transfected with combinations of Kv1.4-1.2, Kv1.4[S2-S3L]1.5, and LMAN2 in 200 µM DTT conditions. Cells underwent a single 300 ms +50 mV pulse. (B) Box plots showing % inactivation of Kv1.4-1.2 + 200 µM DTT (n = 6), Kv1.4-1.2[S2-S3L]1.5 + 200 µM DTT (n = 9), Kv1.4-1.2 + 200 µM DTT + LMAN2 (n = 8), and Kv1.4-1.2[S2-S3L]1.5 + 200 µM DTT + LMAN2 (n = 8). Kruskal-Wallis followed by Dunn's post hoc test was used. Statistical differences relative to Kv1.4-1.2 + 200 µM DTT was indicated with * $p<0.05$. Statistical differences relative to Kv1.4-1.2 + 200 µM DTT + LMAN2 was indicated with # $p<0.05$.

4.3 DISCUSSION

Kv1.2 channels exhibit widely variable activation properties across different studies and expression systems, with the reported voltage-dependence of activation spanning a range of 120 mV (Baronas et al., 2018, 2017; Grissmer et al., 1994; Hite et al., 2014; Rezazadeh et al., 2007; Rodriguez-Menchaca et al., 2012). This level of variability is unique to Kv1.2 within the Kv1 subfamily and has been attributed to Kv1.2 occupancy of multiple gating modes (i.e a "fast" permissive gating mode and

"slow" resistant gating mode with distinct kinetics and voltage-dependence of activation), which can be manipulated with various experimental conditions. Preconditioning pulses transiently shift Kv1.2 to the fast gating mode. In contrast, reducing agents (e.g. DTT or TCEP) and/or co-expression with the transmembrane lectin LMAN2 promotes a slow gating mode (Baronas et al., 2017; Lamothe and Kurata, 2023). Other regulatory proteins also induce prominent shifts in Kv1.2 voltage-dependence, notably co-expression with amino acid transporter Slc7a5 induces a -50 mV shift of $V_{1/2}$ (Baronas et al., 2018). In light of emerging structural studies of ion channel 'mega-complexes' with diverse and unexpected auxiliary proteins/subunits, we have begun to explore the functional outcomes of interactions of regulatory proteins that influence Kv1.2 gating (Lin et al., 2021). In this study, we demonstrated that LMAN2 indirectly influences the onset of N-type inactivation induced by either Kvβ or Kv1.4 α-subunits in Kv1.2 containing channels. A model depicting this relationship is shown in Figure 4.7. The key feature of the model is that reducing conditions and overexpression of LMAN2 decelerates Kv1.2 channel activation, and therefore reduces the apparent rate and extent of inactivation (Figures 4.3,4.4, and 4.6). We demonstrated that this effect is primarily attributed to redox/LMAN2 effects on Kv1.2 gating (rather than direct modulation of N-type inactivation), because prepulses or mutations in the Kv1.2 S2-S3 linker region attenuate the slow gating mode, thus facilitating inactivation (Figures 4.2-4.6). Altogether, these illustrate how the interaction of distinct regulatory mechanisms generate cell-to-cell and moment-to-moment variation of Kv1.2 gating properties.

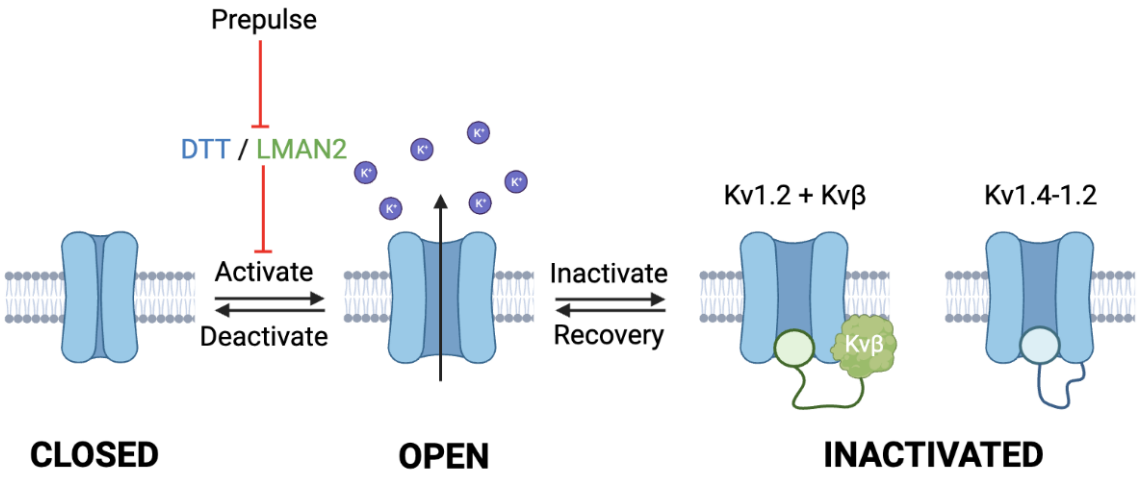


Figure 4.7. Hypothetical model for the interplay between DTT/LMAN2 and Kvβ/Kv1.4 induced N-type inactivation. Upon depolarization, Kv1.2 will be activated to their open states and can subsequently be inactivated by Kvβ or Kv1.4. DTT and LMAN2 inhibit channel opening and thus reduce the extent that N-type inactivation can occur. A prepulse can reverse the inhibitory effects of DTT and LMAN2 thereby encouraging channel activation and inactivation.

The S2-S3 linker region (particularly residues Thr252, Phe251, and Phe250) strongly influence sensitivity of Kv1.2 channels to redox modulation and LMAN2 (Baronas et al., 2016; Lamothe and Kurata, 2023; Rezazadeh et al., 2007). Point mutations at these positions strongly attenuate the gating effects of redox and LMAN2. The Kv1.2[S2-S3L]1.5 chimera, combines the T252R and F251S mutations, and appears sufficient to completely abolish LMAN2 or redox sensitivity. Our report bolsters prior studies demonstrating the pivotal role of this structural element, and clarifies how mutations in this region can indirectly shape the kinetics of inactivation by influencing sensitivity to reducing conditions and LMAN2.

Lamothe et al. demonstrated that both Slc7a5 and Kvβ can simultaneously regulate Kv1.2 channels, leading to Slc7a5-induced disinhibition and hyperpolarizing shifts in $V_{1/2}$, alongside Kvβ1.2-mediated N-type inactivation (Lamothe and Kurata, 2020). Moreover, Slc7a5 was found to accelerate Kvβ-mediated inactivation and prolong recovery from inactivation. These previous findings align with our own observations in hippocampal neurons and heterologous mammalian cells, where both LMAN2-

induced slow gating and Kvβ-induced inactivation were detected (Figure 4.1-4.4). Collectively, these outcomes highlight the possibility of multiple regulatory mechanisms coexisting, resulting in diverse current outcomes. A notable distinction between Slc7a5 and LMAN2 is the mechanism by which they affect inactivation. Slc7a5 directly stabilizes the inactivated state whereas LMAN2 indirectly influences inactivation by inhibiting channel activation. This distinction is exemplified by the behavior of Kv1.2[L298F] mutant channels, which exhibit a similar hyperpolarizing shift of activation as Slc7a5 but fail to replicate the accelerated inactivation or delayed recovery observed with Slc7a5 co-expression. Conversely, the Kv1.2[S2-S3L]1.5 mutant, which eliminates the depolarizing shift of activation, exhibits increased % inactivation, confirming that the impact of LMAN2 and redox on inactivation are due to their modulation on channel activation kinetics. The reason for this difference remains unknown but nevertheless, demonstrates the need for further investigation into how channels can be regulated by Slc7a5, LMAN2, redox and Kvβ.

Shortly after the discovery of N-type inactivation, this process was linked with redox modulation. Both Kvβ and Kv1.4 α-subunits contain N-terminal cysteine residues (C13 and C7/8 respectively) that are reported to be sensitive to oxidation (Rettig et al., 1994; Ruppersberg et al., 1990; Sahoo et al., 2014). In general, conditions that promote cysteine oxidation were reported to decelerate the inactivation rate and decrease the % inactivation. Conversely, these effects could be rescued via the application of reducing agents. This effect may be due to direct structural constraints on the N-terminal inactivating particle. An additional mechanism for redox-sensitivity of N-type inactivation is that Kvβ itself may act as a redox sensor. Structural and electrophysiological studies have confirmed that Kvβ is a functional aldo-keto reductase (AKR) enzyme that catalyzes the reduction of aldehydes to alcohols using an NADPH cofactor (Pan et al., 2011; Weng et al., 2006). This enzymatic process is coupled to channel gating as oxidation of NADPH bound to Kvβ can reduce inactivation. These two effects suggest that reducing conditions would result in accelerated and enhanced Kvβ/Kv1.4 induced N-type inactivation. Our findings lead to a different functional outcome as reducing conditions (200 μM DTT) decreased the apparent extent of inactivation by blunting the peak current achieved upon depolarization (Figures 4.3-4.4, and 4.6). It is

most noteworthy that previous studies of redox sensitivity were not conducted in Kv1.2 channels, but instead used Kv1.4, Kv1.1, or other Shaker subtypes. As mentioned, the redox-sensitive slow gating phenotype described here is exclusive to Kv1.2 (Baronas et al., 2017, 2015). Our findings do not negate the possibility that Kvβ and Kv1.4 α-subunits are redox sensitive, either via a N-terminal cysteine or AKR core domain, but rather when co-expressed with Kv1.2, the Kv1.2 redox-sensitive slow gating phenotype is more dominant. It is also important to recognize that intracellular redox potential is strongly reducing, and thus addition of extracellular reducing agents may not have a large influence on the N-terminal cysteines or AKR activity described for Kvβ (Baronas et al., 2017; Moriarty-Craige and Jones, 2004). This likely explains why DTT fails to accelerate inactivation kinetics in redox-insensitive Kv1.2 mutants such as the Kv1.2[S2-S3L]1.5 and Kv1.4-1.2[S2-S3L]1.5 chimeras.

Overall, there appear to be a variety of potential mechanisms that have evolved for Kv1 channels to respond to redox, and the functional outcomes observed likely will depend on the combination of channel subunits and accessory proteins expressed in a given cell. Our findings provide a mechanistic link between redox/LMAN2 modulation of Kv1.2 α-subunits and other subunits that confer N-type inactivation.

4.4 CONCLUSION

In conclusion, reducing conditions and LMAN2 can attenuate the appearance of N-type inactivation of Kv1.2 channels indirectly by slowing the rate of channel opening. These findings broaden our understanding of how auxiliary proteins and regulatory mechanisms can interact to generate complex patterns of Kv gating. This highlights the importance of further exploration of extrinsic factors that regulate Kv channels.

CHAPTER 5: GENERAL DISCUSSION

5.1 INTERPLAY BETWEEN REDOX, LMAN2, KVβ and KV1.4

The Kv channel family exhibits remarkable diversity in terms of channel kinetics and sensitivities to various signaling pathways. Among these channels, Kv1.2 channels display unique voltage-gating characteristics that vary from both a cell-to-cell and moment-to-moment basis (Baronas et al., 2015; Grissmer et al., 1994; Rezazadeh et al., 2007). This diversity arises from Kv1.2's ability to occupy either a fast permissive gating mode or a slow resistant gating mode, which can be biased by different experimental conditions (Rezazadeh et al., 2007). Depolarizing prepulses can drive the channel to their fast permissive gating mode resulting in hyperpolarizing shifts in $V_{1/2}$, and accelerated activation kinetics (Baronas et al., 2015; Rezazadeh et al., 2007). Alternatively, exposure to reducing agents biases Kv1.2 towards a slow resistant gating mode characterized by depolarizing shifts in $V_{1/2}$, decelerated activation kinetics, and use-dependent activation (Baronas et al., 2017). More recently, LMAN2 was found to replicate the functional outcomes of reducing agents by also shifting Kv1.2 channels to their slow/resistant gating mode (Lamothe and Kurata, 2023).

This diversity is further complicated by the ability of different Kv1 members to assemble together resulting in heteromeric channels with distinct properties and sensitivities to various regulatory pathways (Plane et al., 2005; Po et al., 1993; Ruppersberg et al., 1990; Shamotienko et al., 1997; Sheng et al., 1993). For instance, assembly of Kv1.4 α-subunits with other Kv1 α-subunits leads to heteromeric channels that retain N-type inactivation (Lee et al., 1996). As such, heteromerization contributes to variability in activation, inactivation kinetics, voltage-dependence, and sensitivities to modulation. These findings are also reflected in recordings in which hippocampal neurons exhibit highly variable activation and inactivation kinetics (Figure 4.1) (Baronas et al., 2015). In my thesis, I delved into the underlying mechanisms that give rise to these diverse current outcomes, shedding light on the role of redox and LMAN2 mediated signaling on Kv1.2 slow gating.

In Chapter 3 of my thesis, I provided further evidence supporting the role of reducing agents and LMAN2 as major regulators of the Kv1.2 slow gating phenotype, with converging mechanisms. Exposure of Kv1.2 to either reducing agents or LMAN2 significantly increased the appearance of the slow gating phenotype (Figure 3.1 and 3.3). Moreover, I demonstrated that Kv1.2 is capable of conferring redox and LMAN2 when combined with other Kv1 subunits in a heterotetrameric channel (Figure 3.2-3.5). Channels could only demonstrate sensitivity to both redox and LMAN2 sensitivity, but not exclusively one, thus these two regulators exhibit overlapping subtype specificity. It was previously confirmed through cysteine mutagenesis that Kv1.2 channels lacked intrinsic redox sensitivity and that an extrinsic modulator was likely responsible for the redox-mediated effects on Kv1.2 currents (Baronas et al., 2017). Lamothe et al. proposed that LMAN2 contributes to slow gating modulation, as LMAN2 knockdown greatly diminishes the effects of reducing agents on Kv1.2 channels and overexpression of LMAN2 hyper-sensitizes Kv1.2 channels to redox (Lamothe and Kurata, 2023). Taken together, these findings strongly support the notion that LMAN2 is a regulator of redox sensitivity of Kv1.2 channel function.

Based on these findings, I propose a hypothesis/model that when LMAN2 is in its reduced form, it exhibits stronger interactions (either direct or indirect) with Kv1.2 channels, leading to a shift toward the resistant gating mode. Conversely, when LMAN2 is oxidized, its interaction with Kv1.2 channels is weaker, suggesting that reducing agents indirectly impact Kv1.2 channel function by affecting this auxiliary subunit and mediating slow gating. It is important to note that heterologous LM cells naturally express endogenous levels of LMAN2 (Lamothe and Kurata, 2023). Therefore, even without the overexpression of LMAN2 alongside Kv1.2, the application of reducing agents can induce the transition of endogenous LMAN2 to its reduced state. This shift in LMAN2 leads to subsequent modulation of Kv1.2 channels, driving them towards their slow gating modes.

Expanding on the theme of extrinsic modulators and slow gating of Kv1.2, in Chapter 4, I further demonstrated that LMAN2 and redox can indirectly affect the rate and extent of N-type inactivation induced by Kvβ and Kv1.4 α-subunits. This indirect inhibition occurs because channel inactivation occurs via a stepwise process (C → O → I) (Hoshi et al., 1991). By inhibiting channel opening (C → O), redox

and LMAN2 can indirectly inhibit channel inactivation (O → I). The most compelling evidence supporting this claim arose from the examination of redox and LMAN2 insensitive mutants, Kv1.2[S2-S3L]1.5 and Kv1.4-1.2[S2-S3L]1.5. These mutant channels exhibit notably faster activation kinetics and higher degrees of N-type inactivation (Figures 4.3-4.6). This dramatic change in % inactivation based solely on the mutation of the crucial S2-S3 linker, which is essential for slow gating, demonstrates the significant influence of LMAN2 and redox signaling in governing N-type inactivation in Kv1.2 channels, rather than a direct effect of redox on the Kvβ/Kv1.4 subunits that endow N-type inactivation.

