Distinctive Role of $K_V1.1$ Subunit in the Biology and Functions of Low Threshold K^+ Channels with Implications for Neurological Disease

Authors: Saak V. Ovsepian[1,2], Marie LeBerre[3], Volker Steuber[4], Valerie B. O'Leary[5], Christian Leibold[6] and J. Oliver Dolly[2]

Institute of Bio-Medical Imaging at Helmholtz Zentrum Munich; Neuherberg, Germany (1); International Centre for Neurotherapeutics, Dublin City University, Dublin, Ireland (2); National Centre for Biomedical Engineering, National University of Ireland, Galway, Ireland (3); Science and Technology Research Institute, University of Hertfordshire, United Kingdom (4); Institute of Radiation Biology, Helmholtz Zentrum Munich, Neuherberg, Germany (5); Department Biology, Ludwig II Maximilians University Munich, Martinsried, Germany (6).

Correspondence: Saak V. Ovsepian: saak.ovsepian@gmail.com

Institute of Bio-Medical Imaging, Helmholtz Zentrum Munich, Ingolstadtler Landstrasse 1, 85764 Neuherberg, Munich, Germany

Tel.: +49 (0) 4989 3187 1141

Fax: +49 (0) 4989 3187 1140

Abstract

The diversity of pore-forming subunits of K_V1 channels ($K_V1.1$-$K_V1.8$) affords their physiological versatility and predicts a range of functional impairments resulting from genetic aberrations. Curiously, identified so far human neurological conditions associated with dysfunctions of K_V1 channels have been linked exclusively to mutations in the *KCNA1* gene encoding for the $K_V1.1$ subunit. The absence of phenotypes related to irregularities in other subunits, including the prevalent $K_V1.2$ subunit of neurons is highly perplexing given that deletions of corresponding *kcna2* gene in mouse models precipitate symptoms reminiscent to those of $K_V1.1$ knockouts. Herein, we critically evaluate the molecular and biophysical characteristics of the $K_V1.1$ protein in comparison with others and discuss their role in the greater penetrance of *KCNA1* mutations in humans leading to the neurological signs of episodic ataxia type 1 (EA1). Future research and interpretation of emerging data should afford new insight towards a better understanding of the role of $K_V1.1$ in integrative mechanisms of neurons and synaptic functions under normal and disease conditions.

Keywords: *KCNA1*, low-threshold potassium channel, hetero-tetramer, dendrotoxin – K, synaptic integration, episodic ataxia 1 (EA1)

Table of contents

Abbreviations: AA – amino acid; α-DTX – alpha dendrotoxin; DTX_K – dendrotoxin K; EA-1 – episodic ataxia type -1; ER – endoplasmic reticulum; ERR – endoplasmic reticular retention; FTS – forward trafficking signal; HGNC – HUGO Genetic Nomenclature Committee; IS – initial segment; IUPHAR – International Union of Basic and Clinical Pharmacology; JXP – juxta-paranode; *KCNA1* – human gene encoding K_V1.1 subunit of potassium channel; *kcna2* – mouse gene encoding K_V1.2 subunit of potassium channel; *kcna4* – mouse gene encoding K_V1.4 subunit of potassium channel; K_V – voltage-gated potassium channels; K_Vβ - beta subunit of voltage-gated potassium channels; MBP – myelin basic protein; N – node of Ranvier; PAUP – phylogenetic analysis using parsimony; S – soma; SD – somato-dendritic; T-domain; TM – trans-membrane; $V_{1/2}$ – half activation voltage.

1. Introduction

K_V1 voltage-gated potassium channels are integral membrane proteins, which are of major importance in adjusting the bio-electrical activity of neurons. Through an ion conductive pore, they mediate the outflow of K^+ across the lipid bilayer of the surface membrane in response to depolarization, regulating the resting membrane potential and excitability, timing and frequency of action potentials during repetitive spike trains, and the release of neurotransmitters at axon terminals (Clark, Goldberg, & Rudy, 2009; Hille, 2001; Kuba, Yamada, Ishiguro, & Adachi, 2015; Yellen, 2002). The functional versatility of K_V1 channels arises to a large extent from their molecular diversity and fine regulation. The conductive pore of the channel complex is formed through oligomerization of four α subunits, which are multi-domain proteins composed of six membrane spanning segments (S1-S6) linked via hydrophilic intra- and extra-cellular loops. Since cloning of the first K_V1 channel gene in *Drosophila* affected by *Shaker* mutations (Papazian, Schwarz, Tempel, Jan, & Jan, 1987), eight members of the family ($K_V1.1$-$K_V1.8$) encoded by corresponding *KCNA1-KCNA8* genes have been identified and functionally characterized (Gutman, et al., 2005; Jan & Jan, 2012; Kamb, Tseng-Crank, & Tanouye, 1988; Pongs, et al., 1988; Tempel, Jan, & Jan, 1988) **(FIG. 1)**. In neurons, typically different K_V1 α subunits co-assemble to form hetero-tetramers, with channels made of four identical subunits (homo-tetramers) also described (Coleman, Newcombe, Pryke, & Dolly, 1999; Dolly & Parcej, 1996; Parcej, Scott, & Dolly, 1992; Stuhmer, et al., 1989; Wang, Kunkel, Martin, Schwartzkroin, & Tempel, 1993). Studies of K_V1 homo-tetramers in expression systems, in addition to commonalities have revealed differences in the biophysical and pharmacological properties, which in hetero-

tetramers equilibrate between contributing subunits (Akhtar, Shamotienko, Papakosta, Ali, & Dolly, 2002; Bagchi, et al., 2014; Sokolov, Shamotienko, Dhochartaigh, Sack, & Dolly, 2007). In addition to electrophysiological properties, the molecular composition of K_V1 channels is known to control their mobility and targeting to specific neuronal compartments with surface expression (Heusser & Schwappach, 2005; Manganas & Trimmer, 2000; Manganas, Wang, et al., 2001; Vacher, Mohapatra, Misonou, & Trimmer, 2007; Vacher, Mohapatra, & Trimmer, 2008).

Although in heterologous systems all combinations of K_V1 subunits yield K^+ currents, native channels from crude forebrain extracts and synaptosomes have revealed a predominance of certain subunits and their combinations over others (Coleman, et al., 1999; Koch, et al., 1997; Shamotienko, Parcej, & Dolly, 1997; Wang, Parcej, & Dolly, 1999). These data suggests that the assembly of K_V1 channels within intact neurons is not promiscuous but is tightly regulated, and predict a greater role for molecular aberrations in prevalent subunits in the generation of neurological phenotypes associated with *KCNA* mutations. Surprisingly and notwithstanding of the similar distribution with comparable expression levels of $K_V1.2$, $K_V1.4$, $K_V1.6$ and $K_V1.1$ throughout the mammalian nervous system, linkage studies of human K_V1 channelopathies, which are characterized by bouts of cerebellar ataxia with motor deficits, vertigo and occasions of sporadic seizures (fits of epilepsy), and defined clinically as episodic ataxia type 1 (EA1) have mapped all related mutations to the *KCNA1* gene (12p13) encoding for $K_V1.1$ subunit (Imbrici, D'Adamo, Kullmann, & Pessia, 2006; Kullmann, 2002; Kullmann, Rea, Spauschus, & Jouvenceau, 2001; Rajakulendran, Schorge, Kullmann, & Hanna, 2007). The absence of $K_V1.1$ homo-tetramers in the mammalian brain along with distinct neurological signs in *kcna2* and

kcna4 null mice (Brew, et al., 2007; Brew, Hallows, & Tempel, 2003; London, Wang, Hill, & Bennett, 1998; Smart, et al., 1998) raises the possibility of special traits of $K_V1.1$ subunit, which afford the greater penetrance of *KCNA1* mutations. Because EA1 is a dominantly inherited disease and $K_V1.1$ co-assembles with others to produce channels, it is expected that a defective $K_V1.1$ will interfere with the functions of K_V1 channels to which they contribute. Reports from expression systems showed that co-expression of mutant $K_V1.1$ with wild type yield currents with intermediate biophysical characteristics (D'Adamo, Imbrici, Sponcichetti, & Pessia, 1999; Eunson, et al., 2000; Spauschus, Eunson, Hanna, & Kullmann, 1999; Zerr, Adelman, & Maylie, 1998b; Zuberi, et al., 1999), an observation which confirms not only the ability of the faulty $K_V1.1$ to form channels but also yield anomalous integral membrane currents. Below, we overview the molecular and biophysical properties of the $K_V1.1$ subunit in comparison with others, and the possible mechanistic grounds for the disruptive effects of EA1 mutations on K_V1 channel functions and integrative mechanisms of the brain.

2. Molecular partners of the $K_V1.1$ subunit in native K^+ channels

In neurons, K_V1 channels are produced by oligomerization of four pore-forming α and an equal amount of cytoplasmic $K_V\beta$ ($K_V\beta1$, $\beta2$ and $\beta3$) subunits. Although in expression systems K_V1 α subunits co-assemble randomly to yield K^+ currents, native channels from mammalian brain tissue are known to prefer certain combinations of α subunits over others (Isacoff, Jan, & Jan, 1990; Koch, et al., 1997; Rettig, et al., 1994; Rhodes, et al., 1997; Ruppersberg, et al., 1990; Shamotienko, et al., 1997). Analysis of native K_V1 channels isolated from the total cerebral extracts as well as from various brain structures

with α-dendrotoxin (α-DTX, K_V1.2 specific mamba snake toxin) demonstrated that the predominant fraction of K_V1 channels are represented as hetero-tetramers (Dolly & Parcej, 1996; Parcej, et al., 1992; Scott, et al., 1994). Accordingly, over 85% of the material bound to α-DTX was precipitated by an anti-K_V1.2 antibody, with lesser amounts removed by anti-K_V1.1, -K_V1.6 and -K_V1.4 antibodies (47%, 16% and 8%, respectively) (Dolly & Parcej, 1996; Muniz, Parcej, & Dolly, 1992; Scott, et al., 1994). These data demonstrate that almost half of α-DTX-sensitive K_V1 channels also contained a K_V1.1 subunit and that the vast majority of K_V1.1, K_V1.4 and K_V1.6 proteins oligomerize with K_V1.2 to form functional channels (Dolly & Parcej, 1996; Trimmer & Rhodes, 2004; Vacher, et al., 2008). Importantly, anti-K_V1.1 IgG failed to sequester oligomers from the material not precipitated by anti-K_V1.2 antisera, a finding which infers that in the brain, K_V1.1 always occurs in association with a K_V1.2 subunit. On the other hand, a small fraction of Kv1.4 and Kv1.2 subunits have been shown to form homo-tetramers in central neurons, while on non-myelinated axons at the periphery, K_V1.1 has been occasionally found to form hetero-tetramers with K_V1.4 in the absence of Kv1.2 (Rasband, et al., 2001; Trimmer & Rhodes, 2004). Results of these biochemical and immuno-histochemical studies are in line with the evidence from pharmacological experiments, using specific peptide blockers of K_V1 currents, which showed that in the vast majority of cases, different K_V1 subunits co-assemble to produce functional channels (Bagchi, et al., 2014; Devaux, Gola, Jacquet, & Crest, 2002; Dodson, Barker, & Forsythe, 2002; Dodson, et al., 2003; Johnston, Forsythe, & Kopp-Scheinpflug, 2010; Norris, Foeger, & Nerbonne, 2010; Ovsepian, et al., 2013). Overall, in central neurons, neither K_V1.1, the second most abundant Kv1 α subunit, nor K_V1.6 or the least abundant K_V1.3

form homo-tetramers but always co-assemble with others (mainly $K_V1.2$) to form functional channels, while $K_V1.2$ and $K_V1.4$ in addition to forming hetero-tetramers also occasionally produces homomers (Trimmer & Rhodes, 2004). As noted, the expression of the $K_V1.1$ protein in the absence of other *Shaker* related family members has been documented in a small fraction of thin peripheral axons, but their functionality remains to be shown (Rasband & Shrager, 2000; Rasband, et al., 1998). We have demonstrated recently that in demyelinating axons of the optic nerve in a cuprizone mouse model, the expression of $K_V1.1$ at juxta-paranodes (JPNs) and nodal regions is selectively enhanced, an observation that suggests enrichment of denuded axons with this protein, and perhaps formation of a population of $K_V1.1$ homo-tetramers, functioning alongside with the $K_V1.1$/$K_V1.2$ hetero-tetramers (Bagchi, et al., 2014).

Thus, from the brief overview of selected reports it emerges that in central neurons the majority of $K_V1.1$ co-assemble with other members of the family to produce functional hetero-tetramers, with most containing $K_V1.2$ and $K_V1.4$ subunits. As such, it is expected that the microscopic K_V1 currents in neurons are subject to influence by the functional characteristics of the $K_V1.1$ subunit. Such arrangement, as shown below, is of key importance not only for defining the biophysical profile of integral K_V1 currents, but plays a decisive role in neurological phenotypes associated with EA1 mutations.

3. $K_V1.1$ subunit regulates the mobility and surface expression of K_V1 channels

One of the major insights gained from studies of the biology of K_V1 channels in heterologous systems is that the composition of hetero-tetramers can be biased by the expression levels of individual subunits. Equally important and perhaps more revealing

are the data which suggest that the subunit composition of K_V1 channels determines their intracellular mobility and surface expression competence (Heusser & Schwappach, 2005; Jensen, Rasmussen, & Misonou, 2011; Manganas & Trimmer, 2000; Manganas, Wang, et al., 2001; Vacher, Misonou, & Trimmer, 2007; Vacher, Mohapatra, et al., 2007). It emerges that the assembly and export of functional K_V1 tetramers from the endoplasmic reticulum (ER) and trafficking to the cell surface are controlled by complex and hierarchical mechanisms. Similar to other membrane proteins, the export competence of nascent K_V1 channels is a major rate limiting factor for their surface expression, with the ER retention (ERR) signal encoded in the amino acid residues of the external face (turret region) of the pore region playing an essential role (Lodish & Kong, 1983; Manganas, Wang, et al., 2001; Nagaya & Papazian, 1997; Vacher, Misonou, et al., 2007; Zhu, Watanabe, Gomez, & Thornhill, 2001). Interestingly, the residues in the P-loop, which encode the ERR signal also determine the high affinity binding of $K_V1.1$ to the mamba snake toxin, DTX_K. Thus, K_V1 family members capable of high affinity binding to DTX_K ($K_V1.1$ > $K_V1.2$ > $K_V1.6$) exhibit conforming ER retention, unlike those lacking this signal ($K_V1.3$, $K_V1.4$ and $K_V1.5$) and are prone to inherently strong surface expression (Dolly & Parcej, 1996; Hurst, et al., 1991; Imredy & MacKinnon, 2000; Manganas & Trimmer, 2000; Manganas, Wang, et al., 2001; Tytgat, Debont, Carmeliet, & Daenens, 1995). The notion of the strong ER retention of the $K_V1.1$ subunit is in line with the poor surface expression of $K_V1.1$ homo-tetramers as well as with inhibitory effects of $K_V1.1$ on the expression of hetero-tetramers containing other K_V1 subunits.