These findings provide insights into the variable rates of activation and inactivation observed in Kv1.2-containing currents in primary hippocampal neurons. The expression levels of LMAN2, Kvβ, and Kv1.4 α-subunits, as well as the local redox potential, vary across different neurons, possibly resulting in distinct Kv1.2 current kinetics. Neurons with LMAN2 expression and/or a relatively reducing environment would be expected to produce decelerated Kv1.2 activation kinetics. Conversely, neurons with low expression of LMAN2 and/or a relatively oxidizing environment would be expected to produce accelerated Kv1.2 activation. Additionally, neurons with low levels of LMAN2, an oxidizing environment, and high levels of Kvβ/Kv1.4 α-subunits would exhibit fast activation kinetics and significant degrees of inactivation.

Furthermore, my research highlights the influence a strong depolarizing prepulse and repetitive depolarizations can have by shifting Kv1.2 channels to their fast gating mode. It is suspected that strong and repetitive depolarizations can inhibit the effects mediated by LMAN2. Consequently, channels will then activate more quickly and be more susceptible to N-type inactivation. This explains why preconditioning prepulses resulted in the subsequent pulse exhibiting higher levels of % inactivation (Figures 4.2-4.4).

5.2 KV1.2-LMAN2 INTERACTION SIGNIFICANCE

Redox and LMAN2 have prominent effects on the voltage-dependence and activation kinetics of Kv1.2. Previously described Kv1.2 regulatory mechanisms including glycosylation, PIP2, and sigma-1

receptors elicit comparably modest shifts in voltage dependence (i.e. less than 15 mV shifts) (Abraham et al., 2019; Rodriguez-Menchaca et al., 2012; Watanabe et al., 2000). In comparison, reducing conditions and LMAN2 induce depolarizing shifts exceeding +60 mV (Table 3.1), and this effect is modulated most steeply in the physiological range of extracellular redox potential (Baronas et al., 2017; Moriarty-Craige and Jones, 2004). Under physiological conditions and voltages, this means that channels could be shifted from completely suppressed or completely active depending on the surrounding redox environment or the relative expression of regulatory proteins. These dramatic shifts in activation properties could impact neuronal excitability and firing patterns. For example, a high degree of slow gating (resulting in decelerated activation kinetics and UDA) would suppress activation of Kv1.2-containing currents, potentially leading to prolonged action potential duration and/or increased neuronal firing rate, similar to the effects observed when Kv1.2 channels are blocked pharmacologically (Devaux et al., 2002; Southan and Robertson, 1998; Wu and Barish, 1992; Zhou et al., 2020). Conversely, relief of slow gating in an oxidizing environment would maximize Kv1.2-containing currents, thereby suppressing neuronal excitability. Modulating Kv1.2 channel activity, redox signaling and LMAN2 may play a role in fine-tuning neuronal excitability and shaping action potential firing patterns, thereby allowing the CNS to dynamically respond to various physiological conditions such as stress, and learning and memory (Dunn and Kaczorowski, 2019; Nawreen et al., 2021; Oh and Disterhoft, 2020; Rosenkranz et al., 2010).

Further building on these ideas, Kv1.2 slow gating and use-dependent activity may contribute to the phenomenon of spike-frequency adaptation. Spike-frequency adaptation refers to the gradual decrease in firing rate of neurons subjected to repetitive or prolonged stimuli (Benda and Tabak, 2013). This adaptive response is found in various neurons and is important for preventing excessive excitability and enabling neurons to better detect and adapt to changes in stimuli (Pineda et al., 1999; Benda et al., 2005; Sharpee et al., 2006). While the mechanisms underlying spike-frequency adaptation are diverse, it has been established that certain ion currents, specifically M-type currents (Kv7 currents) and AHP-type currents (calcium-gated potassium currents) can accumulate over time in response to repetitive stimulation (Benda and Tabak, 2013). These currents lead to increased inhibitory effects on neurons,

ultimately culminating in spike-frequency adaptation. It is plausible that Kv1.2 channels could exhibit similar behavior in the context of spike-frequency adaptation. When in their slow resistant gating modes, Kv1.2 would initially produce minimal current in response to the first few stimuli. Over time, Kv1.2 activity would gradually increase resulting in stronger inhibition of the neuron's input. This mechanism could contribute to the decrease in firing rate during repetitive stimulation reported in some neuron types (Pineda et al., 1999; Benda et al., 2005; Sharpee et al., 2006). An experimental approach of verifying this hypothesis could involve recording neurons exposed to tityustoxin. If we observe a lack of spike-frequency adaptation in neurons exposed to tityustoxin while neurons not exposed to tityustoxin exhibit typical adaptation responses, this would further confirm the significance of Kv1.2 slow gating.

The persistence of redox and LMAN2 sensitivity in multiple Kv1.2-containing channels and in the presence of auxiliary subunits is noteworthy. It is well documented that Kv1 channel subtypes assemble heteromerically in various tissues resulting in diverse functional outcomes (Sheng et al., 1993, 1992; Vacher et al., 2008). The modulation of Kv1 channel function by Kvβ subunits further adds to the complexity (Lamothe and Kurata, 2020; Pongs and Schwarz, 2010). Our observation that the gating effect and redox/LMAN2 sensitivity are retained amidst this potential diversity indicates that these effects likely persist in physiological conditions.

Findings here and elsewhere also demonstrate the presence of slow gating in hippocampal and DRG neurons, further providing direct evidence of its occurrence in the CNS and in vivo (Figure 4.1) (Baronas et al., 2015; Lamothe and Kurata, 2023). Kv1.2 channels are extensively expressed in the CNS including in hippocampal and DRG neurons both as homomeric and heteromeric channels and influence membrane potential, action potential firing, and overall excitability (Dodson and Forsythe, 2004; Palani et al., 2010). As mentioned previously in Chapters 3 and 4, Kv1.2 knockout causes complete mortality in mice within three of birth, and Kv1.2 mutants are linked to severe neurodevelopmental delay seizures (Coetzee et al., 1999; Masnada et al., 2017; Robbins and Tempel, 2012; Syrbe et al., 2015; Wang et al., 1994). In conjunction with this, LMAN2 is expressed in the CNS and closely related homologs are associated with epileptic seizures (Alkhater et al., 2019; Bettencourt et al., 2020; Rafiullah et al., 2016).

The slow gating properties observed in hippocampal neurons coupled with the Kv1.2-related pathologies and the expression/disease patterns of LMAN2 provide intriguing hints of a potential interplay between Kv1.2 channels and LMAN2 in the brain.

Despite these findings, the physiological influence of the Kv1.2 slow gating mechanism has yet to be directly established. To address this critical question, further investigations could involve generating mutant mice expressing Kv1.2[S2-S3L]1.5, a channel variant that lacks slow gating. Additionally, creating KO mouse models lacking LMAN2 expression would be another valuable strategy. From there, both electrophysiology studies conducted on neuronal slices and animal testing could be done. My hypothesis would be that these mutant mice models would experience neurodevelopmental delay similar to Kv1.2 KO mice models given the seemingly large influence of redox and LMAN2 on Kv1.2 function.

5.3 REDOX SENSITIVITY AMONG OTHER ION CHANNELS

Aside from Kv1.2, several other ion channels have been found to exhibit redox sensitivity demonstrating the broad involvement of redox signaling in ion channel regulation. Oxidizing agent H_2O_2 was shown to activate TRPC5 and TRPV1 channels through direct modifications to cysteine residues (Kozai et al., 2014). Reducing agents such as DTT and L-cysteine can activate T-type calcium channels in sensory neurons, which is relevant to pain sensation (Todorovic et al., 2001; Todorovic and Jevtovic-Todorovic, 2014). Injection of reducing agents in the peripheral receptive fields of adult rats, thus resulted in hyperalgesia (Todorovic et al., 2001). In the case of voltage-gated potassium channels, oxidizing agents induce Kv2.1 oligomerization, resulting in decreased current amplitude and contributing to neuronal apoptosis (Cotella et al., 2012). In contrast, oxidizing conditions enhance the M-current mediated by Kv7.2, 7.4 and 7.5 channels, leading to decreased neuronal firing and providing neuronal protection against oxidative stress (Gamper et al., 2006). What distinguishes Kv1.2 from these previously mentioned examples is that redox is thought to have direct effects on these channels whereas for Kv1.2, we believe that redox induces its effects indirectly through LMAN2 and other unidentified regulators. Nevertheless, the growing body of literature and the link to physiological mechanisms provide further support for the

significance of the interplay between ion channels and redox signaling. It highlights the intricate relationship between cellular redox state and ion channel function, which has implications for various physiological and pathological processes.

5.4 EVIDENCE FOR NOVEL KV1 AUXILIARY SUBUNITS BEYOND LMAN2

Throughout my thesis, I propose that LMAN2 is a significant contributor to the slow gating mechanism of Kv1.2, but I hope to emphasize that it is likely not the sole regulator. Instead, I suggest that Kv1.2 channels interact with numerous proteins to form a complex. There are multiple lines of evidence to support this view.

First, and perhaps the biggest piece of support, is that Kv1.2 channels do not exhibit slow gating or use-dependent activation in *Xenopus laevis* oocytes even when co-expressed with LMAN2 (data not shown). This differs from the strong effects of LMAN2 co-expression seen in mammalian cell lines and suggests the involvement of other auxiliary proteins or regulatory elements (such as lipids) that may be essential for this mechanism. Secondly, while redox and LMAN2 can explain the extreme depolarized shifts in voltage-dependence, it cannot explain the hyperpolarized shifts in voltage-dependence. This seems to be primarily attributed to Slc7a5 which can induce prominent leftward shifts in $V_{1/2}$ when coexpressed alongside Kv1.2 channels. This suggests the presence of a minimum of two proteins regulating Kv1.2 gating, with the possibility of additional regulators. Thirdly, in Chapter 4 of my thesis, I demonstrated that LMAN2 and Kvβ can simultaneously regulate Kv1.2 channels to produce intermediate effects and combined effects in both primary and heterologous cells. Similarly, Slc7a5 affects Kvβ-mediated regulation of Kv1.2 channels and vice versa, indicating competition and synchronous interactions among regulators (Lamothe and Kurata, 2020). Fourth, as shown with the Kv1.2-1.1 heterodimer, overexpression of LMAN2 was unable to recapitulate as high of a shift in $V_{1/2}$ compared to reducing conditions (Figures 3.2 and 3.5). The differences between the shifts induced solely by LMAN2 overexpression versus changes in redox could be attributed to currently unknown regulators.

To identify novel interacting proteins, cross-linking and immunoprecipitation of Kv1.2 channel complexes were performed, followed by mass spectrometry analysis, and electrophysiological screening. This approach was used to identify LMAN2, Slc7a5, and TMEM33 as potential regulators of Kv1.2 (Baronas et al., 2018). A limitation of this approach is that because the screening approaches used are based upon examining electrophysiological outcomes, it may miss potential regulators which elicit small functional changes but are nevertheless essential for slow gating. To address this limitation, a comparative analysis of potential regulators between Kv1.2 and Kv1.2[S2-S3L]1.5 could be conducted. Given that the Kv1.2[S2-S3L]1.5 chimera exhibits no slow gating behavior, this may indicate the loss of interactions with key regulators. Comparing protein levels between the WT and chimera would help identify essential proteins for slow gating.

5.5 FUTURE DIRECTION

LMAN2 cysteine mutants

In a previous study, Kv1.2 cysteine mutants were individually tested for redox sensitivity (Baronas et al., 2017). None of these cysteine mutants affected the response to DTT, indicating the involvement of an unknown redox sensitive extrinsic binding partner. Since then, we have identified LMAN2 as a potential regulator of Kv1.2 slow gating that likely contributes to redox modulation of Kv1.2. To confirm this hypothesis, we intend to generate cysteine mutants (Cys → Ala) of LMAN2. In our working-hypothesis, the LMAN2-mediated signaling pathway is strongest when LMAN2 is reduced and weakest when LMAN2 is oxidized (Figure 4.7). I would therefore predict that a relevant cysteine mutation would prevent the formation of a disulfide bond thereby enabling LMAN2 to always be in their reduced form. Consequently, this would then strengthen the Kv1.2 slow gating mechanism.

If the relevant cysteine can be identified successfully, it could offer valuable insights into the mechanism of action of LMAN2 regulation of Kv1.2. This cysteine residue could potentially serve as the interaction site between LMAN2 and Kv1.2, or it might be involved in the recruitment of additional auxiliary subunits by LMAN2. Another possibility is that LMAN2 undergoes conformational changes

dependent on the redox state of this cysteine group. Regardless, investigating this cysteine through experimental approaches would provide a solid foundation for further understanding the mechanism by which LMAN2 influences slow gating.

Impact of Kv1.2 on LMAN2 function

While my thesis shows that LMAN2 can vastly impact Kv1.2 function, we have yet to determine the effects of Kv1.2 on LMAN2. As mentioned previously in Chapters 1 and 3, the impact of LMAN2 on ion channels differs from its more commonly studied roles in vesicular transport through the ER and Golgi (Füllekrug et al., 1999; Kwon et al., 2016; Reiterer et al., 2010). It would be interesting to explore whether Kv1.2 can impact the localization of LMAN2 as well as its ability to transport various cargo proteins.

Patch-sequencing Protocol

One limitation of my thesis is the lack of direct evidence linking specific regulators to the production of slow activating and inactivating properties in hippocampal neurons. To address this gap, I propose patch-sequencing as a viable strategy. By combining both whole-cell patch clamp recordings with single-cell RNA-sequencing, we can simultaneously determine the electrophysiological properties and the gene profile of individual neurons. This approach would allow us to compare the gene expression patterns of neurons with slow activation kinetics to those with fast activation and inactivation rates, potentially identifying specific regulators associated with these different phenotypes.

It is important to acknowledge, however, that patch-seq is a labor-intensive and time-consuming procedure that demands a high level of technical skill. Performing successful patch-clamp recordings, which already has a steep learning curve, is just one aspect of the process. Another crucial consideration is the prevention of RNA degradation, which requires an RNAse-free environment and maintaining cell integrity during recordings to avoid exposure to external RNAse enzymes. These challenges have posed difficulties even for experienced electrophysiologists (Cadwell et al., 2017). Furthermore, the

heterogeneity of hippocampal neurons characterized by diverse gene expressions and electrophysiological phenotypes may require a large number of neuronal recordings to be done to draw meaningful conclusions and capture the full spectrum of hippocampal diversity (Mallory and Giocomo, 2018). Consequently, this further adds to the effort and time required to complete such a project.

5.5 CONCLUSION

Voltage-gated ion channels generate a remarkable range of current diversity, partially stemming from their ability to heteromerically assemble and be regulated by auxiliary subunits. This thesis contributes to the ongoing exploration of these factors by demonstrating the regulatory role of redox and lectin protein LMAN2 in both homomeric and heteromeric Kv1 channels, independently and in conjunction with Kvβ and Kv1.4 inactivating subunits. By unveiling the intricate interplay between these modulators and ion channels, these findings ultimately shed light on the complex processes governing neuronal excitability.