Although the export code of K_V1 α subunits can be shared among different family members, it is not transferable to non-*Shaker*-related channels (Trimmer, 2015; Vacher,

Misonou, et al., 2007; Zhu, Gomez, Watanabe, & Thornhill, 2005). Analysis of the molecular determinants of the ERR through the use of chimeric K_V1 α subunits showed that swapping of the turret region (P-domain) of $K_V1.1$ with $K_V1.4$ greatly reduces the mobility and surface expression of the $K_V1.4$ subunit, with its retention to the ER. Conversely, the transfer of the turret region of $K_V1.4$ onto $K_V1.1$ promotes the surface expression of the latter (Manganas, Akhtar, et al., 2001; Vacher, Misonou, et al., 2007). Amongst other key regulators of the mobility and surface expression of K_V1 channels, cytoplasmic C-terminal VXXSL forward trafficking signal (FTS) and $K_V\beta2$ auxiliary subunit have been widely discussed (Li, Takimoto, & Levitan, 2000; Shi, et al., 1996). It is important to note that ERR of $K_V1.1$ is dominant over these additional regulatory signals and is capable of overriding their effects. Indeed, the cytoplasmic FTS motif has been shown to be recessive to the turret ERR signal, as evident from studies of $K_V1.4$ chimeras containing the turret region of $K_V1.1$ subunit, which show strongly reduced surface expression and retention in the ER (Li, et al., 2000; Zhu, Watanabe, Gomez, & Thornhill, 2003). On the other hand, possession of FTS by $K_V1.4$ lacking the ERR signal renders its surface expression highly efficient. Finally, $K_V1.1$ appears to be capable of neutralizing the augmenting effects of $K_V\beta2$ on surface expression of K_V1 channels. While promoting the expression of $K_V1.2$ homo-tetramers, $K_V\beta2$ falls short in its similar effects on channels containing $K_V1.1$ or $K_V1.4$ proteins (Shi, et al., 1996; Vacher, Misonou, et al., 2007). Interestingly, the failure of $K_V\beta2$ to facilitate surface expression of $K_V1.4$ has been viewed as proof of the maximal inherent propensity of the latter for surface expression, while the lack of effects on the $K_V1.1$ subunit infers the dominance of the ERR signal (Vacher, Misonou, et al., 2007). Thus, it emerges that $K_V1.1$ plays a key

role in controlling the intracellular mobility and surface expression of K_V1 channels with important implications for the biology and functions of neurons.

4. Distribution of $K_V1.1$ subunit throughout the mammalian brain

In central neurons, K_V1 channels are located on the soma, axons, synaptic terminals and dendrites **(FIG. 2)**. Differential expression of K_V1 subunits with their precise targeting to various neuronal compartments and fine regulation renders K_V1 channels particularly important in governing an array of neuronal processes and functions (Rasband & Shrager, 2000; Robbins & Tempel, 2012; Trimmer, 2015; Trimmer & Rhodes, 2004; Wang, et al., 1993). Pull-down experiments with biochemical analysis of native K_V1 channels with α-DTX ($K_V1.2$ > $K_V1.1$-selective) from bovine cerebellum, hippocampus, cerebral cortex, corpus striatum and brainstem revealed their strong enrichment with the $K_V1.2$ protein (Dolly & Parcej, 1996; Scott, et al., 1994). Importantly, considerable variability in the relative expression levels of different subunits throughout the mammalian nervous system have also been shown using quantitative biochemistry, with levels of $K_V1.1$ being highest in brainstem nuclei and white matter and lowest in the cerebellum and hippocampus, while $K_V1.4$ > $K_V1.2$ represent the main K_V1 subunits in the hippocampus (Scott, et al., 1994). Of note, the expression of $K_V1.6$ or $K_V1.2$ throughout various compartments of the brain is maintained fairly evenly. The relatively low levels of $K_V1.1$ in the cerebellum and hippocampus reflect the low copy number of this protein in hetero-tetramers within these structures. As a result, both neuronal activity and synaptic transmission are likely to be more susceptible to the molecular aberrations in the $K_V1.1$ subunit. The results of immuno-fluorescence reports are consistent with biochemical data, and show that

throughout the brain K_V1.1 is expressed predominantly in two channel populations: (*1*) together with K_V1.2 or K_V1.4 in the hippocampus and with K_V1.4 in striatal efferents of pallidial neurons as well as in neurons of pars reticulata of the substantia nigra, and (*2*) in association with K_V1.2 (without K_V1.4 and K_V1.6) at the pinceau of cerebellar basket neurons, somata of deep cerebellar nuclear neurons, brainstem nuclei including the octopus cells of ventral cochlear nucleus, medial nucleus of the trapezoid body as well as JPNs of myelinated axons within the white matter of the brain (McNamara, Averill, Wilkin, Dolly, & Priestley, 1996; McNamara, Muniz, Wilkin, & Dolly, 1993; Ovsepian, et al., 2013; Rasband, Trimmer, Peles, Levinson, & Shrager, 1999; Rhodes, et al., 1996; Rhodes, et al., 1997; Sheng, Tsaur, Jan, & Jan, 1992; Trimmer & Rhodes, 2004; H. Wang, et al., 1993; H. Wang, Kunkel, Schwartzkroin, & Tempel, 1994). Considerable variability in the expression of K_V1 subunits within various brain regions has also been reported. In the hippocampus for instance, Kv1.1, Kv1.2 and Kv1.4 expression reaches the highest levels in the axon terminals of perforant projections, in hilar interneurons as well as in terminals of mossy fibers and Schaffer collaterals within the CA3 and CA1 subfields, respectively (Monaghan, Trimmer, & Rhodes, 2001; Rhodes, Keilbaugh, Barrezueta, Lopez, & Trimmer, 1995; Sheng, et al., 1992; Sheng, Tsaur, Jan, & Jan, 1994; Veh, et al., 1995; Wang, et al., 1993; Wang, et al., 1994). Within the middle third of the molecular layer of the dentate gyrus, K_V1.1 subunits co-localizes with K_V1.2 and K_V1.4 in presynaptic terminals of perforante pathway axons. Similar results have been reported in CA1 Schaffer collaterals, whereas within the CA3 subfield K_V1.1 is expressed in mossy fibers together with K_V1.4 in the absence of the Kv1.2 subunit (Cooper, Milroy, Jan, Jan, & Lowenstein, 1998; Rasband, et al., 1999; Sheng, et al., 1992, 1994; Veh, et

al., 1995; Wang, et al., 1993; Wang, et al., 1994). It should be noted that the data demonstrating co-localization of K_V1 subunits obtained through immuno-histochemistry and light microscopy should be taken with a great deal of caution even when the data highlights strong overlap of the labelling. Veh and colleagues (Veh, et al., 1995) for instance, in their light microscopic study concluded that the majority of K_V1 immuno-reactivity in the dentate gyrus and CA subfields is associated with the dendrites of granule and pyramidal cells, while Sheng and others (Sheng, et al., 1994) assigned intense $K_V1.2$ immunoreactivity to the apical dendritic arbors of hippocampal pyramidal neurons. As demonstrated by subsequent lesion studies, in the hippocampus, channels enriched with the $K_V1.2$ subunit are largely concentrated at axon terminals (Cooper, et al., 1998; Monaghan, et al., 2001). Of note, ablation of entorhinal projections had distinct effects on the distribution of $K_V1.2$ and $K_V1.4$ subunits, an observation which suggests that these two proteins may co-localize on different subsets of axon terminals despite their apparent overlap at light microscopic levels (Monaghan, et al., 2001).