REFERENCES

Abraham, M.J., Fleming, K.L., Raymond, S., Wong, A.Y.C., Bergeron, R., 2019. The sigma-1 receptor behaves as an atypical auxiliary subunit to modulate the functional characteristics of Kv1.2 channels expressed in HEK293 cells. Physiol. Rep. 7, e14147. https://doi.org/10.14814/phy2.14147

Accili, E.A., Kiehn, J., Wible, B.A., Brown, A.M., 1997. Interactions among Inactivating and Noninactivating Kvβ Subunits, and Kvα1.2, Produce Potassium Currents with Intermediate Inactivation*. J. Biol. Chem. 272, 28232–28236. https://doi.org/10.1074/jbc.272.45.28232

Alkhater, R.A., Wang, P., Ruggieri, A., Israelian, L., Walker, S., Scherer, S.W., Smith, M.L., Minassian, B.A., 2019. Dominant LMAN2L mutation causes intellectual disability with remitting epilepsy. Ann. Clin. Transl. Neurol. 6, 807–811. https://doi.org/10.1002/acn3.727

Allen, N.M., Conroy, J., Shahwan, A., Lynch, B., Correa, R.G., Pena, S.D.J., McCreary, D., Magalhães, T.R., Ennis, S., Lynch, S.A., King, M.D., 2016. Unexplained early onset epileptic encephalopathy: Exome screening and phenotype expansion. Epilepsia 57, e12-17. https://doi.org/10.1111/epi.13250

Arancibia-Carcamo, I.L., Attwell, D., 2014. The node of Ranvier in CNS pathology. Acta Neuropathol. (Berl.) 128, 161–175. https://doi.org/10.1007/s00401-014-1305-z

Archer, S.L., Souil, E., Dinh-Xuan, A.T., Schremmer, B., Mercier, J.C., Yaagoubi, A.E., Nguyen-Huu, L., Reeve, H.L., Hampl, V., 1998. Molecular identification of the role of voltage-gated K+ channels, Kv1.5 and Kv2.1, in hypoxic pulmonary vasoconstriction and control of resting membrane potential in rat pulmonary artery myocytes. J. Clin. Invest. 101, 2319–2330. https://doi.org/10.1172/JCI333

Baculis, B.C., Zhang, J., Chung, H.J., 2020. The Role of Kv7 Channels in Neural Plasticity and Behavior. Front. Physiol. 11, 568667. https://doi.org/10.3389/fphys.2020.568667

Bähring, R., Milligan, C.J., Vardanyan, V., Engeland, B., Young, B.A., Dannenberg, J., Waldschutz, R., Edwards, J.P., Wray, D., Pongs, O., 2001. Coupling of voltage-dependent potassium channel inactivation and oxidoreductase active site of Kvbeta subunits. J. Biol. Chem. 276, 22923–22929. https://doi.org/10.1074/jbc.M100483200

Bähring, R., Vardanyan, V., Pongs, O., 2004. Differential modulation of Kv1 channel-mediated currents by co-expression of Kvbeta3 subunit in a mammalian cell-line. Mol. Membr. Biol. 21, 19–25. https://doi.org/10.1080/09687680310001597749

Barnes-Davies, M., Barker, M.C., Osmani, F., Forsythe, I.D., 2004. Kv1 currents mediate a gradient of principal neuron excitability across the tonotopic axis in the rat lateral superior olive. Eur. J. Neurosci. 19, 325–333. https://doi.org/10.1111/j.0953-816x.2003.03133.x

Baronas, V.A., McGuinness, B.R., Brigidi, G.S., Gomm Kolisko, R.N., Vilin, Y.Y., Kim, R.Y., Lynn, F.C., Bamji, S.X., Yang, R., Kurata, H.T., 2015. Use-Dependent Activation of Neuronal Kv1.2 Channel Complexes. J. Neurosci. 35, 3515–3524. https://doi.org/10.1523/JNEUROSCI.4518-13.2015

Baronas, V.A., Yang, R., Vilin, Y.Y., Kurata, H.T., 2016. Determinants of frequency-dependent regulation of Kv1.2-containing potassium channels. Channels Austin Tex 10, 158–166. https://doi.org/10.1080/19336950.2015.1120390

Baronas, V.A., Yang, R.Y., Kurata, H.T., 2017. Extracellular redox sensitivity of Kv1.2 potassium channels. Sci. Rep. 7, 9142. https://doi.org/10.1038/s41598-017-08718-z

Baronas, V.A., Yang, R.Y., Morales, L.C., Sipione, S., Kurata, H.T., 2018. Slc7a5 regulates Kv1.2 channels and modifies functional outcomes of epilepsy-linked channel mutations. Nat. Commun. 9. https://doi.org/10.1038/s41467-018-06859-x

Barry, D.M., Trimmer, J.S., Merlie, J.P., Nerbonne, J.M., 1995. Differential Expression of Voltage-Gated K+ Channel Subunits in Adult Rat Heart. Circ. Res. 77, 361–369. https://doi.org/10.1161/01.RES.77.2.361

Baukrowitz, T., Yellen, G., 1996. Use-dependent blockers and exit rate of the last ion from the multi-ion

pore of a K+ channel. Science 271, 653–656. https://doi.org/10.1126/science.271.5249.653
Bean, B.P., 2007. The action potential in mammalian central neurons. Nat. Rev. Neurosci. 8, 451–465. https://doi.org/10.1038/nrn2148
Bekkers, J.M., Delaney, A.J., 2001. Modulation of Excitability by α-Dendrotoxin-Sensitive Potassium Channels in Neocortical Pyramidal Neurons. J. Neurosci. 21, 6553–6560. https://doi.org/10.1523/JNEUROSCI.21-17-06553.2001
Benda, J., Longtin, A., Maler, L., 2005. Spike-Frequency Adaptation Separates Transient Communication Signals from Background Oscillations. J. Neurosci. 25, 2312–2321. https://doi.org/10.1523/JNEUROSCI.4795-04.2005
Benda, J., Tabak, J., 2013. Spike-Frequency Adaptation, in: Jaeger, D., Jung, R. (Eds.), Encyclopedia of Computational Neuroscience. Springer, New York, NY, pp. 1–12. https://doi.org/10.1007/978-1-4614-7320-6_339-1
Bertaso, F., Sharpe, C.C., Hendry, B.M., James, A.F., 2002. Expression of voltage-gated K+ channels in human atrium. Basic Res. Cardiol. 97, 424–433. https://doi.org/10.1007/s00395-002-0377-4
Bettencourt, C., Foti, S.C., Miki, Y., Botia, J., Chatterjee, A., Warner, T.T., Revesz, T., Lashley, T., Balazs, R., Viré, E., Holton, J.L., 2020. White matter DNA methylation profiling reveals deregulation of HIP1, LMAN2, MOBP, and other loci in multiple system atrophy. Acta Neuropathol. (Berl.) 139, 135–156. https://doi.org/10.1007/s00401-019-02074-0
Bezanilla, F., 2018. Gating currents. J. Gen. Physiol. 150, 911–932. https://doi.org/10.1085/jgp.201812090
Bezanilla, F., 2002. Voltage sensor movements. J. Gen. Physiol. 120, 465–473. https://doi.org/10.1085/jgp.20028660
Bezanilla, F., 2000. The Voltage Sensor in Voltage-Dependent Ion Channels. Physiol. Rev. 80, 555–592. https://doi.org/10.1152/physrev.2000.80.2.555
Bixby, K.A., Nanao, M.H., Shen, N.V., Kreusch, A., Bellamy, H., Pfaffinger, P.J., Choe, S., 1999. Zn2+-binding and molecular determinants of tetramerization in voltage-gated K+ channels. Nat. Struct. Biol. 6, 38–43. https://doi.org/10.1038/4911
Börjesson, S.I., Elinder, F., 2008. Structure, Function, and Modification of the Voltage Sensor in Voltage-Gated Ion Channels. Cell Biochem. Biophys. 52, 149–174. https://doi.org/10.1007/s12013-008-9032-5
Bos, R., Harris-Warrick, R.M., Brocard, C., Demianenko, L.E., Manuel, M., Zytnicki, D., Korogod, S.M., Brocard, F., 2018. Kv1.2 Channels Promote Nonlinear Spiking Motoneurons for Powering Up Locomotion. Cell Rep. 22, 3315–3327. https://doi.org/10.1016/j.celrep.2018.02.093
Brew, H.M., Gittelman, J.X., Silverstein, R.S., Hanks, T.D., Demas, V.P., Robinson, L.C., Robbins, C.A., McKee-Johnson, J., Chiu, S.Y., Messing, A., Tempel, B.L., 2007. Seizures and reduced life span in mice lacking the potassium channel subunit Kv1.2, but hypoexcitability and enlarged Kv1 currents in auditory neurons. J. Neurophysiol. 98, 1501–1525. https://doi.org/10.1152/jn.00640.2006
Browne, D.L., Gancher, S.T., Nutt, J.G., Brunt, E.R.P., Smith, E.A., Kramer, P., Litt, M., 1994. Episodic ataxia/myokymia syndrome is associated with point mutations in the human potassium channel gene, KCNA1. Nat. Genet. 8, 136–140. https://doi.org/10.1038/ng1094-136
Brunetti, O., Imbrici, P., Botti, F.M., Pettorossi, V.E., D'Adamo, M.C., Valentino, M., Zammit, C., Mora, M., Gibertini, S., Di Giovanni, G., Muscat, R., Pessia, M., 2012. Kv1.1 knock-in ataxic mice exhibit spontaneous myokymic activity exacerbated by fatigue, ischemia and low temperature. Neurobiol. Dis. 47, 310–321. https://doi.org/10.1016/j.nbd.2012.05.002
Cachero, T.G., Morielli, A.D., Peralta, E.G., 1998. The Small GTP-Binding Protein RhoA Regulates a Delayed Rectifier Potassium Channel. Cell 93, 1077–1085. https://doi.org/10.1016/S0092-8674(00)81212-X
Cadwell, C.R., Sandberg, R., Jiang, X., Tolias, A.S., 2017. Q&A: using Patch-seq to profile single cells. BMC Biol. 15, 58. https://doi.org/10.1186/s12915-017-0396-0
Cahalan, M.D., Chandy, K.G., DeCoursey, T.E., Gupta, S., 1985. A voltage-gated potassium channel in

human T lymphocytes. J. Physiol. 358, 197–237.
Catterall, W.A., 2013. Ion Channel Protein Superfamily, in: Lennarz, W.J., Lane, M.D. (Eds.), Encyclopedia of Biological Chemistry (Second Edition). Academic Press, Waltham, pp. 648–652. https://doi.org/10.1016/B978-0-12-378630-2.00133-X
Catterall, W.A., 2010. Ion Channel Voltage Sensors: Structure, Function, and Pathophysiology. Neuron 67, 915–928. https://doi.org/10.1016/j.neuron.2010.08.021
Cha, A., Bezanilla, F., 1997. Characterizing voltage-dependent conformational changes in the Shaker K+ channel with fluorescence. Neuron 19, 1127–1140. https://doi.org/10.1016/s0896-6273(00)80403-1
Chanda, B., Kwame Asamoah, O., Blunck, R., Roux, B., Bezanilla, F., 2005. Gating charge displacement in voltage-gated ion channels involves limited transmembrane movement. Nature 436, 852–856. https://doi.org/10.1038/nature03888
Chen, L.Q., Santarelli, V., Horn, R., Kallen, R.G., 1996. A unique role for the S4 segment of domain 4 in the inactivation of sodium channels. J. Gen. Physiol. 108, 549–556. https://doi.org/10.1085/jgp.108.6.549
Chen, X., Wang, Q., Ni, F., Ma, J., 2010. Structure of the full-length Shaker potassium channel Kv1.2 by normal-mode-based X-ray crystallographic refinement. Proc. Natl. Acad. Sci. 107, 11352–11357. https://doi.org/10.1073/pnas.1000142107
Choi, K.L., Aldrich, R.W., Yellen, G., 1991. Tetraethylammonium blockade distinguishes two inactivation mechanisms in voltage-activated K+ channels. Proc. Natl. Acad. Sci. U. S. A. 88, 5092–5095. https://doi.org/10.1073/pnas.88.12.5092
Coetzee, W.A., Amarillo, Y., Chiu, J., Chow, A., Lau, D., McCORMACK, T., Morena, H., Nadal, M.S., Ozaita, A., Pountney, D., Saganich, M., De Miera, E.V.-S., Rudy, B., 1999. Molecular Diversity of K+ Channels. Ann. N. Y. Acad. Sci. 868, 233–255. https://doi.org/10.1111/j.1749-6632.1999.tb11293.x
Coleman, S.K., Newcombe, J., Pryke, J., Dolly, J.O., 1999. Subunit Composition of Kv1 Channels in Human CNS. J. Neurochem. 73, 849–858. https://doi.org/10.1046/j.1471-4159.1999.0730849.x
Cotella, D., Hernandez-Enriquez, B., Wu, X., Li, R., Pan, Z., Leveille, J., Link, C.D., Oddo, S., Sesti, F., 2012. Toxic role of K+ channel oxidation in mammalian brain. J. Neurosci. Off. J. Soc. Neurosci. 32, 4133–4144. https://doi.org/10.1523/JNEUROSCI.6153-11.2012
Covarrubias, M., Wei, A., Salkoff, L., 1991. Shaker, Shal, Shab, and Shaw express independent K+ current systems. Neuron 7, 763–773. https://doi.org/10.1016/0896-6273(91)90279-9
Cushman, S.J., Nanao, M.H., Jahng, A.W., DeRubeis, D., Choe, S., Pfaffinger, P.J., 2000. Voltage dependent activation of potassium channels is coupled to T1 domain structure. Nat. Struct. Biol. 7, 403–407. https://doi.org/10.1038/75185
D'Adamo, M.C., Liantonio, A., Rolland, J.-F., Pessia, M., Imbrici, P., 2020. Kv1.1 Channelopathies: Pathophysiological Mechanisms and Therapeutic Approaches. Int. J. Mol. Sci. 21, 2935. https://doi.org/10.3390/ijms21082935
Delint-Ramirez, I., Garcia-Oscos, F., Segev, A., Kourrich, S., 2020. Cocaine engages a non-canonical, dopamine-independent, mechanism that controls neuronal excitability in the nucleus accumbens. Mol. Psychiatry 25, 680–691. https://doi.org/10.1038/s41380-018-0092-7
Demos, M.K., Macri, V., Farrell, K., Nelson, T.N., Chapman, K., Accili, E., Armstrong, L., 2009. A novel KCNA1 mutation associated with global delay and persistent cerebellar dysfunction. Mov. Disord. Off. J. Mov. Disord. Soc. 24, 778–782. https://doi.org/10.1002/mds.22467
Deutsch, C., Chen, L.Q., 1993. Heterologous expression of specific K+ channels in T lymphocytes: functional consequences for volume regulation. Proc. Natl. Acad. Sci. U. S. A. 90, 10036–10040.
Devaux, J., Gola, M., Jacquet, G., Crest, M., 2002. Effects of K+ channel blockers on developing rat myelinated CNS axons: identification of four types of K+ channels. J. Neurophysiol. 87, 1376–1385. https://doi.org/10.1152/jn.00646.2001
Dodson, P.D., Barker, M.C., Forsythe, I.D., 2002. Two heteromeric Kv1 potassium channels differentially regulate action potential firing. J. Neurosci. Off. J. Soc. Neurosci. 22, 6953–6961.