Overall, the results from immuno-fluorescence and biochemical studies indicate that although the prevalent K_V1 subunits are ubiquitously present throughout the mammalian nervous system, both the density and the topography of their distribution varies widely between different brain regions. The latter is likely to reflect the functional significance of individual subunits and, possibly also the level of their redundancy. Relatively low levels of $K_V1.1$ in the hippocampus and cerebellum infer its lower copy number in functional channels. Such an arrangement, as discussed below, would most likely contribute towards the special vulnerability of these two brain regions to mutations in the *KCNA1* gene.

5. K_V1.1 defines the activation threshold and kinetics of the K_V1 current

In the absence of the $K_V\beta$1.1 subunit, most of the K_V1 homo-tetramers (K_V1.1, K_V1.2, K_V1.5 and K_V1.6 subunits) mediate delayed rectifier (non-inactivating) outward currents, with others showing inactivation (K_V1.3, K_V1.4 and K_V1.7). Detailed analysis of the biophysical profiles of K_V1 homo-tetramers, in addition to differences in their inactivation types and kinetics (N-, C-type inactivation and non-inactivating delayed rectifier currents) (Ashcroft, 2000; Hoshi & Armstrong, 2013; Hoshi, Zagotta, & Aldrich, 1990), also revealed subtle but important variations in their activation threshold and kinetics **(TABLE 1)**. Although the physiological significance of these variations between homo-tetramers remain to be established, under certain conditions they are likely to play a decisive role in regulating neuronal activity and synaptic transmission, given that co-assembly of K_V1 subunits in hetero-tetramers yields integral currents with functional characteristics that are somewhat intermediate from there contributing subunits (Akhtar, et al., 2002; Christie, North, Osborne, Douglass, & Adelman, 1990; Grissmer, et al., 1994; Gutman, et al., 2005; Hopkins, Allen, Houamed, & Tempel, 1994; O. Shamotienko, et al., 1999; Stuhmer, et al., 1989) **(FIG. 3)**. Importantly, features of K_V1.1 such as the especially low activation threshold (K_V1.1 $V_{1/2}$= -35 mV < K_V1.6 $V_{1/2}$= -20 mV < K_V1.2 $V_{1/2}$= 5-27 mV < K_V1.4 $V_{1/2}$= 22-34 mV) and fastest activation kinetics (K_V1.1 τ = 5 ms < K_V1.2 τ = 6 ms < K_V1.6 τ = 6-8 ms < K_V1.4 τ = 16.5 ms) (Cox, 2005; Grissmer, et al., 1994; Gutman, et al., 2005; Sokolov, et al., 2007) would be of critical importance in regulating neuronal excitability and responsiveness to fast depolarizing inputs. Indeed, the hierarchy of activation threshold and kinetics entails that during

depolarization, channels enriched with the $K_V1.1$ subunit would be the first to switch on stabilizing outward currents counterbalancing depolarizing inputs and preventing their excessive excitation. It is worth noting that considerable variability in the major functional parameters of K_V1 subunits have been reported, depending on the experimental conditions and expression system, possibly reflective of their differential regulation (Grissmer, et al., 1994; Grupe, et al., 1990; Hatton, et al., 2001; Hulme, Coppock, Felipe, Martens, & Tamkun, 1999; Jeong, Yoon, & Hahn, 2012; Sprunger, Stewig, & O'Grady, 1996; Stuhmer, et al., 1989; Swanson, et al., 1990). Complementary observations were also made with concatenated dimers or tetramers, with the presence of the $K_V1.1$ subunit defining both the activation threshold and kinetics of macroscopic currents (Bagchi, et al., 2014; Sokolov, et al., 2007). Analysis of currents mediated by concatenated $K_V1.1$ / $K_V1.2$ hetero-dimers or hetero-tetramers showed that an increase in the number of $K_V1.1$ subunits in tetramers dose-dependently accelerated the activation kinetics of macroscopic currents and shifted the $V_{1/2}$ towards more negative potentials (Bagchi, et al., 2014; Sokolov, et al., 2007). To define how these rate-limiting traits of $K_V1.1$ could influence the profiles of K_V1 currents and membrane voltage dynamics of myelinated axons and hippocampal pyramidal cells, we used multi-compartmental models (**SUPPL. FIG. 1**). As illustrated, the enrichment of K_V1 channels with $K_V1.1$ alters the activation threshold and kinetics of K^+ currents in favor of reduced electro-responsiveness of the soma and myelinated axons. Interestingly, along with well-known regulation of excitability and conductivity, the $K_V1.1$ subunit also appears to adjust the coupling of the axon initial segment to the soma of neurons, with DTX_K ($K_V1.1$ selective) promoting the invasion of antidromic spikes from the axon initial segment to the somato-

dendritc compartment of the cerebellar projection neurons (Ovsepian, et al., 2013). Thus, in addition to major effects on intracellular mobility and surface expression of K_V1 channels, recruitment of the $K_V1.1$ protein into hetero-tetramers appears to tune their major biophysical characteristics, lowering the activation threshold and accelerating the onset rate of integral K^+ currents.

6. Molecular aberrations in the $K_V1.1$ subunit and related neurological disorders

EA1 is a broad clinical term defining a dominantly inherited multi-faceted neurological disease manifested through a range of disorders, including attacks of cerebellar ataxia triggered by stress, startle or exertion, tremor or cramps of motor groups, vertigo, nystagmus with diplopia and episodes of sporadic seizures. Since the pioneering work by Browne and colleagues that led to the discovery of four *KCNA1* mutations in four different families affected by EA1 (Browne, et al., 1994), more than a dozen mutations in this gene have been reported (D'Adamo, et al., 1999; Herson, et al., 2003; Klein, Lennon, Aston, McKeon, & Pittock, 2012; Poujois, Antoine, Combes, & Touraine, 2006; Rajakulendran, et al., 2007; Schaffer, et al., 1998; Shook, Mamsa, Jen, Baloh, & Zhou, 2008; Spauschus, et al., 1999; Tomlinson, et al., 2010; Zerr, Adelman, & Maylie, 1998a; Zerr, et al., 1998b; Zuberi, et al., 1999). The majority of these are point mutations of conserved residues of the $K_V1.1$ subunit **(FIG. 4)**, with a range of effects on macroscopic K_V1 currents (Adelman, Bond, Pessia, & Maylie, 1995; Boland, Price, & Jackson, 1999; D'Adamo, et al., 1999; Eunson, et al., 2000; Spauschus, et al., 1999; Zerr, et al., 1998a, 1998b; Zuberi, et al., 1999). It is noteworthy that changes of the K_V1 current amplitude do not seem to be the sole cause of neurological deficits in EA1, with rising evidence