https://doi.org/10.1523/JNEUROSCI.22-16-06953.2002
Dodson, P.D., Billups, B., Rusznák, Z., Szûcs, G., Barker, M.C., Forsythe, I.D., 2003. Presynaptic rat Kv1.2 channels suppress synaptic terminal hyperexcitability following action potential invasion. J. Physiol. 550, 27–33. https://doi.org/10.1113/jphysiol.2003.046250
Dodson, P.D., Forsythe, I.D., 2004. Presynaptic K+ channels: electrifying regulators of synaptic terminal excitability. Trends Neurosci. 27, 210–217. https://doi.org/10.1016/j.tins.2004.02.012
Doyle, D.A., Cabral, J.M., Pfuetzner, R.A., Kuo, A., Gulbis, J.M., Cohen, S.L., Chait, B.T., MacKinnon, R., 1998. The Structure of the Potassium Channel: Molecular Basis of K+ Conduction and Selectivity. Science 280, 69–77. https://doi.org/10.1126/science.280.5360.69
Du, X., Gamper, N., 2013. Potassium Channels in Peripheral Pain Pathways: Expression, Function and Therapeutic Potential. Curr. Neuropharmacol. 11, 621–640. https://doi.org/10.2174/1570159X113119990042
Duflocq, A., Chareyre, F., Giovannini, M., Couraud, F., Davenne, M., 2011. Characterization of the axon initial segment (AIS) of motor neurons and identification of a para-AIS and a juxtapara-AIS, organized by protein 4.1B. BMC Biol. 9, 66. https://doi.org/10.1186/1741-7007-9-66
Dunn, A.R., Kaczorowski, C.C., 2019. Regulation of intrinsic excitability: Roles for learning and memory, aging and Alzheimer's disease, and genetic diversity. Neurobiol. Learn. Mem. 164, 107069. https://doi.org/10.1016/j.nlm.2019.107069
Durell, S.R., Guy, H.R., 1992. Atomic scale structure and functional models of voltage-gated potassium channels. Biophys. J. 62, 238–247; discussion 247-250. https://doi.org/10.1016/S0006-3495(92)81809-X
El Ansari, R., Craze, M.L., Miligy, I., Diez-Rodriguez, M., Nolan, C.C., Ellis, I.O., Rakha, E.A., Green, A.R., 2018. The amino acid transporter SLC7A5 confers a poor prognosis in the highly proliferative breast cancer subtypes and is a key therapeutic target in luminal B tumours. Breast Cancer Res. 20, 21. https://doi.org/10.1186/s13058-018-0946-6
Eom, K., Lee, H.R., Hyun, J.H., Ryu, H.-H., Lee, Y.-S., Lee, I., Ho, W.-K., Lee, S.-H., 2020. Kv1.2 contributes to pattern separation by regulating the hippocampal CA3 neuronal ensemble size. https://doi.org/10.1101/2020.07.21.213538
Escande, D., Loisance, D., Planche, C., Coraboeuf, E., 1985. Age-related changes of action potential plateau shape in isolated human atrial fibers. Am. J. Physiol. 249, H843-850. https://doi.org/10.1152/ajpheart.1985.249.4.H843
Fedida, D., Wible, B., Wang, Z., Fermini, B., Faust, F., Nattel, S., Brown, A.M., 1993. Identity of a novel delayed rectifier current from human heart with a cloned K+ channel current. Circ. Res. 73, 210–216. https://doi.org/10.1161/01.RES.73.1.210
Feria Pliego, J.A., Pedroarena, C.M., 2020. Kv1 potassium channels control action potential firing of putative GABAergic deep cerebellar nuclear neurons. Sci. Rep. 10, 6954. https://doi.org/10.1038/s41598-020-63583-7
Finol-Urdaneta, R.K., Remedi, M.S., Raasch, W., Becker, S., Clark, R.B., Strüver, N., Pavlov, E., Nichols, C.G., French, R.J., Terlau, H., 2012. Block of Kv1.7 potassium currents increases glucose-stimulated insulin secretion. EMBO Mol. Med. 4, 424–434. https://doi.org/10.1002/emmm.201200218
Fischl, M.J., Burger, R.M., Schmidt-Pauly, M., Alexandrova, O., Sinclair, J.L., Grothe, B., Forsythe, I.D., Kopp-Scheinpflug, C., 2016. Physiology and anatomy of neurons in the medial superior olive of the mouse. J. Neurophysiol. 116, 2676–2688. https://doi.org/10.1152/jn.00523.2016
Foster, R.E., Connors, B.W., Waxman, S.G., 1982. Rat optic nerve: electrophysiological, pharmacological and anatomical studies during development. Brain Res. 255, 371–386. https://doi.org/10.1016/0165-3806(82)90005-0
Füllekrug, J., Scheiffele, P., Simons, K., 1999. VIP36 localisation to the early secretory pathway. J. Cell Sci. 112 (Pt 17), 2813–2821. https://doi.org/10.1242/jcs.112.17.2813
Gamper, N., Zaika, O., Li, Y., Martin, P., Hernandez, C.C., Perez, M.R., Wang, A.Y.C., Jaffe, D.B., Shapiro, M.S., 2006. Oxidative modification of M-type K+ channels as a mechanism of

cytoprotective neuronal silencing. EMBO J. 25, 4996–5004. https://doi.org/10.1038/sj.emboj.7601374
Gandhi, C.S., Isacoff, E.Y., 2002. Molecular Models of Voltage Sensing. J. Gen. Physiol. 120, 455–463. https://doi.org/10.1085/jgp.20028678
Gao, K., Lin, Z., Wen, S., Jiang, Y., 2022. Potassium channels and epilepsy. Acta Neurol. Scand. 146, 699–707. https://doi.org/10.1111/ane.13695
Gautier, M., Hyvelin, J.-M., de Crescenzo, V., Eder, V., Bonnet, P., 2007. Heterogeneous Kv1 function and expression in coronary myocytes from right and left ventricles in rats. Am. J. Physiol.-Heart Circ. Physiol. 292, H475–H482. https://doi.org/10.1152/ajpheart.00774.2005
Ghelardini, C., Galeotti, N., Bartolini, A., 1998. Influence of potassium channel modulators on cognitive processes in mice. Br. J. Pharmacol. 123, 1079–1084. https://doi.org/10.1038/sj.bjp.0701709
Gittelman, J.X., Tempel, B.L., 2006. Kv1.1-containing channels are critical for temporal precision during spike initiation. J. Neurophysiol. 96, 1203–1214. https://doi.org/10.1152/jn.00092.2005
Glasscock, E., Voigt, N., McCauley, M.D., Sun, Q., Li, N., Chiang, D.Y., Zhou, X.-B., Molina, C.E., Thomas, D., Schmidt, C., Skapura, D.G., Noebels, J.L., Dobrev, D., Wehrens, X.H.T., 2015. Expression and function of Kv1.1 potassium channels in human atria from patients with atrial fibrillation. Basic Res. Cardiol. 110, 505. https://doi.org/10.1007/s00395-015-0505-6
Glasscock, E., Yoo, J.W., Chen, T.T., Klassen, T.L., Noebels, J.L., 2010. Kv1.1 potassium channel deficiency reveals brain-driven cardiac dysfunction as a candidate mechanism for sudden unexplained death in epilepsy. J. Neurosci. Off. J. Soc. Neurosci. 30, 5167–5175. https://doi.org/10.1523/JNEUROSCI.5591-09.2010
Glazebrook, P.A., Ramirez, A.N., Schild, J.H., Shieh, C.-C., Doan, T., Wible, B.A., Kunze, D.L., 2002. Potassium channels Kv1.1, Kv1.2 and Kv1.6 influence excitability of rat visceral sensory neurons. J. Physiol. 541, 467–482. https://doi.org/10.1113/jphysiol.2001.018333
Goldberg, E.M., Clark, B.D., Zagha, E., Nahmani, M., Erisir, A., Rudy, B., 2008. K+ channels at the axon initial segment dampen near-threshold excitability of neocortical fast-spiking GABAergic interneurons. Neuron 58, 387–400. https://doi.org/10.1016/j.neuron.2008.03.003
Graves, T.D., Rajakulendran, S., Zuberi, S.M., Morris, H.R., Schorge, S., Hanna, M.G., Kullmann, D.M., 2010. Nongenetic factors influence severity of episodic ataxia type 1 in monozygotic twins(Video). Neurology 75, 367–372. https://doi.org/10.1212/WNL.0b013e3181ea9ee3
Grissmer, S., Nguyen, A.N., Aiyar, J., Hanson, D.C., Mather, R.J., Gutman, G.A., Karmilowicz, M.J., Auperin, D.D., Chandy, K.G., 1994. Pharmacological characterization of five cloned voltage-gated K+ channels, types Kv1.1, 1.2, 1.3, 1.5, and 3.1, stably expressed in mammalian cell lines. Mol. Pharmacol. 45, 1227–1234.
Grizel, A.V., Glukhov, G.S., Sokolova, O.S., 2014. Mechanisms of Activation of Voltage-Gated Potassium Channels. Acta Naturae 6, 10–26.
Grupe, A., Schröter, K.H., Ruppersberg, J.P., Stocker, M., Drewes, T., Beckh, S., Pongs, O., 1990. Cloning and expression of a human voltage-gated potassium channel. A novel member of the RCK potassium channel family. EMBO J. 9, 1749–1756.
Gu, C., Jan, Y.N., Jan, L.Y., 2003. A conserved domain in axonal targeting of Kv1 (Shaker) voltage-gated potassium channels. Science 301, 646–649. https://doi.org/10.1126/science.1086998
Guan, D., Lee, J.C.F., Higgs, M.H., Spain, W.J., Foehring, R.C., 2007. Functional roles of Kv1 channels in neocortical pyramidal neurons. J. Neurophysiol. 97, 1931–1940. https://doi.org/10.1152/jn.00933.2006
Guan, D., Pathak, D., Foehring, R.C., 2018. Functional roles of Kv1-mediated currents in genetically identified subtypes of pyramidal neurons in layer 5 of mouse somatosensory cortex. J. Neurophysiol. 120, 394–408. https://doi.org/10.1152/jn.00691.2017
Guéguinou, M., Chantôme, A., Fromont, G., Bougnoux, P., Vandier, C., Potier-Cartereau, M., 2014. KCa and Ca2+ channels: The complex thought. Biochim. Biophys. Acta BBA - Mol. Cell Res., Calcium Signaling in Health and Disease 1843, 2322–2333. https://doi.org/10.1016/j.bbamcr.2014.02.019

Gulbis, J.M., Mann, S., MacKinnon, R., 1999. Structure of a Voltage-Dependent K+ Channel β Subunit. Cell 97, 943–952. https://doi.org/10.1016/S0092-8674(00)80805-3
Gulbis, J.M., Zhou, M., Mann, S., MacKinnon, R., 2000. Structure of the Cytoplasmic β Subunit--T1 Assembly of Voltage-Dependent K+ Channels. Science 289, 123–127. https://doi.org/10.1126/science.289.5476.123
Gutman, G.A., Chandy, K.G., Grissmer, S., Lazdunski, M., McKinnon, D., Pardo, L.A., Robertson, G.A., Rudy, B., Sanguinetti, M.C., Stühmer, W., Wang, X., 2005. International Union of Pharmacology. LIII. Nomenclature and molecular relationships of voltage-gated potassium channels. Pharmacol. Rev. 57, 473–508. https://doi.org/10.1124/pr.57.4.10
Han, M., Zhang, J.Z.H., 2008. Molecular Dynamic Simulation of the Kv1.2 Voltage-Gated Potassium Channel in Open and Closed State Conformations. J. Phys. Chem. B 112, 16966–16974. https://doi.org/10.1021/jp807905p
Hattan, D., Nesti, E., Cachero, T.G., Morielli, A.D., 2002. Tyrosine phosphorylation of Kv1.2 modulates its interaction with the actin-binding protein cortactin. J. Biol. Chem. 277, 38596–38606. https://doi.org/10.1074/jbc.M205005200
Hayashi, T., Su, T.-P., 2007. Sigma-1 receptor chaperones at the ER-mitochondrion interface regulate Ca(2+) signaling and cell survival. Cell 131, 596–610. https://doi.org/10.1016/j.cell.2007.08.036
Heginbotham, L., Lu, Z., Abramson, T., MacKinnon, R., 1994. Mutations in the K+ channel signature sequence. Biophys. J. 66, 1061–1067. https://doi.org/10.1016/S0006-3495(94)80887-2
Heinemann, S.H., Rettig, J., Graack, H.R., Pongs, O., 1996. Functional characterization of Kv channel beta-subunits from rat brain. J. Physiol. 493, 625–633.
Heinemann, S.H., Rettig, J., Wunder, F., Pongs, O., 1995. Molecular and functional characterization of a rat brain Kv β3 potassium channel subunit. FEBS Lett. 377, 383–389. https://doi.org/10.1016/0014-5793(95)01377-6
Helbig, K.L., Hedrich, U.B.S., Shinde, D.N., Krey, I., Teichmann, A.-C., Hentschel, J., Schubert, J., Chamberlin, A.C., Huether, R., Lu, H.-M., Alcaraz, W.A., Tang, S., Jungbluth, C., Dugan, S.L., Vainionpää, L., Karle, K.N., Synofzik, M., Schöls, L., Schüle, R., Lehesjoki, A.-E., Helbig, I., Lerche, H., Lemke, J.R., 2016. A recurrent mutation in KCNA2 as a novel cause of hereditary spastic paraplegia and ataxia. Ann. Neurol. 80. https://doi.org/10.1002/ana.24762
Herson, P.S., Virk, M., Rustay, N.R., Bond, C.T., Crabbe, J.C., Adelman, J.P., Maylie, J., 2003. A mouse model of episodic ataxia type-1. Nat. Neurosci. 6, 378–383. https://doi.org/10.1038/nn1025
Hite, R.K., Butterwick, J.A., MacKinnon, R., 2014. Phosphatidic acid modulation of Kv channel voltage sensor function. eLife 3, e04366. https://doi.org/10.7554/eLife.04366
Hivert, B., Pinatel, D., Labasque, M., Tricaud, N., Goutebroze, L., Faivre-Sarrailh, C., 2016. Assembly of juxtaparanodes in myelinating DRG culture: Differential clustering of the Kv1/Caspr2 complex and scaffolding protein 4.1B. Glia 64, 840–852. https://doi.org/10.1002/glia.22968
Hodgkin, A.L., Huxley, A.F., 1952. A quantitative description of membrane current and its application to conduction and excitation in nerve. J. Physiol. 117, 500–544. https://doi.org/10.1113/jphysiol.1952.sp004764
Hoshi, T., Zagotta, W.N., Aldrich, R.W., 1991. Two types of inactivation in Shaker K+ channels: effects of alterations in the carboxy-terminal region. Neuron 7, 547–556. https://doi.org/10.1016/0896-6273(91)90367-9
Hoshi, T., Zagotta, W.N., Aldrich, R.W., 1990. Biophysical and molecular mechanisms of Shaker potassium channel inactivation. Science 250, 533–538. https://doi.org/10.1126/science.2122519
Huang, C.Y.-M., Rasband, M.N., 2018. Axon initial segments: structure, function, and disease. Ann. N. Y. Acad. Sci. 1420, 46–61. https://doi.org/10.1111/nyas.13718
Huang, J., Han, S., Sun, Q., Zhao, Y., Liu, J., Yuan, X., Mao, W., Peng, B., Liu, W., Yin, J., He, X., 2017. Kv1.3 channel blocker (ImKTx88) maintains blood-brain barrier in experimental autoimmune encephalomyelitis. Cell Biosci. 7, 31. https://doi.org/10.1186/s13578-017-0158-2
Huang, X.Y., Morielli, A.D., Peralta, E.G., 1994. Molecular basis of cardiac potassium channel stimulation by protein kinase A. Proc. Natl. Acad. Sci. U. S. A. 91, 624–628.

Huang, X.Y., Morielli, A.D., Peralta, E.G., 1993. Tyrosine kinase-dependent suppression of a potassium channel by the G protein-coupled m1 muscarinic acetylcholine receptor. Cell 75, 1145–1156. https://doi.org/10.1016/0092-8674(93)90324-j
Hurst, R.S., Toro, L., Stefani, E., 1996. Molecular determinants of external barium block in Shaker potassium channels. FEBS Lett. 388, 59–65. https://doi.org/10.1016/0014-5793(96)00516-9
Hyndman, D., Bauman, D.R., Heredia, V.V., Penning, T.M., 2003. The aldo-keto reductase superfamily homepage. Chem. Biol. Interact. 143–144, 621–631. https://doi.org/10.1016/s0009-2797(02)00193-x
Imbrici, P., D'Adamo, M.C., Cusimano, A., Pessia, M., 2007. Episodic ataxia type 1 mutation F184C alters Zn2+-induced modulation of the human K+ channel Kv1.4-Kv1.1/Kvβ1.1. Am. J. Physiol.-Cell Physiol. 292, C778–C787. https://doi.org/10.1152/ajpcell.00259.2006
Ishida, I.G., Rangel-Yescas, G.E., Carrasco-Zanini, J., Islas, L.D., 2015. Voltage-dependent gating and gating charge measurements in the Kv1.2 potassium channel. J. Gen. Physiol. 145, 345–358. https://doi.org/10.1085/jgp.201411300
Jan, L.Y., Jan, Y.N., 2012. Voltage-gated potassium channels and the diversity of electrical signalling. J. Physiol. 590, 2591–2599. https://doi.org/10.1113/jphysiol.2011.224212
Jeevaratnam, K., Chadda, K.R., Huang, C.L.-H., Camm, A.J., 2018. Cardiac Potassium Channels: Physiological Insights for Targeted Therapy. J. Cardiovasc. Pharmacol. Ther. 23, 119–129. https://doi.org/10.1177/1074248417729880
Jen, J.C., Graves, T.D., Hess, E.J., Hanna, M.G., Griggs, R.C., Baloh, R.W., CINCH investigators, 2007. Primary episodic ataxias: diagnosis, pathogenesis and treatment. Brain J. Neurol. 130, 2484–2493. https://doi.org/10.1093/brain/awm126
Jiang, Y., Lee, A., Chen, J., Ruta, V., Cadene, M., Chait, B.T., MacKinnon, R., 2003a. X-ray structure of a voltage-dependent K+ channel. Nature 423, 33–41. https://doi.org/10.1038/nature01580
Jiang, Y., Ruta, V., Chen, J., Lee, A., MacKinnon, R., 2003b. The principle of gating charge movement in a voltage-dependent K+ channel. Nature 423, 42–48. https://doi.org/10.1038/nature01581
Jogini, V., Roux, B., 2007. Dynamics of the Kv1.2 Voltage-Gated K+ Channel in a Membrane Environment. Biophys. J. 93, 3070–3082. https://doi.org/10.1529/biophysj.107.112540
Kalman, K., Nguyen, A., Tseng-Crank, J., Dukes, I.D., Chandy, G., Hustad, C.M., Copeland, N.G., Jenkins, N.A., Mohrenweiser, H., Brandriff, B., Cahalan, M., Gutman, G.A., Chandy, K.G., 1998. Genomic Organization, Chromosomal Localization, Tissue Distribution, and Biophysical Characterization of a Novel MammalianShaker-related Voltage-gated Potassium Channel, Kv1.7 *. J. Biol. Chem. 273, 5851–5857. https://doi.org/10.1074/jbc.273.10.5851
Kamiya, Y., Kamiya, D., Yamamoto, K., Nyfeler, B., Hauri, H.-P., Kato, K., 2008. Molecular Basis of Sugar Recognition by the Human L-type Lectins ERGIC-53, VIPL, and VIP36*. J. Biol. Chem. 283, 1857–1861. https://doi.org/10.1074/jbc.M709384200
Kamiya, Y., Yamaguchi, Y., Takahashi, N., Arata, Y., Kasai, K.-I., Ihara, Y., Matsuo, I., Ito, Y., Yamamoto, K., Kato, K., 2005. Sugar-binding properties of VIP36, an intracellular animal lectin operating as a cargo receptor. J. Biol. Chem. 280, 37178–37182. https://doi.org/10.1074/jbc.M505757200
Kanai, Y., Segawa, H., Miyamoto, K., Uchino, H., Takeda, E., Endou, H., 1998. Expression Cloning and Characterization of a Transporter for Large Neutral Amino Acids Activated by the Heavy Chain of 4F2 Antigen (CD98) *. J. Biol. Chem. 273, 23629–23632. https://doi.org/10.1074/jbc.273.37.23629
Kashuba, V.I., Kvasha, S.M., Protopopov, A.I., Gizatullin, R.Z., Rynditch, A.V., Wahlestedt, C., Wasserman, W.W., Zabarovsky, E.R., 2001. Initial isolation and analysis of the human Kv1.7 (KCNA7) gene, a member of the voltage-gated potassium channel gene family. Gene 268, 115–122. https://doi.org/10.1016/s0378-1119(01)00423-1
Kerr, P.M., Clément-Chomienne, O., Thorneloe, K.S., Chen, T.T., Ishii, K., Sontag, D.P., Walsh, M.P., Cole, W.C., 2001. Heteromultimeric Kv1.2-Kv1.5 Channels Underlie 4-Aminopyridine-Sensitive Delayed Rectifier K+ Current of Rabbit Vascular Myocytes. Circ. Res. 89, 1038–1044.

https://doi.org/10.1161/hh2301.100803
Keynes, R.D., Elinder, F., 1999. The screw-helical voltage gating of ion channels. Proc. Biol. Sci. 266, 843–852. https://doi.org/10.1098/rspb.1999.0714
Kim, D.M., Nimigean, C.M., 2016. Voltage-Gated Potassium Channels: A Structural Examination of Selectivity and Gating. Cold Spring Harb. Perspect. Biol. 8, a029231. https://doi.org/10.1101/cshperspect.a029231
Kim, S.-J., Choi, W.S., Han, J.S.M., Warnock, G., Fedida, D., McIntosh, C.H.S., 2005. A novel mechanism for the suppression of a voltage-gated potassium channel by glucose-dependent insulinotropic polypeptide: protein kinase A-dependent endocytosis. J. Biol. Chem. 280, 28692–28700. https://doi.org/10.1074/jbc.M504913200
Kobertz, W.R., Williams, C., Miller, C., 2000. Hanging gondola structure of the T1 domain in a voltage-gated K(+) channel. Biochemistry 39, 10347–10352. https://doi.org/10.1021/bi001292j
Kocsis, J.D., Waxman, S.G., 1980. Absence of potassium conductance in central myelinated axons. Nature 287, 348–349. https://doi.org/10.1038/287348a0
Kole, M.H.P., Letzkus, J.J., Stuart, G.J., 2007. Axon initial segment Kv1 channels control axonal action potential waveform and synaptic efficacy. Neuron 55, 633–647. https://doi.org/10.1016/j.neuron.2007.07.031
Kontis, K.J., Goldin, A.L., 1997. Sodium channel inactivation is altered by substitution of voltage sensor positive charges. J. Gen. Physiol. 110, 403–413. https://doi.org/10.1085/jgp.110.4.403
Kopp-Scheinpflug, C., Fuchs, K., Lippe, W.R., Tempel, B.L., Rübsamen, R., 2003. Decreased temporal precision of auditory signaling in Kcna1-null mice: an electrophysiological study in vivo. J. Neurosci. Off. J. Soc. Neurosci. 23, 9199–9207. https://doi.org/10.1523/JNEUROSCI.23-27-09199.2003
Koschak, A., Koch, R.O., Liu, J., Kaczorowski, G.J., Reinhart, P.H., Garcia, M.L., Knaus, H.G., 1997. [125I]Iberiotoxin-D19Y/Y36F, the first selective, high specific activity radioligand for high-conductance calcium-activated potassium channels. Biochemistry 36, 1943–1952. https://doi.org/10.1021/bi962074m
Kourrich, S., Hayashi, T., Chuang, J.-Y., Tsai, S.-Y., Su, T.-P., Bonci, A., 2013. Dynamic interaction between sigma-1 receptor and Kv1.2 shapes neuronal and behavioral responses to cocaine. Cell 152, 236–247. https://doi.org/10.1016/j.cell.2012.12.004
Kourrich, S., Klug, J.R., Mayford, M., Thomas, M.J., 2012a. AMPAR-Independent Effect of Striatal αCaMKII Promotes the Sensitization of Cocaine Reward. J. Neurosci. 32, 6578–6586. https://doi.org/10.1523/JNEUROSCI.6391-11.2012
Kourrich, S., Su, T.-P., Fujimoto, M., Bonci, A., 2012b. The sigma-1 receptor: roles in neuronal plasticity and disease. Trends Neurosci. 35, 762–771. https://doi.org/10.1016/j.tins.2012.09.007
Kozai, D., Ogawa, N., Mori, Y., 2014. Redox Regulation of Transient Receptor Potential Channels. Antioxid. Redox Signal. 21, 971–986. https://doi.org/10.1089/ars.2013.5616
Krames, E.S., 2014. The Role of the Dorsal Root Ganglion in the Development of Neuropathic Pain. Pain Med. 15, 1669–1685. https://doi.org/10.1111/pme.12413
Kruse, M., Hammond, G.R.V., Hille, B., 2012. Regulation of voltage-gated potassium channels by PI(4,5)P2. J. Gen. Physiol. 140, 189–205. https://doi.org/10.1085/jgp.201210806
Kruse, M., Hille, B., 2013. The phosphoinositide sensitivity of the KV channel family. Channels 7, 530–536. https://doi.org/10.4161/chan.25816
Kuang, Q., Purhonen, P., Hebert, H., 2015. Structure of potassium channels. Cell. Mol. Life Sci. CMLS 72, 3677–3693. https://doi.org/10.1007/s00018-015-1948-5
Kuo, M.M.-C., Haynes, W.J., Loukin, S.H., Kung, C., Saimi, Y., 2005. Prokaryotic K(+) channels: from crystal structures to diversity. FEMS Microbiol. Rev. 29, 961–985. https://doi.org/10.1016/j.femsre.2005.03.003
Kurata, H.T., Fedida, D., 2006. A structural interpretation of voltage-gated potassium channel inactivation. Prog. Biophys. Mol. Biol. 92, 185–208. https://doi.org/10.1016/j.pbiomolbio.2005.10.001

Kwon, S.-H., Oh, S., Nacke, M., Mostov, K.E., Lipschutz, J.H., 2016. Adaptor Protein CD2AP and L-type Lectin LMAN2 Regulate Exosome Cargo Protein Trafficking through the Golgi Complex. J. Biol. Chem. 291, 25462–25475. https://doi.org/10.1074/jbc.M116.729202
Labro, A., Snyders, D., 2012. Being Flexible: The Voltage-Controllable Activation Gate of Kv Channels. Front. Pharmacol. 3.
Labro, A.J., Raes, A.L., Bellens, I., Ottschytsch, N., Snyders, D.J., 2003. Gating of Shaker-type Channels Requires the Flexibility of S6 Caused by Prolines *. J. Biol. Chem. 278, 50724–50731. https://doi.org/10.1074/jbc.M306097200
Lamothe, S.M., Kurata, H.T., 2023. Regulation of Kv1.2 redox-sensitive gating by the transmembrane lectin LMAN2. Manuscr. Prep.
Lamothe, S.M., Kurata, H.T., 2020. Slc7a5 alters Kvβ-mediated regulation of Kv1.2. J. Gen. Physiol. 152, e201912524. https://doi.org/10.1085/jgp.201912524
Lancaster, Eunjoo, Burnor, E., Zhang, J., Lancaster, Eric, 2019. ADAM23 is a negative regulator of Kv1.1/Kv1.4 potassium currents. Neurosci. Lett. 704, 159–163. https://doi.org/10.1016/j.neulet.2019.04.012
Lang, R., Lee, G., Liu, W., Tian, S., Rafi, H., Orias, M., Segal, A.S., Desir, G.V., 2000. KCNA10: a novel ion channel functionally related to both voltage-gated potassium and CNG cation channels. Am. J. Physiol.-Ren. Physiol. 278, F1013–F1021. https://doi.org/10.1152/ajprenal.2000.278.6.F1013
Lee, J.-K., Nishiyama, A., Kambe, F., Seo, H., Takeuchi, S., Kamiya, K., Kodama, I., Toyama, J., 1999. Downregulation of voltage-gated K+ channels in rat heart with right ventricular hypertrophy. Am. J. Physiol.-Heart Circ. Physiol. 277, H1725–H1731. https://doi.org/10.1152/ajpheart.1999.277.5.H1725
Lee, S.I., Conrad, T., Jones, S.M., Lagziel, A., Starost, M.F., Belyantseva, I.A., Friedman, T.B., Morell, R.J., 2013. A null mutation of mouse Kcna10 causes significant vestibular and mild hearing dysfunction. Hear. Res. 300, 1–9. https://doi.org/10.1016/j.heares.2013.02.009
Lee, T.E., Philipson, L.H., Kuznetsov, A., Nelson, D.J., 1994. Structural determinant for assembly of mammalian K+ channels. Biophys. J. 66, 667–673.
Lee, T.E., Philipson, L.H., Nelson, D.J., 1996. N-type Inactivation in the Mammalian Shaker K+ Channel Kv1.4. J. Membr. Biol. 151, 225–235. https://doi.org/10.1007/s002329900073
Li, M., Jan, Y.N., Jan, L.Y., 1992. Specification of subunit assembly by the hydrophilic amino-terminal domain of the Shaker potassium channel. Science 257, 1225–1230. https://doi.org/10.1126/science.1519059
Liang, L., Gu, X., Zhao, J.-Y., Wu, S., Miao, X., Xiao, J., Mo, K., Zhang, J., Lutz, B.M., Bekker, A., Tao, Y.-X., 2016. G9a participates in nerve injury-induced Kcna2 downregulation in primary sensory neurons. Sci. Rep. 6, 37704. https://doi.org/10.1038/srep37704
Lim, C.H., Zain, S.M., Reynolds, G.P., Zain, M.A., Roffeei, S.N., Zainal, N.Z., Kanagasundram, S., Mohamed, Z., 2014. Genetic association of LMAN2L gene in schizophrenia and bipolar disorder and its interaction with ANK3 gene polymorphism. Prog. Neuropsychopharmacol. Biol. Psychiatry 54, 157–162. https://doi.org/10.1016/j.pnpbp.2014.05.017
Lin, S., Ke, M., Zhang, Y., Yan, Z., Wu, J., 2021. Structure of a mammalian sperm cation channel complex. Nature 595, 746–750. https://doi.org/10.1038/s41586-021-03742-6
Long, S.B., Campbell, E.B., Mackinnon, R., 2005. Crystal structure of a mammalian voltage-dependent Shaker family K+ channel. Science 309, 897–903. https://doi.org/10.1126/science.1116269
Long, S.B., Tao, X., Campbell, E.B., MacKinnon, R., 2007. Atomic structure of a voltage-dependent K+ channel in a lipid membrane-like environment. Nature 450, 376–382. https://doi.org/10.1038/nature06265
Loots, E., Isacoff, E.Y., 1998. Protein Rearrangements Underlying Slow Inactivation of the Shaker K+ Channel. J. Gen. Physiol. 112, 377–389. https://doi.org/10.1085/jgp.112.4.377
López-Barneo, J., Hoshi, T., Heinemann, S.H., Aldrich, R.W., 1993. Effects of external cations and mutations in the pore region on C-type inactivation of Shaker potassium channels. Receptors

Channels 1, 61–71.
Lorincz, A., Nusser, Z., 2008. Cell-Type-Dependent Molecular Composition of the Axon Initial Segment. J. Neurosci. 28, 14329–14340. https://doi.org/10.1523/JNEUROSCI.4833-08.2008
Ma, Z., Lavebratt, C., Almgren, M., Portwood, N., Forsberg, L.E., Bränström, R., Berglund, E., Falkmer, S., Sundler, F., Wierup, N., Björklund, A., 2011. Evidence for Presence and Functional Effects of Kv1.1 Channels in β-Cells: General Survey and Results from mceph/mceph Mice. PLOS ONE 6, e18213. https://doi.org/10.1371/journal.pone.0018213
MacDonald, P.E., Ha, X.F., Wang, J., Smukler, S.R., Sun, A.M., Gaisano, H.Y., Salapatek, A.M., Backx, P.H., Wheeler, M.B., 2001. Members of the Kv1 and Kv2 voltage-dependent K(+) channel families regulate insulin secretion. Mol. Endocrinol. Baltim. Md 15, 1423–1435. https://doi.org/10.1210/mend.15.8.0685
MacDonald, P.E., Sewing, S., Wang, Jianli, Joseph, J.W., Smukler, S.R., Sakellaropoulos, G., Wang, Jing, Saleh, M.C., Chan, C.B., Tsushima, R.G., Salapatek, A.M.F., Wheeler, M.B., 2002. Inhibition of Kv2.1 voltage-dependent K+ channels in pancreatic beta-cells enhances glucose-dependent insulin secretion. J. Biol. Chem. 277, 44938–44945. https://doi.org/10.1074/jbc.M205532200
Mallory, C.S., Giocomo, L.M., 2018. Heterogeneity in hippocampal place coding. Curr. Opin. Neurobiol. 49, 158–167. https://doi.org/10.1016/j.conb.2018.02.014
Mandal, K., 2020. Review of PIP2 in Cellular Signaling, Functions and Diseases. Int. J. Mol. Sci. 21, 8342. https://doi.org/10.3390/ijms21218342
Mannuzzu, L.M., Moronne, M.M., Isacoff, E.Y., 1996. Direct physical measure of conformational rearrangement underlying potassium channel gating. Science 271, 213–216. https://doi.org/10.1126/science.271.5246.213
Masnada, S., Hedrich, U.B.S., Gardella, E., Schubert, J., Kaiwar, C., Klee, E.W., Lanpher, B.C., Gavrilova, R.H., Synofzik, M., Bast, T., Gorman, K., King, M.D., Allen, N.M., Conroy, J., Ben Zeev, B., Tzadok, M., Korff, C., Dubois, F., Ramsey, K., Narayanan, V., Serratosa, J.M., Giraldez, B.G., Helbig, I., Marsh, E., O'Brien, M., Bergqvist, C.A., Binelli, A., Porter, B., Zaeyen, E., Horovitz, D.D., Wolff, M., Marjanovic, D., Caglayan, H.S., Arslan, M., Pena, S.D.J., Sisodiya, S.M., Balestrini, S., Syrbe, S., Veggiotti, P., Lemke, J.R., Møller, R.S., Lerche, H., Rubboli, G., 2017. Clinical spectrum and genotype–phenotype associations of KCNA2-related encephalopathies. Brain 140, 2337–2354. https://doi.org/10.1093/brain/awx184
McCormack, T., McCormack, K., 1994. Shaker K+ channel β subunits belong to an NAD(P)H-dependent oxidoreductase superfamily. Cell 79, 1133–1135. https://doi.org/10.1016/0092-8674(94)90004-3
Minor, D.L., Lin, Y.-F., Mobley, B.C., Avelar, A., Jan, Y.N., Jan, L.Y., Berger, J.M., 2000. The Polar T1 Interface Is Linked to Conformational Changes that Open the Voltage-Gated Potassium Channel. Cell 102, 657–670. https://doi.org/10.1016/S0092-8674(00)00088-X
Monaghan, M.M., Trimmer, J.S., Rhodes, K.J., 2001. Experimental localization of Kv1 family voltage-gated K+ channel alpha and beta subunits in rat hippocampal formation. J. Neurosci. Off. J. Soc. Neurosci. 21, 5973–5983. https://doi.org/10.1523/JNEUROSCI.21-16-05973.2001
Moore, J.K., 2000. Organization of the human superior olivary complex. Microsc. Res. Tech. 51, 403–412. https://doi.org/10.1002/1097-0029(20001115)51:4<403::AID-JEMT8>3.0.CO;2-Q
Morales, M.J., Castellino, R.C., Crews, A.L., Rasmusson, R.L., Strauss, H.C., 1995. A novel beta subunit increases rate of inactivation of specific voltage-gated potassium channel alpha subunits. J. Biol. Chem. 270, 6272–6277. https://doi.org/10.1074/jbc.270.11.6272
Moriarty-Craige, S.E., Jones, D.P., 2004. Extracellular thiols and thiol/disulfide redox in metabolism. Annu. Rev. Nutr. 24, 481–509. https://doi.org/10.1146/annurev.nutr.24.012003.132208
Nascimento, A.I., Mar, F.M., Sousa, M.M., 2018. The intriguing nature of dorsal root ganglion neurons: Linking structure with polarity and function. Prog. Neurobiol. 168, 86–103. https://doi.org/10.1016/j.pneurobio.2018.05.002
Nattel, S., Yue, L., Wang, Z., 1999. Cardiac ultrarapid delayed rectifiers: a novel potassium current family o f functional similarity and molecular diversity. Cell. Physiol. Biochem. Int. J. Exp. Cell.