pinpointing also the possible role for other biophysical parameters, including activation threshold, gating properties or alterations of the activation and deactivation kinetics (Adelman, et al., 1995; Boland, et al., 1999; D'Adamo, et al., 1999; Eunson, et al., 2000; Spauschus, et al., 1999; Zerr, et al., 1998a, 1998b; Zuberi, et al., 1999). Moreover, electrophysiological studies in heterologous expression systems showed that for some EA1 mutations, the extent of changes in certain characteristics of macroscopic K_V1 currents correlate better with the severity of the neurological phenotypes, implying a possible direct mechanistic link between the alteration of the specific parameters of K_V1 currents and neurological signs (Eunson, et al., 2000; Kullmann, et al., 2001; Rea, Spauschus, Eunson, Hanna, & Kullmann, 2002). Given the importance of K_V1 currents in shaping the bioelectrical activity of neurons and rate-limiting characteristics of the $K_V1.1$ subunit, molecular aberrations associated with EA1 mutations are likely to exert disruptive effects on several important neuronal functions, including their excitability and transmission of electro-chemical signals. In addition to changes of biophysical properties of K_V1 currents, impairments of intracellular mobility and surface expression the $K_V1.1$ protein may also contribute towards the development of EA1 signs (Eunson, et al., 2000; Manganas, Akhtar, et al., 2001; Rea, et al., 2002; Zhu, Alsaber, Zhao, Ribeiro-Hurley, & Thornhill, 2012). It is important to note that despite the considerable overlap in the biophysical profiles between various K_V1 subunits and well-recognized cross-compensation and plasticity formation of tetramers (Kirchheim, Tinnes, Haas, Stegen, & Wolfart, 2013; Wolfart & Laker, 2015), the unique traits of the $K_V1.1$ subunit such as the exceptionally low activation threshold and fast onset rate render the compensation of their functional loss by others problematic. Because no homo-tetrameric $K_V1.1$ channel

has been found in the mammalian nervous system and its expression throughout the brain closely replicates the distribution of the $K_V1.4$ and especially the $K_V1.2$ subunit, neurological signs of EA1 associated with *KCNA1* mutations cannot be attributed to the deficit of $K_V1.1$ homo-tetramers, but imply the disruptive effects of faulty $K_V1.1$ on hetero-tetramers to which they contribute. This notion is in line with the greater vulnerability of cerebellar and hippocampal functions to EA1 mutations, two brain regions with the lowest relative expression of $K_V1.1$ protein. Conceivably, sparse representation of $K_V1.1$ in hetero-tetramers curbs the chances of compensation of the functional deficit of the faulty $K_V1.1$ by the regular partner, rendering the functions of hippocampal and cerebellar neurons especially vulnerable to EA1 mutations. With hippocampal seizures proposed to originate from neurons exhibiting especially low after-discharge threshold (Handforth & Ackermann, 1995; McIntyre & Gilby, 2008; Robbins & Tempel, 2012), deficits of $K_V1.1$ at entorhinal inputs or mossy fibers would most certainly promote the generation of seizures and their spread over the wider limbic areas and other brain regions. Likewise, the pinceau of basket cell axons and soma of deep cerebellar nuclear neurons enriched with $K_V1.1$ could serve as a primary locus of the effects of *KCAN1* mutations in the cerebellum, leading to balance impairments and motor deficit. As proposed earlier, the relatively low levels of $K_V1.1$ (as compared to $K_V1.2$ and $K_V1.4$) in these structures is likely to contribute towards their lower functional reserve and stronger contribution to the neurological phenotypes of EA1.

7. Concluding remarks

Linkage of all human K_V1 channel disorders to mutations in the *KCNA1* gene is surprising given that other members of the *Shaker*-related family, including $K_V1.2$, $K_V1.4$ and $K_V1.6$, are equally or even more widely represented throughout the nervous system. In this review, we have discussed the molecular and biophysical properties of prevalent K_V1 subunits in comparison with $K_V1.1$ and presented evidence suggestive of a critical role for the latter in defining the functional limits of integral K_V1 currents, and possibly contributing to the greater penetrance of *KCNA1* mutations with neurological signs of EA1. The abundance of $K_V1.1$ in a variety of body tissues including cardiomyocytes, retina, skeletal muscles, pancreatic tissue and chromaffin cells (Glasscock, et al., 2015; Gutman, et al., 2005), without overt non-neurological signs in EA1 patients are in line with disruption of neuron-specific functions of $K_V1.1$. We propose that the higher degree of redundancy amongst other members of the *Shaker* family with a closer overlap of biophysical profiles affords a better functional cross-compensation and plasticity under taxing conditions. The situation is different in knockouts of the K_V1 subunits (e.g. -/- *kcna2*), which manifest in mouse models by severe neurological signs. The latter could be perhaps explained (*1*) by complete absence of a sub-population of functional $K_V1.2$ homo-tetramers in -/- *kcna2* mice, which are normally present throughout the nervous system and (*2*) failure of other family members to substitute the role of $K_V1.2$ as a principal partner in the formation of hetero-tetramers. The evidence discussed here implies that the rate-limiting properties of $K_V1.1$ with its low functional reserve, due to its sparse representation in hetero-tetramers in the hippocampus and cerebellum, render these two brain structures especially vulnerable to its functional deficits. The unique molecular and biophysical properties of $K_V1.1$ not only are of major importance in

defining the parameters of K_V1 currents, but also provide appealing targets for developing restorative therapies towards normalizing neuronal functions. Auspiciously, nature provided a valuable model for successful targeting $K_V1.1$ with DTX_K, one of the deadliest of all known toxins, which selectively binds and block channels containing this protein. Highlighted here are the facets of biology and physiology of the $K_V1.1$ subunit which deserve further research, an endeavor with major potential rewards.

FIGURE LEGENDS

FIGURE 1: Family of *Shaker*-related K_V1 channels: an overview. (A) Phylogenetic tree of the gene family of K_V1 channel subunits: amino acid sequence alignment of the human K_V1 channel proteins were generated using CLUSTALW and analyzed by maximum parsimony with PAUP. The IUPHAR and HGNC names are shown together with the genes chromosomal localization. (B) Schematic illustration of the structure of the K_V1 α subunit (top) with crystal structure of $K_V1.2$ - β_2 subunit complex: stereo-view of a ribbon representation from the side (bottom). Four K_V1 α subunits assembled into the channel pore (including the T-domain (T1)) and four associated cytoplasmic β_2 subunits are presented in different color. TM corresponds to the integral membrane component of the complex (adapted with permission from Long et al., 2005). (C) Representative recordings of K_V1 currents mediated via $K_V1.1$ – $K_V1.8$ proteins expressed in heterologous expression systems adapted with permission from (Finol-Urdaneta, Struver, & Terlau, 2006; Heinemann, Rettig, Graack, & Pongs, 1996; Tian, et al., 2002). Current amplitude units – μA.

FIGURE 2: Sub-cellular distribution and functionalities of K_V1 channels in central neurons. (A1-D1) Fluorescence micrographs illustrating the enrichment of the $K_V1.1$ subunit at the presynaptic terminals of a basket cell of the cerebellum (A1); juxta-paranodes (JPN) of optic nerve axons (B1); axonal initial segment of hippocampal pyramidal cells (C1) and soma of the deep cerebellar nuclear neurons (D1). ML – PCL – and GL – molecular, Purkinje cell and granule cell layers, respectively (A1); NR – node of Ranvier (B1); SO – SP and SR – strata oriens, pyramidale and radiatum, respectively

(C1); DN – and IPN – dentate and interpositus nuclei, respectively (D1). (A2-D2) Schematic illustration of the localization and electrophysiological processes (traces below) in neurons involving prevalent subunits of K_V1 channels. At the terminal segment of an axon (TSA), K_V1 subunits regulate parameters of action potentials and release of transmitters from synaptic boutons (SB) onto the soma or dendrites (SD) of postsynaptic neurons (A2). At Ranvier nodes of myelinated axons, JPN K_V1 channels control salutatory propagation of action potentials: PN – and NR – paranode and node of Ranvier (B2). At axon initial segment (AIS) or somato-dendritic (SD) compartments of neurons, K_V1 channels control the generation of action potentials, integration of synaptic inputs and firing precision of neurons, respectively (C2-D2).