Physiol. Biochem. Pharmacol. 9, 217–226. https://doi.org/10.1159/000016318
Nawreen, N., Baccei, M.L., Herman, J.P., 2021. Single Prolonged Stress Reduces Intrinsic Excitability and Excitatory Synaptic Drive Onto Pyramidal Neurons in the Infralimbic Prefrontal Cortex of Adult Male Rats. Front. Cell. Neurosci. 15.
Nesti, E., Everill, B., Morielli, A.D., 2004. Endocytosis as a Mechanism for Tyrosine Kinase-dependent Suppression of a Voltage-gated Potassium Channel. Mol. Biol. Cell 15, 4073–4088. https://doi.org/10.1091/mbc.E03-11-0788
Nicola, S.M., Taha, S.A., Kim, S.W., Fields, H.L., 2005. Nucleus accumbens dopamine release is necessary and sufficient to promote the behavioral response to reward-predictive cues. Neuroscience 135, 1025–1033. https://doi.org/10.1016/j.neuroscience.2005.06.088
Nielsen, N.H., Winkel, B.G., Kanters, J.K., Schmitt, N., Hofman-Bang, J., Jensen, H.S., Bentzen, B.H., Sigurd, B., Larsen, L.A., Andersen, P.S., Haunsø, S., Kjeldsen, K., Grunnet, M., Christiansen, M., Olesen, S.-P., 2007. Mutations in the Kv1.5 channel gene KCNA5 in cardiac arrest patients. Biochem. Biophys. Res. Commun. 354, 776–782. https://doi.org/10.1016/j.bbrc.2007.01.048
Nilsson, M., Lindström, S.H., Kaneko, M., Wang, K., Minguez-Viñas, T., Angelini, M., Steccanella, F., Holder, D., Ottolia, M., Olcese, R., Pantazis, A., 2022. An epilepsy-associated KV1.2 charge-transfer-center mutation impairs KV1.2 and KV1.4 trafficking. Proc. Natl. Acad. Sci. 119, e2113675119. https://doi.org/10.1073/pnas.2113675119
Nitabach, M.N., Llamas, D.A., Araneda, R.C., Intile, J.L., Thompson, I.J., Zhou, Y.I., Holmes, T.C., 2001. A mechanism for combinatorial regulation of electrical activity: Potassium channel subunits capable of functioning as Src homology 3-dependent adaptors. Proc. Natl. Acad. Sci. 98, 705–710. https://doi.org/10.1073/pnas.98.2.705
Niwa, N., Nerbonne, J.M., 2010. Molecular Determinants of Cardiac Transient Outward Potassium Current (Ito) Expression and Regulation. J. Mol. Cell. Cardiol. 48, 12. https://doi.org/10.1016/j.yjmcc.2009.07.013
Noda, M., Shimizu, S., Tanabe, T., Takai, T., Kayano, T., Ikeda, T., Takahashi, H., Nakayama, H., Kanaoka, Y., Minamino, N., 1984. Primary structure of Electrophorus electricus sodium channel deduced from cDNA sequence. Nature 312, 121–127. https://doi.org/10.1038/312121a0
Ogawa, Y., Horresh, I., Trimmer, J.S., Bredt, D.S., Peles, E., Rasband, M.N., 2008. Postsynaptic density-93 clusters Kv1 channels at axon initial segments independently of Caspr2. J. Neurosci. Off. J. Soc. Neurosci. 28, 5731–5739. https://doi.org/10.1523/JNEUROSCI.4431-07.2008
Oh, M.M., Disterhoft, J.F., 2020. Learning and Aging Affect Neuronal Excitability and Learning. Neurobiol. Learn. Mem. 167, 107133. https://doi.org/10.1016/j.nlm.2019.107133
Olcese, R., Latorre, R., Toro, L., Bezanilla, F., Stefani, E., 1997. Correlation between Charge Movement and Ionic Current during Slow Inactivation in Shaker K+ Channels. J. Gen. Physiol. 110, 579–589. https://doi.org/10.1085/jgp.110.5.579
Olson, T.M., Alekseev, A.E., Liu, X.K., Park, S., Zingman, L.V., Bienengraeber, M., Sattiraju, S., Ballew, J.D., Jahangir, A., Terzic, A., 2006. Kv1.5 channelopathy due to KCNA5 loss-of-function mutation causes human atrial fibrillation. Hum. Mol. Genet. 15, 2185–2191. https://doi.org/10.1093/hmg/ddl143
Pal, S., Takimoto, K., Aizenman, E., Levitan, E., 2006. Apoptotic surface delivery of K+ channels. Cell Death Differ. 13, 661–667. https://doi.org/10.1038/sj.cdd.4401792
Palani, D., Baginskas, A., Raastad, M., 2010. Bursts and hyperexcitability in non-myelinated axons of the rat hippocampus. Neuroscience 167, 1004–1013. https://doi.org/10.1016/j.neuroscience.2010.03.021
Pan, Y., Weng, J., Levin, E.J., Zhou, M., 2011. Oxidation of NADPH on Kvbeta1 inhibits ball-and-chain type inactivation by restraining the chain. Proc. Natl. Acad. Sci. U. S. A. 108, 5885–5890. https://doi.org/10.1073/pnas.1100316108
Park, K.-S., Yang, J.-W., Seikel, E., Trimmer, J.S., 2008. Potassium Channel Phosphorylation in Excitable Cells: Providing Dynamic Functional Variability to a Diverse Family of Ion Channels. Physiology 23, 49–57. https://doi.org/10.1152/physiol.00031.2007

Pena, S. d. j., Coimbra, R. l. m., 2015. Ataxia and myoclonic epilepsy due to a heterozygous new mutation in KCNA2: proposal for a new channelopathy. Clin. Genet. 87, e1–e3. https://doi.org/10.1111/cge.12542
Pineda, J.C., Galarraga, E., Foehring, R.C., 1999. Different Ca2+ source for slow AHP in completely adapting and repetitive firing pyramidal neurons. Neuroreport 10, 1951–1956. https://doi.org/10.1097/00001756-199906230-00029
Plane, F., Johnson, R., Kerr, P., Wiehler, W., Thorneloe, K., Ishii, K., Chen, T., Cole, W., 2005. Heteromultimeric Kv1 Channels Contribute to Myogenic Control of Arterial Diameter. Circ. Res. 96, 216–224. https://doi.org/10.1161/01.RES.0000154070.06421.25
Pless, S.A., Galpin, J.D., Niciforovic, A.P., Kurata, H.T., Ahern, C.A., 2013. Hydrogen bonds as molecular timers for slow inactivation in voltage-gated potassium channels. eLife 2, e01289. https://doi.org/10.7554/eLife.01289
Po, S., Roberds, S., Snyders, D.J., Tamkun, M.M., Bennett, P.B., 1993. Heteromultimeric assembly of human potassium channels. Molecular basis of a transient outward current? Circ. Res. 72, 1326–1336. https://doi.org/10.1161/01.res.72.6.1326
Poliak, S., Salomon, D., Elhanany, H., Sabanay, H., Kiernan, B., Pevny, L., Stewart, C.L., Xu, X., Chiu, S.-Y., Shrager, P., Furley, A.J.W., Peles, E., 2003. Juxtaparanodal clustering of Shaker-like K+ channels in myelinated axons depends on Caspr2 and TAG-1. J. Cell Biol. 162, 1149–1160. https://doi.org/10.1083/jcb.200305018
Poncet, N., Halley, P.A., Lipina, C., Gierliński, M., Dady, A., Singer, G.A., Febrer, M., Shi, Y.-B., Yamaguchi, T.P., Taylor, P.M., Storey, K.G., 2020. Wnt regulates amino acid transporter Slc7a5 and so constrains the integrated stress response in mouse embryos. EMBO Rep. 21, e48469. https://doi.org/10.15252/embr.201948469
Poncet, N., Mitchell, F.E., Ibrahim, A.F.M., McGuire, V.A., English, G., Arthur, J.S.C., Shi, Y.-B., Taylor, P.M., 2014. The catalytic subunit of the system L1 amino acid transporter (slc7a5) facilitates nutrient signalling in mouse skeletal muscle. PloS One 9, e89547. https://doi.org/10.1371/journal.pone.0089547
Pongs, O., Schwarz, J.R., 2010. Ancillary Subunits Associated With Voltage-Dependent K+ Channels. Physiol. Rev. 90, 755–796. https://doi.org/10.1152/physrev.00020.2009
Pozeg, Z.I., Michelakis, E.D., McMurtry, M.S., Thébaud, B., Wu, X.-C., Dyck, J.R.B., Hashimoto, K., Wang, S., Moudgil, R., Harry, G., Sultanian, R., Koshal, A., Archer, S.L., 2003. In vivo gene transfer of the O2-sensitive potassium channel Kv1.5 reduces pulmonary hypertension and restores hypoxic pulmonary vasoconstriction in chronically hypoxic rats. Circulation 107, 2037–2044. https://doi.org/10.1161/01.CIR.0000062688.76508.B3
Rafiullah, R., Aslamkhan, M., Paramasivam, N., Thiel, C., Mustafa, G., Wiemann, S., Schlesner, M., Wade, R.C., Rappold, G.A., Berkel, S., 2016. Homozygous missense mutation in the LMAN2L gene segregates with intellectual disability in a large consanguineous Pakistani family. J. Med. Genet. 53, 138–144. https://doi.org/10.1136/jmedgenet-2015-103179
Rama, S., Zbili, M., Fékété, A., Tapia, M., Benitez, M.J., Boumedine, N., Garrido, J.J., Debanne, D., 2017. The role of axonal Kv1 channels in CA3 pyramidal cell excitability. Sci. Rep. 7, 315. https://doi.org/10.1038/s41598-017-00388-1
Ranjan, R., Logette, E., Marani, M., Herzog, M., Tâche, V., Scantamburlo, E., Buchillier, V., Markram, H., 2019. A Kinetic Map of the Homomeric Voltage-Gated Potassium Channel (Kv) Family. Front. Cell. Neurosci. 13.
Rasband, M.N., 2010. The axon initial segment and the maintenance of neuronal polarity. Nat. Rev. Neurosci. 11, 552–562. https://doi.org/10.1038/nrn2852
Rasband, M.N., Park, E.W., Vanderah, T.W., Lai, J., Porreca, F., Trimmer, J.S., 2001. Distinct potassium channels on pain-sensing neurons. Proc. Natl. Acad. Sci. U. S. A. 98, 13373–13378. https://doi.org/10.1073/pnas.231376298
Rasband, M.N., Trimmer, J.S., Peles, E., Levinson, S.R., Shrager, P., 1999. K+ channel distribution and clustering in developing and hypomyelinated axons of the optic nerve. J. Neurocytol. 28, 319–

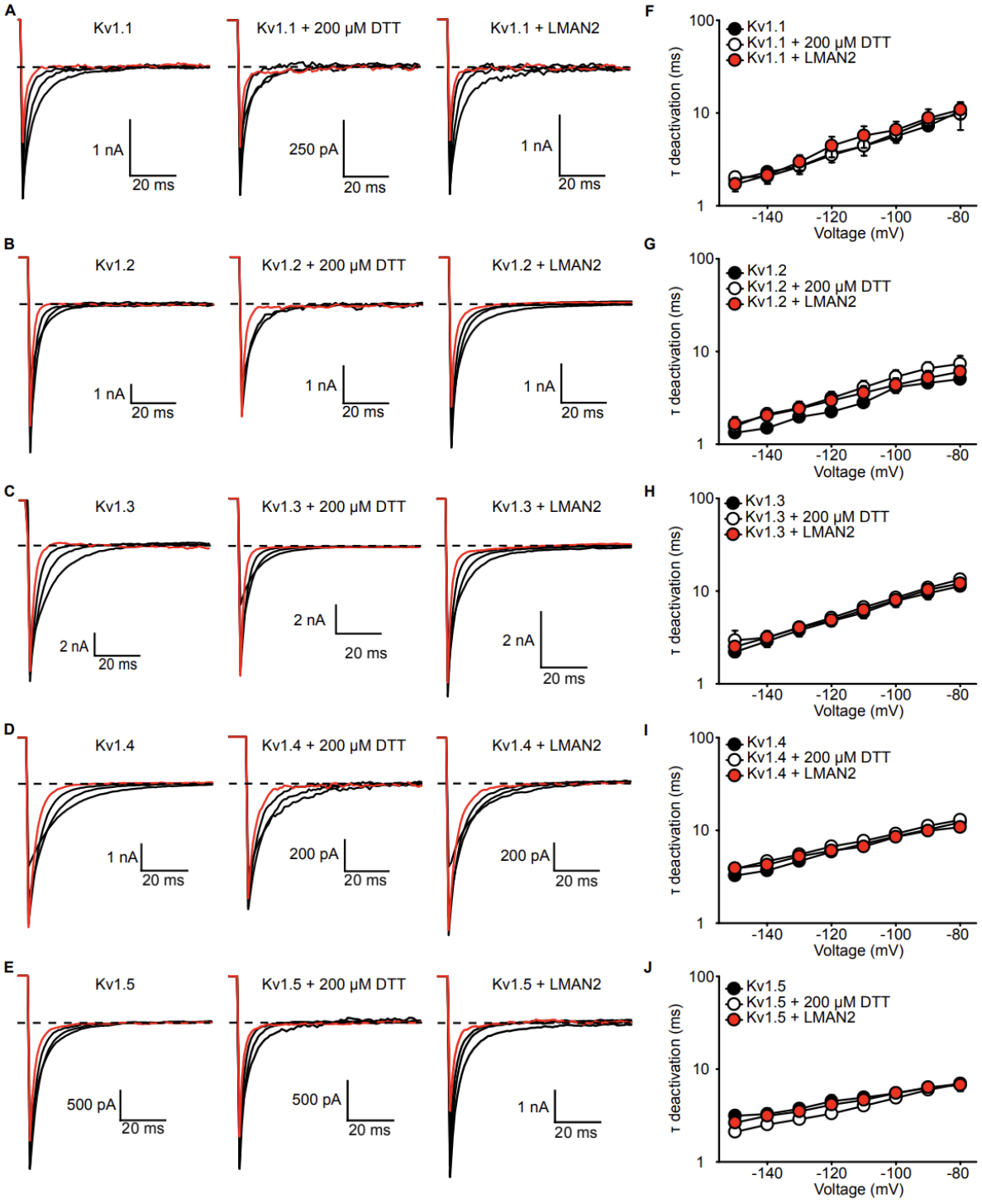
331. https://doi.org/10.1023/a:1007057512576
Rasband, M.N., Trimmer, J.S., Schwarz, T.L., Levinson, S.R., Ellisman, M.H., Schachner, M., Shrager, P., 1998. Potassium channel distribution, clustering, and function in remyelinating rat axons. J. Neurosci. Off. J. Soc. Neurosci. 18, 36–47. https://doi.org/10.1523/JNEUROSCI.18-01-00036.1998
Rasmusson, R.L., Morales, M.J., Wang, S., Liu, S., Campbell, D.L., Brahmajothi, M.V., Strauss, H.C., 1998. Inactivation of voltage-gated cardiac K+ channels. Circ. Res. 82, 739–750. https://doi.org/10.1161/01.res.82.7.739
Reddi, R., Matulef, K., Riederer, E.A., Whorton, M.R., Valiyaveetil, F.I., 2022. Structural basis for C-type inactivation in a Shaker family voltage-gated K+ channel. Sci. Adv. 8, eabm8804. https://doi.org/10.1126/sciadv.abm8804
Reiterer, V., Nyfeler, B., Hauri, H.-P., 2010. Role of the lectin VIP36 in post-ER quality control of human alpha1-antitrypsin. Traffic Cph. Den. 11, 1044–1055. https://doi.org/10.1111/j.1600-0854.2010.01078.x
Rettig, J., Heinemann, S.H., Wunder, F., Lorra, C., Parcej, D.N., Oliver Dolly, J., Pongs, O., 1994. Inactivation properties of voltage-gated K+ channels altered by presence of β-subunit. Nature 369, 289–294. https://doi.org/10.1038/369289a0
Rezazadeh, S., Kurata, H.T., Claydon, T.W., Kehl, S.J., Fedida, D., 2007. An activation gating switch in Kv1.2 is localized to a threonine residue in the S2-S3 linker. Biophys. J. 93, 4173–4186. https://doi.org/10.1529/biophysj.107.116160
Rhodes, K.J., Keilbaugh, S.A., Barrezueta, N.X., Lopez, K.L., Trimmer, J.S., 1995. Association and colocalization of K+ channel alpha- and beta-subunit polypeptides in rat brain. J. Neurosci. Off. J. Soc. Neurosci. 15, 5360–5371. https://doi.org/10.1523/JNEUROSCI.15-07-05360.1995
Robbins, C.A., Tempel, B.L., 2012. Kv1.1 and Kv1.2: similar channels, different seizure models. Epilepsia 53 Suppl 1. https://doi.org/10.1111/j.1528-1167.2012.03484.x
Rodriguez-Menchaca, A.A., Adney, S.K., Tang, Q.-Y., Meng, X.-Y., Rosenhouse-Dantsker, A., Cui, M., Logothetis, D.E., 2012. PIP2 controls voltage-sensor movement and pore opening of Kv channels through the S4–S5 linker. Proc. Natl. Acad. Sci. 109, E2399–E2408. https://doi.org/10.1073/pnas.1207901109
Rosenkranz, J.A., Venheim, E.R., Padival, M., 2010. Chronic stress causes amygdala hyperexcitability in rodents. Biol. Psychiatry 67, 1128–1136. https://doi.org/10.1016/j.biopsych.2010.02.008
Ruppersberg, J.P., Schröter, K.H., Sakmann, B., Stocker, M., Sewing, S., Pongs, O., 1990. Heteromultimeric channels formed by rat brain potassium-channel proteins. Nature 345, 535–537. https://doi.org/10.1038/345535a0
Sahoo, N., Hoshi, T., Heinemann, S.H., 2014. Oxidative modulation of voltage-gated potassium channels. Antioxid. Redox Signal. 21, 933–952. https://doi.org/10.1089/ars.2013.5614
Saifetiarova, J., Liu, X., Taylor, A.M., Li, J., Bhat, M.A., 2017. Axonal Domain Disorganization in Caspr1 and Caspr2 Mutant Myelinated Axons Affects Neuromuscular Junction Integrity Leading to Muscle Atrophy. J. Neurosci. Res. 95, 1373–1390. https://doi.org/10.1002/jnr.24052
Salpietro, V., Galassi Deforie, V., Efthymiou, S., O'Connor, E., Marcé-Grau, A., Maroofian, R., Striano, P., Zara, F., Morrow, M.M., SYNAPS Study Group, Reich, A., Blevins, A., Sala-Coromina, J., Accogli, A., Fortuna, S., Alesandrini, M., Au, P.Y.B., Singhal, N.S., Cogne, B., Isidor, B., Hanna, M.G., Macaya, A., Kullmann, D.M., Houlden, H., Männikkö, R., 2023. De novo KCNA6 variants with attenuated KV 1.6 channel deactivation in patients with epilepsy. Epilepsia 64, 443–455. https://doi.org/10.1111/epi.17455
Sánchez-Ponce, D., DeFelipe, J., Garrido, J.J., Muñoz, A., 2012. Developmental expression of Kv potassium channels at the axon initial segment of cultured hippocampal neurons. PloS One 7, e48557. https://doi.org/10.1371/journal.pone.0048557
Sanders, S.S., Hernandez, L.M., Soh, H., Karnam, S., Walikonis, R.S., Tzingounis, A.V., Thomas, G.M., 2020. The palmitoyl acyltransferase ZDHHC14 controls Kv1-family potassium channel clustering at the axon initial segment. eLife 9, e56058. https://doi.org/10.7554/eLife.56058

Scalise, M., Galluccio, M., Console, L., Pochini, L., Indiveri, C., 2018. The Human SLC7A5 (LAT1): The Intriguing Histidine/Large Neutral Amino Acid Transporter and Its Relevance to Human Health. Front. Chem. 6. https://doi.org/10.3389/fchem.2018.00243
Scholle, A., Dugarmaa, S., Zimmer, T., Leonhardt, M., Koopmann, R., Engeland, B., Pongs, O., Benndorf, K., 2004. Rate-limiting Reactions Determining Different Activation Kinetics of Kv1.2and Kv2.1 Channels. J. Membr. Biol. 198, 103–112. https://doi.org/10.1007/s00232-004-0664-0
Schulte, U., Thumfart, J.-O., Klöcker, N., Sailer, C.A., Bildl, W., Biniossek, M., Dehn, D., Deller, T., Eble, S., Abbass, K., Wangler, T., Knaus, H.-G., Fakler, B., 2006. The epilepsy-linked Lgi1 protein assembles into presynaptic Kv1 channels and inhibits inactivation by Kvbeta1. Neuron 49, 697–706. https://doi.org/10.1016/j.neuron.2006.01.033
Shamotienko, O.G., Parcej, D.N., Dolly, J.O., 1997. Subunit Combinations Defined for K+ Channel Kv1 Subtypes in Synaptic Membranes from Bovine Brain. Biochemistry 36, 8195–8201. https://doi.org/10.1021/bi970237g
Sharpee, T.O., Sugihara, H., Kurgansky, A.V., Rebrik, S.P., Stryker, M.P., Miller, K.D., 2006. Adaptive filtering enhances information transmission in visual cortex. Nature 439, 936–942. https://doi.org/10.1038/nature04519
Shen, N.V., Chen, X., Boyer, M.M., Pfaffinger, P.J., 1993. Deletion analysis of K+ channel assembly. Neuron 11, 67–76. https://doi.org/10.1016/0896-6273(93)90271-r
Shen, N.V., Pfaffinger, P.J., 1995. Molecular recognition and assembly sequences involved in the subfamily-specific assembly of voltage-gated K+ channel subunit proteins. Neuron 14, 625–633. https://doi.org/10.1016/0896-6273(95)90319-4
Sheng, M., Liao, Y.J., Jan, Y.N., Jan, L.Y., 1993. Presynaptic A-current based on heteromultimeric K+ channels detected in vivo. Nature 365, 72–75. https://doi.org/10.1038/365072a0
Sheng, M., Tsaur, M.L., Jan, Y.N., Jan, L.Y., 1994. Contrasting subcellular localization of the Kv1.2 K+ channel subunit in different neurons of rat brain. J. Neurosci. Off. J. Soc. Neurosci. 14, 2408–2417. https://doi.org/10.1523/JNEUROSCI.14-04-02408.1994
Sheng, M., Tsaur, M.L., Jan, Y.N., Jan, L.Y., 1992. Subcellular segregation of two A-type K+ channel proteins in rat central neurons. Neuron 9, 271–284. https://doi.org/10.1016/0896-6273(92)90166-b
Shi, G., Nakahira, K., Hammond, S., Rhodes, K.J., Schechter, L.E., Trimmer, J.S., 1996. Beta subunits promote K+ channel surface expression through effects early in biosynthesis. Neuron 16, 843–852. https://doi.org/10.1016/s0896-6273(00)80104-x
Shi, G., Trimmer, J.S., 1999. Differential Asparagine-Linked Glycosylation of Voltage-Gated K+ Channels in Mammalian Brain and in Transfected Cells. J. Membr. Biol. 168, 265–273. https://doi.org/10.1007/s002329900515
Shu, Y., Yu, Y., Yang, J., McCormick, D.A., 2007. Selective control of cortical axonal spikes by a slowly inactivating K+ current. Proc. Natl. Acad. Sci. U. S. A. 104, 11453–11458. https://doi.org/10.1073/pnas.0702041104
Snyders, D.J., 1999. Structure and function of cardiac potassium channels. Cardiovasc. Res. 42, 377–390. https://doi.org/10.1016/s0008-6363(99)00071-1
Sokolov, A.M., Holmberg, J.C., Feliciano, D.M., 2020. The amino acid transporter Slc7a5 regulates the mTOR pathway and is required for granule cell development. Hum. Mol. Genet. 29, 3003–3013. https://doi.org/10.1093/hmg/ddaa186
Southan, A.P., Robertson, B., 1998. Patch-Clamp Recordings from Cerebellar Basket Cell Bodies and Their Presynaptic Terminals Reveal an Asymmetric Distribution of Voltage-Gated Potassium Channels. J. Neurosci. 18, 948–955. https://doi.org/10.1523/JNEUROSCI.18-03-00948.1998
Stirling, L., Williams, M.R., Morielli, A.D., 2009. Dual Roles for RHOA/RHO-Kinase In the Regulated Trafficking of a Voltage-sensitive Potassium Channel. Mol. Biol. Cell 20, 2991–3002. https://doi.org/10.1091/mbc.E08-10-1074
Stühmer, W., Conti, F., Suzuki, H., Wang, X.D., Noda, M., Yahagi, N., Kubo, H., Numa, S., 1989.

Structural parts involved in activation and inactivation of the sodium channel. Nature 339, 597–603. https://doi.org/10.1038/339597a0
Suárez-Delgado, E., Rangel-Sandín, T.G., Ishida, I.G., Rangel-Yescas, G.E., Rosenbaum, T., Islas, L.D., 2020. KV1.2 channels inactivate through a mechanism similar to C-type inactivation. J. Gen. Physiol. 152, e201912499. https://doi.org/10.1085/jgp.201912499
Suh, B.-C., Hille, B., 2008. PIP2 is a necessary cofactor for ion channel function: How and why? Annu. Rev. Biophys. 37, 175–195. https://doi.org/10.1146/annurev.biophys.37.032807.125859
Syrbe, S., Hedrich, U.B.S., Riesch, E., Djémié, T., Müller, S., Møller, R.S., Maher, B., Hernandez-Hernandez, L., Synofzik, M., Caglayan, H.S., Arslan, M., Serratosa, J.M., Nothnagel, M., May, P., Krause, R., Löffler, H., Detert, K., Dorn, T., Vogt, H., Krämer, G., Schöls, L., Mullis, P.E., Linnankivi, T., Lehesjoki, A.-E., Sterbova, K., Craiu, D.C., Hoffman-Zacharska, D., Korff, C.M., Weber, Y.G., Steinlin, M., Gallati, S., Bertsche, A., Bernhard, M.K., Merkenschlager, A., Kiess, W., Gonzalez, M., Züchner, S., Palotie, A., Suls, A., Craiu, D.C., De Jonghe, P., Helbig, I., Biskup, S., Wolff, M., Maljevic, S., Schüle, R., Sisodiya, S.M., Weckhuysen, S., Lerche, H., Lemke, J.R., 2015. De novo loss- or gain-of-function mutations in KCNA2 cause epileptic encephalopathy. Nat. Genet. 47, 393–399. https://doi.org/10.1038/ng.3239
Taha, S.A., Fields, H.L., 2006. Inhibitions of Nucleus Accumbens Neurons Encode a Gating Signal for Reward-Directed Behavior. J. Neurosci. 26, 217–222. https://doi.org/10.1523/JNEUROSCI.3227-05.2006
Tan, X.-F., Bae, C., Stix, R., Fernández-Mariño, A.I., Huffer, K., Chang, T.-H., Jiang, J., Faraldo-Gómez, J.D., Swartz, K.J., 2022. Structure of the Shaker Kv channel and mechanism of slow C-type inactivation. Sci. Adv. 8, eabm7814. https://doi.org/10.1126/sciadv.abm7814
Tanner, M.R., Tajhya, R.B., Huq, R., Gehrmann, E.J., Rodarte, K.E., Atik, M.A., Norton, R.S., Pennington, M.W., Beeton, C., 2017. Prolonged immunomodulation in inflammatory arthritis using the selective Kv1.3 channel blocker HsTX1[R14A] and its PEGylated analog. Clin. Immunol. Orlando Fla 180, 45–57. https://doi.org/10.1016/j.clim.2017.03.014
Tărlungeanu, D.C., Deliu, E., Dotter, C.P., Kara, M., Janiesch, P.C., Scalise, M., Galluccio, M., Tesulov, M., Morelli, E., Sonmez, F.M., Bilguvar, K., Ohgaki, R., Kanai, Y., Johansen, A., Esharif, S., Ben-Omran, T., Topcu, M., Schlessinger, A., Indiveri, C., Duncan, K.E., Caglayan, A.O., Gunel, M., Gleeson, J.G., Novarino, G., 2016. Impaired Amino Acid Transport at the Blood Brain Barrier Is a Cause of Autism Spectrum Disorder. Cell 167, 1481-1494.e18. https://doi.org/10.1016/j.cell.2016.11.013
Thayer, D.A., Yang, S., Jan, Y.N., Jan, L.Y., 2016. N-linked glycosylation of Kv1.2 voltage-gated potassium channel facilitates cell surface expression and enhances the stability of internalized channels. J. Physiol. 594, 6701–6713. https://doi.org/10.1113/JP272394
Tipparaju, S.M., Liu, S.-Q., Barski, O.A., Bhatnagar, A., 2007. NADPH binding to beta-subunit regulates inactivation of voltage-gated K(+) channels. Biochem. Biophys. Res. Commun. 359, 269–276. https://doi.org/10.1016/j.bbrc.2007.05.102
Todorovic, S.M., Jevtovic-Todorovic, V., 2014. Redox Regulation of Neuronal Voltage-Gated Calcium Channels. Antioxid. Redox Signal. 21, 880–891. https://doi.org/10.1089/ars.2013.5610
Todorovic, S.M., Jevtovic-Todorovic, V., Meyenburg, A., Mennerick, S., Perez-Reyes, E., Romano, C., Olney, J.W., Zorumski, C.F., 2001. Redox Modulation of T-Type Calcium Channels in Rat Peripheral Nociceptors. Neuron 31, 75–85. https://doi.org/10.1016/S0896-6273(01)00338-5
Toldi, G., Vásárhelyi, B., Kaposi, A., Mészáros, G., Pánczél, P., Hosszufalusi, N., Tulassay, T., Treszl, A., 2010. Lymphocyte activation in type 1 diabetes mellitus: The increased significance of Kv1.3 potassium channels. Immunol. Lett. 133, 35–41. https://doi.org/10.1016/j.imlet.2010.06.009
Traka, M., Goutebroze, L., Denisenko, N., Bessa, M., Nifli, A., Havaki, S., Iwakura, Y., Fukamauchi, F., Watanabe, K., Soliven, B., Girault, J.-A., Karagogeos, D., 2003. Association of TAG-1 with Caspr2 is essential for the molecular organization of juxtaparanodal regions of myelinated fibers. J. Cell Biol. 162, 1161–1172. https://doi.org/10.1083/jcb.200305078
Treptow, W., Tarek, M., 2006. Environment of the Gating Charges in the Kv1.2 Shaker Potassium