FIGURE 3: Hetero-tetramers exhibit membrane currents with biophysical characteristics intermediate of contributing K_V1 subunits. (A) Representative current traces elicited by depolarizing pulses to + 40 mV from – 80 mV; $K_V1.2$, $K_V1.4$ and $K_V1.2$-$K_V1.4$ tandem channels. Scale 1 μA/100 ms adapted with permission from (Ishii, Nunoki, Yamagishi, Okada, & Taira, 2001). Representative current traces elicited by depolarizing pulses to – 50 mV from – 80 mV; $K_V1.1$, $K_V1.2$ and $K_V1.1$ - $K_V1.2$ tandem channels. Scale 100 pA/50 ms: adapted with permission from (Bagchi, et al., 2014). (C, D) Conductance – voltage relation graphs of macroscopic currents: mean and S.E.M. values. Conductance at various command potentials were normalized and fitted with Boltzmann function, with differences of values for $K_V1.1$ and $K_V1.2$ homo-tetramers reaching statistical significance from – 55 mV onward: adapted with permission from (Bagchi, et al., 2014).

FIGURE 4: Human EA1 mutations in the $K_V1.1$ subunit and brain structures with highest vulnerability to *KCNA1* mutations. (A) Schematic illustration of the structure of human $K_V1.1$, indicating the sites of identified mutations associated with EA1. All mutations are at highly conserved residues. With the exception of R417X that results in truncation of the C-terminus, which contains a consensus sequence implicated in anchoring channels, all others are point mutations with AA substitutions, with the 226 position known to be affected by three mutations. (B) A drawing of the human brain (coronal plane), with outlined hippocampus and cerebellum, two structures with the highest susceptibility to EA1 mutations.

TABLE 1: Major biophysical characteristics of K_V1 currents mediated by the various subunits of the *Shaker*-related family. Numerical values are taken from:(Cox, 2005; Gutman, et al., 2005).

Acknowledgments

The authors apologize for not citing all relevant literature on the topic because of space constraints. This work was supported by the Science Foundation of Ireland PI grant (to JOD) and Program for Research in Third Level Institutions Cycle 4 grant from the Irish Higher Educational Authority for the Neuroscience section of 'Targeted-driven therapeutics and theranostics' (JOD and SVO).

Conflict of interest statement

The authors declare that there are no conflicts of interest.

REFERENCES

Adelman, J. P., Bond, C. T., Pessia, M., & Maylie, J. (1995). Episodic ataxia results from voltage-dependent potassium channels with altered functions. *Neuron, 15*, 1449-1454.

Akhtar, S., Shamotienko, O., Papakosta, M., Ali, F., & Dolly, J. O. (2002). Characteristics of brain Kv1 channels tailored to mimic native counterparts by tandem linkage of alpha subunits: implications for K+ channelopathies. *J Biol Chem, 277*, 16376-16382.

Ashcroft, F. M. (2000). *Ion channels and disease : channelopathies*. San Diego: Academic Press.

Bagchi, B., Al-Sabi, A., Kaza, S., Scholz, D., O'Leary, V. B., Dolly, J. O., & Ovsepian, S. V. (2014). Disruption of myelin leads to ectopic expression of K(V)1.1 channels with abnormal conductivity of optic nerve axons in a cuprizone-induced model of demyelination. *PLoS One, 9*, e87736.

Boland, L. M., Price, D. L., & Jackson, K. A. (1999). Episodic ataxia/myokymia mutations functionally expressed in the Shaker potassium channel. *Neuroscience, 91*, 1557-1564.

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.

Brew, H. M., Hallows, J. L., & Tempel, B. L. (2003). Hyperexcitability and reduced low threshold potassium currents in auditory neurons of mice lacking the channel subunit Kv1.1. *J Physiol, 548*, 1-20.

Browne, D. L., Gancher, S. T., Nutt, J. G., Brunt, E. R., 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.

Christie, M. J., North, R. A., Osborne, P. B., Douglass, J., & Adelman, J. P. (1990). Heteropolymeric potassium channels expressed in Xenopus oocytes from cloned subunits. *Neuron, 4*, 405-411.

Clark, B. D., Goldberg, E. M., & Rudy, B. (2009). Electrogenic tuning of the axon initial segment. *Neuroscientist, 15*, 651-668.

Coleman, S. K., Newcombe, J., Pryke, J., & Dolly, J. O. (1999). Subunit composition of Kv1 channels in human CNS. *J Neurochem, 73*, 849-858.

Cooper, E. C., Milroy, A., Jan, Y. N., Jan, L. Y., & Lowenstein, D. H. (1998). Presynaptic localization of Kv1.4-containing A-type potassium channels near excitatory synapses in the hippocampus. *J Neurosci, 18*, 965-974.

Cox, R. H. (2005). Molecular determinants of voltage-gated potassium currents in vascular smooth muscle. *Cell Biochem Biophys, 42*, 167-195.

D'Adamo, M. C., Imbrici, P., Sponcichetti, F., & Pessia, M. (1999). Mutations in the KCNA1 gene associated with episodic ataxia type-1 syndrome impair heteromeric voltage-gated K(+) channel function. *FASEB J, 13*, 1335-1345.

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.

Dodson, P. D., Barker, M. C., & Forsythe, I. D. (2002). Two heteromeric Kv1 potassium channels differentially regulate action potential firing. *J Neurosci, 22*, 6953-6961.

Dodson, P. D., Billups, B., Rusznak, Z., Szucs, 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.

Dolly, J. O., & Parcej, D. N. (1996). Molecular properties of voltage-gated K+ channels. *J Bioenerg Biomembr, 28*, 231-253.

Eunson, L. H., Rea, R., Zuberi, S. M., Youroukos, S., Panayiotopoulos, C. P., Liguori, R., Avoni, P., McWilliam, R. C., Stephenson, J. B., Hanna, M. G., Kullmann, D. M., & Spauschus, A. (2000). Clinical, genetic, and expression studies of mutations in the potassium channel gene KCNA1 reveal new phenotypic variability. *Ann Neurol, 48*, 647-656.

Finol-Urdaneta, R. K., Struver, N., & Terlau, H. (2006). Molecular and Functional Differences between Heart mKv1.7 Channel Isoforms. *J Gen Physiol, 128*, 133-145.

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. (2015). Expression and function of Kv1.1 potassium channels in human atria from patients with atrial fibrillation. *Basic Res Cardiol, 110*, 505.

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.

Grupe, A., Schroter, 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.

Gutman, G. A., Chandy, K. G., Grissmer, S., Lazdunski, M., McKinnon, D., Pardo, L. A., Robertson, G. A., Rudy, B., Sanguinetti, M. C., Stuhmer, W., & Wang, X. (2005). International Union of Pharmacology. LIII. Nomenclature and molecular relationships of voltage-gated potassium channels. *Pharmacol Rev, 57*, 473-508.

Handforth, A., & Ackermann, R. F. (1995). Mapping of limbic seizure progressions utilizing the electrogenic status epilepticus model and the 14C-2-deoxyglucose method. *Brain Res Brain Res Rev, 20*, 1-23.

Hatton, W. J., Mason, H. S., Carl, A., Doherty, P., Latten, M. J., Kenyon, J. L., Sanders, K. M., & Horowitz, B. (2001). Functional and molecular expression of a voltage-dependent K(+) channel (Kv1.1) in interstitial cells of Cajal. *J Physiol, 533*, 315-327.

Heinemann, S. H., Rettig, J., Graack, H. R., & Pongs, O. (1996). Functional characterization of Kv channel beta-subunits from rat brain. *J Physiol, 493 (Pt 3)*, 625-633.

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.

Heusser, K., & Schwappach, B. (2005). Trafficking of potassium channels. *Curr Opin Neurobiol, 15*, 364-369.

Hille, B. (2001). *Ion channels of excitable membranes* (3rd ed.). Sunderland, Mass.: Sinauer.

Hopkins, W. F., Allen, M. L., Houamed, K. M., & Tempel, B. L. (1994). Properties of voltage-gated K+ currents expressed in Xenopus oocytes by mKv1.1, mKv1.2 and their heteromultimers as revealed by mutagenesis of the dendrotoxin-binding site in mKv1.1. *Pflugers Arch, 428*, 382-390.

Hoshi, T., & Armstrong, C. M. (2013). C-type inactivation of voltage-gated K+ channels: pore constriction or dilation? *J Gen Physiol, 141*, 151-160.

Hoshi, T., Zagotta, W. N., & Aldrich, R. W. (1990). Biophysical and molecular mechanisms of Shaker potassium channel inactivation. *Science, 250*, 533-538.

Hulme, J. T., Coppock, E. A., Felipe, A., Martens, J. R., & Tamkun, M. M. (1999). Oxygen sensitivity of cloned voltage-gated K(+) channels expressed in the pulmonary vasculature. *Circ Res, 85*, 489-497.

Hurst, R. S., Busch, A. E., Kavanaugh, M. P., Osborne, P. B., North, R. A., & Adelman, J. P. (1991). Identification of amino acid residues involved in dendrotoxin block of rat voltage-dependent potassium channels. *Mol Pharmacol, 40*, 572-576.

Imbrici, P., D'Adamo, M. C., Kullmann, D. M., & Pessia, M. (2006). Episodic ataxia type 1 mutations in the KCNA1 gene impair the fast inactivation properties of the human potassium channels Kv1.4-1.1/Kvbeta1.1 and Kv1.4-1.1/Kvbeta1.2. *Eur J Neurosci, 24*, 3073-3083.

Imredy, J. P., & MacKinnon, R. (2000). Energetic and structural interactions between delta-dendrotoxin and a voltage-gated potassium channel. *J Mol Biol, 296*, 1283-1294.

Isacoff, E. Y., Jan, Y. N., & Jan, L. Y. (1990). Evidence for the formation of heteromultimeric potassium channels in Xenopus oocytes. *Nature, 345*, 530-534.

Ishii, K., Nunoki, K., Yamagishi, T., Okada, H., & Taira, N. (2001). Differential sensitivity of Kv1.4, Kv1.2, and their tandem channel to acidic pH: involvement of a histidine residue in high sensitivity to acidic pH. *J Pharmacol Exp Ther, 296*, 405-411.

Jan, L. Y., & Jan, Y. N. (2012). Voltage-gated potassium channels and the diversity of electrical signalling. *J Physiol, 590*, 2591-2599.

Jensen, C. S., Rasmussen, H. B., & Misonou, H. (2011). Neuronal trafficking of voltage-gated potassium channels. *Mol Cell Neurosci, 48*, 288-297.

Jeong, I., Yoon, S. H., & Hahn, S. J. (2012). Effects of dapoxetine on cloned Kv1.5 channels expressed in CHO cells. *Naunyn Schmiedebergs Arch Pharmacol, 385*, 707-716.

Johnston, J., Forsythe, I. D., & Kopp-Scheinpflug, C. (2010). Going native: voltage-gated potassium channels controlling neuronal excitability. *J Physiol, 588*, 3187-3200.

Kamb, A., Tseng-Crank, J., & Tanouye, M. A. (1988). Multiple products of the Drosophila Shaker gene may contribute to potassium channel diversity. *Neuron, 1*, 421-430.

Kirchheim, F., Tinnes, S., Haas, C. A., Stegen, M., & Wolfart, J. (2013). Regulation of action potential delays via voltage-gated potassium Kv1.1 channels in dentate granule cells during hippocampal epilepsy. *Front Cell Neurosci, 7*, 248.

Klein, C. J., Lennon, V. A., Aston, P. A., McKeon, A., & Pittock, S. J. (2012). Chronic pain as a manifestation of potassium channel-complex autoimmunity. *Neurology, 79*, 1136-1144.

Koch, R. O., Wanner, S. G., Koschak, A., Hanner, M., Schwarzer, C., Kaczorowski, G. J., Slaughter, R. S., Garcia, M. L., & Knaus, H. G. (1997). Complex subunit assembly of neuronal voltage-gated K+ channels. Basis for high-affinity toxin interactions and pharmacology. *J Biol Chem, 272*, 27577-27581.

Kuba, H., Yamada, R., Ishiguro, G., & Adachi, R. (2015). Redistribution of Kv1 and Kv7 enhances neuronal excitability during structural axon initial segment plasticity. *Nat Commun, 6*, 8815.

Kullmann, D. M. (2002). The neuronal channelopathies. *Brain, 125*, 1177-1195.

Kullmann, D. M., Rea, R., Spauschus, A., & Jouvenceau, A. (2001). The inherited episodic ataxias: how well do we understand the disease mechanisms? *Neuroscientist, 7*, 80-88.

Li, D., Takimoto, K., & Levitan, E. S. (2000). Surface expression of Kv1 channels is governed by a C-terminal motif. *J Biol Chem, 275*, 11597-11602.

Lodish, H. F., & Kong, N. (1983). Reversible block in intracellular transport and budding of mutant vesicular stomatitis virus glycoproteins. *Virology, 125*, 335-348.

London, B., Wang, D. W., Hill, J. A., & Bennett, P. B. (1998). The transient outward current in mice lacking the potassium channel gene Kv1.4. *J Physiol, 509 (Pt 1)*, 171-182.

Manganas, L. N., Akhtar, S., Antonucci, D. E., Campomanes, C. R., Dolly, J. O., & Trimmer, J. S. (2001). Episodic ataxia type-1 mutations in the Kv1.1 potassium channel display distinct folding and intracellular trafficking properties. *J Biol Chem, 276*, 49427-49434.

Manganas, L. N., & Trimmer, J. S. (2000). Subunit composition determines Kv1 potassium channel surface expression. *J Biol Chem, 275*, 29685-29693.

Manganas, L. N., Wang, Q., Scannevin, R. H., Antonucci, D. E., Rhodes, K. J., & Trimmer, J. S. (2001). Identification of a trafficking determinant localized to the Kv1 potassium channel pore. *Proc Natl Acad Sci U S A, 98*, 14055-14059.

McIntyre, D. C., & Gilby, K. L. (2008). Mapping seizure pathways in the temporal lobe. *Epilepsia, 49 Suppl 3*, 23-30.

McNamara, N. M., Averill, S., Wilkin, G. P., Dolly, J. O., & Priestley, J. V. (1996). Ultrastructural localization of a voltage-gated K+ channel alpha subunit (KV 1.2) in the rat cerebellum. *Eur J Neurosci, 8*, 688-699.

McNamara, N. M., Muniz, Z. M., Wilkin, G. P., & Dolly, J. O. (1993). Prominent location of a K+ channel containing the alpha subunit Kv 1.2 in the basket cell nerve terminals of rat cerebellum. *Neuroscience, 57*, 1039-1045.

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, 21*, 5973-5983.

Muniz, Z. M., Parcej, D. N., & Dolly, J. O. (1992). Characterization of monoclonal antibodies against voltage-dependent K+ channels raised using alpha-dendrotoxin acceptors purified from bovine brain. *Biochemistry, 31*, 12297-12303.