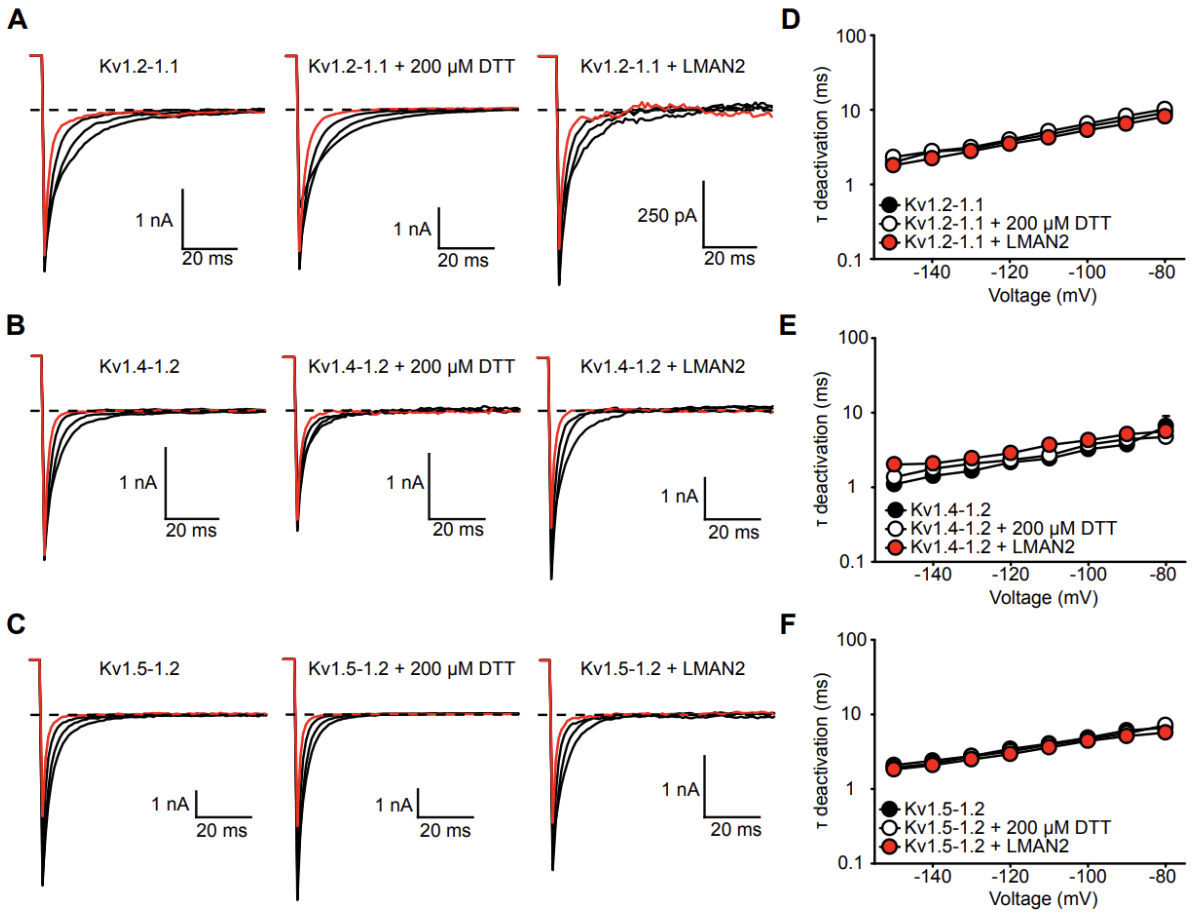
Channel. Biophys. J. 90, L64–L66. https://doi.org/10.1529/biophysj.106.080754
Trimmer, J.S., 2015. Subcellular localization of K+ channels in mammalian brain neurons: remarkable precision in the midst of extraordinary complexity. Neuron 85, 238–256. https://doi.org/10.1016/j.neuron.2014.12.042
Trimmer, J.S., Rhodes, K.J., 2004. Localization of voltage-gated ion channels in mammalian brain. Annu. Rev. Physiol. 66, 477–519. https://doi.org/10.1146/annurev.physiol.66.032102.113328
Vabnick, I., Trimmer, J.S., Schwarz, T.L., Levinson, S.R., Risal, D., Shrager, P., 1999. Dynamic potassium channel distributions during axonal development prevent aberrant firing patterns. J. Neurosci. Off. J. Soc. Neurosci. 19, 747–758. https://doi.org/10.1523/JNEUROSCI.19-02-00747.1999
Vacher, H., Mohapatra, D.P., Trimmer, J.S., 2008. Localization and Targeting of Voltage-Gated Ion Channels in Mammalian Central Neurons. Physiol. Rev. 88, 1407–1447. https://doi.org/10.1152/physrev.00002.2008
Vydyanathan, A., Wu, Z.-Z., Chen, S.-R., Pan, H.-L., 2005. A-type voltage-gated K+ currents influence firing properties of isolectin B4-positive but not isolectin B4-negative primary sensory neurons. J. Neurophysiol. 93, 3401–3409. https://doi.org/10.1152/jn.01267.2004
Wang, H., Kunkel, D.D., Martin, T.M., Schwartzkroin, P.A., Tempel, B.L., 1993. Heteromultimeric K+ channels in terminal and juxtaparanodal regions of neurons. Nature 365, 75–79. https://doi.org/10.1038/365075a0
Wang, H., Kunkel, D.D., Schwartzkroin, P.A., Tempel, B.L., 1994. Localization of Kv1.1 and Kv1.2, two K channel proteins, to synaptic terminals, somata, and dendrites in the mouse brain. J. Neurosci. Off. J. Soc. Neurosci. 14, 4588–4599. https://doi.org/10.1523/JNEUROSCI.14-08-04588.1994
Wang, J., Juhaszova, M., Rubin, L.J., Yuan, X.J., 1997. Hypoxia inhibits gene expression of voltage-gated K+ channel alpha subunits in pulmonary artery smooth muscle cells. J. Clin. Invest. 100, 2347–2353. https://doi.org/10.1172/JCI119774
Wang, Q., Curran, M.E., Splawski, I., Burn, T.C., Millholland, J.M., VanRaay, T.J., Shen, J., Timothy, K.W., Vincent, G.M., de Jager, T., Schwartz, P.J., Toubin, J.A., Moss, A.J., Atkinson, D.L., Landes, G.M., Connors, T.D., Keating, M.T., 1996. Positional cloning of a novel potassium channel gene: KVLQT1 mutations cause cardiac arrhythmias. Nat. Genet. 12, 17–23. https://doi.org/10.1038/ng0196-17
Wang, X., Li, G., Guo, J., Zhang, Z., Zhang, S., Zhu, Y., Cheng, J., Yu, L., Ji, Y., Tao, J., 2020. Kv1.3 Channel as a Key Therapeutic Target for Neuroinflammatory Diseases: State of the Art and Beyond. Front. Neurosci. 13.
Wang, Z., Fermini, B., Nattel, S., 1993. Sustained depolarization-induced outward current in human atrial myocytes. Evidence for a novel delayed rectifier K+ current similar to Kv1.5 cloned channel currents. Circ. Res. 73, 1061–1076. https://doi.org/10.1161/01.res.73.6.1061
Watanabe, H., Nagata, E., Kosakai, A., Nakamura, M., Yokoyama, M., Tanaka, K., Sasai, H., 2000. Disruption of the epilepsy KCNQ2 gene results in neural hyperexcitability. J. Neurochem. 75, 28–33. https://doi.org/10.1046/j.1471-4159.2000.0750028.x
Watanabe, I., Zhu, J., Sutachan, J.J., Gottschalk, A., Recio-Pinto, E., Thornhill, W.B., 2007. The glycosylation state of Kv1.2 potassium channels affects trafficking, gating, and simulated action potentials. Brain Res. 1144, 1–18. https://doi.org/10.1016/j.brainres.2007.01.092
Weng, J., Cao, Y., Moss, N., Zhou, M., 2006. Modulation of voltage-dependent Shaker family potassium channels by an aldo-keto reductase. J. Biol. Chem. 281, 15194–15200. https://doi.org/10.1074/jbc.M513809200
Wettwer, E., Hála, O., Christ, T., Heubach, J.F., Dobrev, D., Knaut, M., Varró, A., Ravens, U., 2004. Role of IKur in Controlling Action Potential Shape and Contractility in the Human Atrium. Circulation 110, 2299–2306. https://doi.org/10.1161/01.CIR.0000145155.60288.71
Willis, M., Leitner, I., Seppi, K., Trieb, M., Wietzorrek, G., Marksteiner, J., Knaus, H.-G., 2018. Shaker-related voltage-gated potassium channels Kv1 in human hippocampus. Brain Struct. Funct. 223, 2663–2671. https://doi.org/10.1007/s00429-018-1653-x

Wu, R.L., Barish, M.E., 1992. Two pharmacologically and kinetically distinct transient potassium currents in cultured embryonic mouse hippocampal neurons. J. Neurosci. Off. J. Soc. Neurosci. 12, 2235–2246. https://doi.org/10.1523/JNEUROSCI.12-06-02235.1992
Wulff, H., Castle, N.A., Pardo, L.A., 2009. Voltage-gated potassium channels as therapeutic targets. Nat. Rev. Drug Discov. 8, 982–1001. https://doi.org/10.1038/nrd2983
Xie, C., Markesbery, W.R., Lovell, M.A., 2000. Survival of hippocampal and cortical neurons in a mixture of MEM+ and B27-supplemented neurobasal medium. Free Radic. Biol. Med. 28, 665–672. https://doi.org/10.1016/S0891-5849(99)00268-3
Xu, J., Yu, W., Jan, Y.N., Jan, L.Y., Li, M., 1995. Assembly of voltage-gated potassium channels. Conserved hydrophilic motifs determine subfamily-specific interactions between the alpha-subunits. J. Biol. Chem. 270, 24761–24768. https://doi.org/10.1074/jbc.270.42.24761
Xu, J., Yu, W., Wright, J.M., Raab, R.W., Li, M., 1998. Distinct functional stoichiometry of potassium channel beta subunits. Proc. Natl. Acad. Sci. U. S. A. 95, 1846–1851. https://doi.org/10.1073/pnas.95.4.1846
Yang, E.-K., Takimoto, K., Hayashi, Y., de Groat, W.C., Yoshimura, N., 2004. Altered expression of potassium channel subunit mRNA and alpha-dendrotoxin sensitivity of potassium currents in rat dorsal root ganglion neurons after axotomy. Neuroscience 123, 867–874. https://doi.org/10.1016/j.neuroscience.2003.11.014
Yang, J.-W., Vacher, H., Park, K.-S., Clark, E., Trimmer, J.S., 2007. Trafficking-dependent phosphorylation of Kv1.2 regulates voltage-gated potassium channel cell surface expression. Proc. Natl. Acad. Sci. 104, 20055–20060. https://doi.org/10.1073/pnas.0708574104
Yang, Y., Yan, Y., Sigworth, F.J., 1997. How Does the W434F Mutation Block Current in Shaker Potassium Channels? J. Gen. Physiol. 109, 779–789.
Yarov-Yarovoy, V., DeCaen, P.G., Westenbroek, R.E., Pan, C.-Y., Scheuer, T., Baker, D., Catterall, W.A., 2012. Structural basis for gating charge movement in the voltage sensor of a sodium channel. Proc. Natl. Acad. Sci. 109, E93–E102. https://doi.org/10.1073/pnas.1118434109
Yellen, G., 1998. The moving parts of voltage-gated ion channels. Q. Rev. Biophys. 31, 239–295. https://doi.org/10.1017/S0033583598003448
Yu, F.H., Yarov-Yarovoy, V., Gutman, G.A., Catterall, W.A., 2005. Overview of Molecular Relationships in the Voltage-Gated Ion Channel Superfamily. Pharmacol. Rev. 57, 387–395. https://doi.org/10.1124/pr.57.4.13
Yuan, X.-J., Wang, J., Juhaszova, M., Golovina, V.A., Rubin, L.J., 1998. Molecular basis and function of voltage-gated K+ channels in pulmonary arterial smooth muscle cells. Am. J. Physiol.-Lung Cell. Mol. Physiol. 274, L621–L635. https://doi.org/10.1152/ajplung.1998.274.4.L621
Zhang, J., Rong, L., Shao, J., Zhang, Y., Liu, Y., Zhao, S., Li, L., Yu, W., Zhang, M., Ren, X., Zhao, Q., Zhu, C., Luo, H., Zang, W., Cao, J., 2021. Epigenetic restoration of voltage-gated potassium channel Kv1.2 alleviates nerve injury-induced neuropathic pain. J. Neurochem. 156, 367–378. https://doi.org/10.1111/jnc.15117
Zhao, J.-Y., Liang, L., Gu, X., Li, Z., Wu, S., Sun, L., Atianjoh, F.E., Feng, J., Mo, K., Jia, S., Lutz, B.M., Bekker, A., Nestler, E.J., Tao, Y.-X., 2017. DNA methyltransferase DNMT3a contributes to neuropathic pain by repressing Kcna2 in primary afferent neurons. Nat. Commun. 8, 14712. https://doi.org/10.1038/ncomms14712
Zhou, J., Brown, A.M., Lackey, E.P., Arancillo, M., Lin, T., Sillitoe, R.V., 2020. Purkinje cell neurotransmission patterns cerebellar basket cells into zonal modules defined by distinct pinceau sizes. eLife 9, e55569. https://doi.org/10.7554/eLife.55569
Zhou, Q.-L., Wang, T.-Y., Li, M., Shang, Y.-X., 2018. Alleviating airway inflammation by inhibiting ERK-NF-κB signaling pathway by blocking Kv1.3 channels. Int. Immunopharmacol. 63, 110–118. https://doi.org/10.1016/j.intimp.2018.07.009



Supplementary Figure 1. Redox and LMAN2 do not influence deactivation kinetics of Kv1 homomeric channels. (A-E) Sample traces LM fibroblast cells expressing Kv1 homomeric channels in control, 200 µM DTT, and LMAN2 conditions being depolarized to +60 mV for 600 ms from a -100 mV holding potential and then being repolarized to -80 to -150 mV (10 mV steps) for 500 ms. Sweeps at -90,

-110, -130, and -150 mV are shown with the highlighted red sweeps representing the -150 mV depolarization. (F-J) Time constants of deactivation (τ deactivation) was measured by fitting deactivation curves with a single exponential function as mentioned in Figure 3.6. Either a one-way ANOVA or Kruskal-Wallis ANOVA was run, depending on if normality and equal variance assumptions were fulfilled, to compare between groups. Neither DTT nor LMAN2 produced significant differences relative to control in any of the channels tested. n = 4-10 individual cells.



Supplementary Figure 2. Redox and LMAN2 do not influence deactivation kinetics of Kv1 heteromeric channels. (A-C) Sample traces LM fibroblast cells expressing Kv1.2-1.1, Kv1.4-1.2, and Kv1.5-1.2 heteromeric channels in control, 200 µM DTT, and LMAN2 conditions being depolarized to +60 mV for 600 ms from a -100 mV holding potential and then being repolarized to -80 to -150 mV (10 mV steps) for 500 ms. Sweeps at -90, -110, -130, and -150 mV are shown with the highlighted red sweeps representing the -150 mV depolarization. (D-F) Time constants of deactivation (τ deactivation) was measured by fitting deactivation curves with a single exponential function as mentioned in Figure 3.6. Either a one-way ANOVA or Kruskal-Wallis ANOVA was run to determine the effect of different conditions on the same channel. Neither DTT nor LMAN2 produced significant differences relative to control in any of the channels tested. n = 4-7 individual cells.