Nagaya, N., & Papazian, D. M. (1997). Potassium channel alpha and beta subunits assemble in the endoplasmic reticulum. *J Biol Chem, 272*, 3022-3027.

Norris, A. J., Foeger, N. C., & Nerbonne, J. M. (2010). Neuronal voltage-gated K+ (Kv) channels function in macromolecular complexes. *Neurosci Lett, 486*, 73-77.

Ovsepian, S. V., Steuber, V., Le Berre, M., O'Hara, L., O'Leary, V. B., & Dolly, J. O. (2013). A defined heteromeric KV1 channel stabilizes the intrinsic pacemaking and regulates the output of deep cerebellar nuclear neurons to thalamic targets. *J Physiol, 591*, 1771-1791.

Papazian, D. M., Schwarz, T. L., Tempel, B. L., Jan, Y. N., & Jan, L. Y. (1987). Cloning of genomic and complementary DNA from Shaker, a putative potassium channel gene from Drosophila. *Science, 237*, 749-753.

Parcej, D. N., Scott, V. E., & Dolly, J. O. (1992). Oligomeric properties of alpha-dendrotoxin-sensitive potassium ion channels purified from bovine brain. *Biochemistry, 31*, 11084-11088.

Pongs, O., Kecskemethy, N., Muller, R., Krah-Jentgens, I., Baumann, A., Kiltz, H. H., Canal, I., Llamazares, S., & Ferrus, A. (1988). Shaker encodes a family of putative potassium channel proteins in the nervous system of Drosophila. *EMBO J, 7*, 1087-1096.

Poujois, A., Antoine, J. C., Combes, A., & Touraine, R. L. (2006). Chronic neuromyotonia as a phenotypic variation associated with a new mutation in the KCNA1 gene. *J Neurol, 253*, 957-959.

Rajakulendran, S., Schorge, S., Kullmann, D. M., & Hanna, M. G. (2007). Episodic ataxia type 1: a neuronal potassium channelopathy. *Neurotherapeutics, 4*, 258-266.

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.

Rasband, M. N., & Shrager, P. (2000). Ion channel sequestration in central nervous system axons. *J Physiol, 525 Pt 1*, 63-73.

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.

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, 18*, 36-47.

Rea, R., Spauschus, A., Eunson, L. H., Hanna, M. G., & Kullmann, D. M. (2002). Variable K(+) channel subunit dysfunction in inherited mutations of KCNA1. *J Physiol, 538*, 5-23.

Rettig, J., Heinemann, S. H., Wunder, F., Lorra, C., Parcej, D. N., Dolly, J. O., & Pongs, O. (1994). Inactivation properties of voltage-gated K+ channels altered by presence of beta-subunit. *Nature, 369*, 289-294.

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, 15*, 5360-5371.

Rhodes, K. J., Monaghan, M. M., Barrezueta, N. X., Nawoschik, S., Bekele-Arcuri, Z., Matos, M. F., Nakahira, K., Schechter, L. E., & Trimmer, J. S. (1996). Voltage-gated K+ channel beta subunits: expression and distribution of Kv beta 1 and Kv beta 2 in adult rat brain. *J Neurosci, 16*, 4846-4860.

Rhodes, K. J., Strassle, B. W., Monaghan, M. M., Bekele-Arcuri, Z., Matos, M. F., & Trimmer, J. S. (1997). Association and colocalization of the Kvbeta1 and Kvbeta2 beta-subunits with Kv1 alpha-subunits in mammalian brain K+ channel complexes. *J Neurosci, 17*, 8246-8258.

Robbins, C. A., & Tempel, B. L. (2012). Kv1.1 and Kv1.2: similar channels, different seizure models. *Epilepsia, 53 Suppl 1*, 134-141.

Ruppersberg, J. P., Schroter, K. H., Sakmann, B., Stocker, M., Sewing, S., & Pongs, O. (1990). Heteromultimeric channels formed by rat brain potassium-channel proteins. *Nature, 345*, 535-537.

Schaffer, P., Pelzmann, B., Bernhart, E., Lang, P., Lokebo, J. E., Machler, H., Rigler, B., & Koidl, B. (1998). Estimation of outward currents in isolated human atrial myocytes using inactivation time course analysis. *Pflugers Arch, 436*, 457-468.

Scott, V. E., Muniz, Z. M., Sewing, S., Lichtinghagen, R., Parcej, D. N., Pongs, O., & Dolly, J. O. (1994). Antibodies specific for distinct Kv subunits unveil a heterooligomeric basis for subtypes of alpha-dendrotoxin-sensitive K+ channels in bovine brain. *Biochemistry, 33*, 1617-1623.

Shamotienko, O., Akhtar, S., Sidera, C., Meunier, F. A., Ink, B., Weir, M., & Dolly, J. O. (1999). Recreation of neuronal Kv1 channel oligomers by expression in mammalian cells using Semliki Forest virus. *Biochemistry, 38*, 16766-16776.

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.

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.

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, 14*, 2408-2417.

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.

Shook, S. J., Mamsa, H., Jen, J. C., Baloh, R. W., & Zhou, L. (2008). Novel mutation in KCNA1 causes episodic ataxia with paroxysmal dyspnea. *Muscle Nerve, 37*, 399-402.

Smart, S. L., Lopantsev, V., Zhang, C. L., Robbins, C. A., Wang, H., Chiu, S. Y., Schwartzkroin, P. A., Messing, A., & Tempel, B. L. (1998). Deletion of the K(V)1.1 potassium channel causes epilepsy in mice. *Neuron, 20*, 809-819.

Sokolov, M. V., Shamotienko, O., Dhochartaigh, S. N., Sack, J. T., & Dolly, J. O. (2007). Concatemers of brain Kv1 channel alpha subunits that give similar K+ currents

yield pharmacologically distinguishable heteromers. *Neuropharmacology, 53*, 272-282.

Spauschus, A., Eunson, L., Hanna, M. G., & Kullmann, D. M. (1999). Functional characterization of a novel mutation in KCNA1 in episodic ataxia type 1 associated with epilepsy. *Ann N Y Acad Sci, 868*, 442-446.

Sprunger, L. K., Stewig, N. J., & O'Grady, S. M. (1996). Effects of charybdotoxin on K+ channel (KV1.2) deactivation and inactivation kinetics. *Eur J Pharmacol, 314*, 357-364.

Stuhmer, W., Ruppersberg, J. P., Schroter, K. H., Sakmann, B., Stocker, M., Giese, K. P., Perschke, A., Baumann, A., & Pongs, O. (1989). Molecular basis of functional diversity of voltage-gated potassium channels in mammalian brain. *EMBO J, 8*, 3235-3244.

Swanson, R., Marshall, J., Smith, J. S., Williams, J. B., Boyle, M. B., Folander, K., Luneau, C. J., Antanavage, J., Oliva, C., Buhrow, S. A., & et al. (1990). Cloning and expression of cDNA and genomic clones encoding three delayed rectifier potassium channels in rat brain. *Neuron, 4*, 929-939.

Tempel, B. L., Jan, Y. N., & Jan, L. Y. (1988). Cloning of a probable potassium channel gene from mouse brain. *Nature, 332*, 837-839.

Tian, S., Liu, W., Wu, Y., Rafi, H., Segal, A. S., & Desir, G. V. (2002). Regulation of the voltage-gated K+ channel KCNA10 by KCNA4B, a novel beta-subunit. *Am J Physiol Renal Physiol, 283*, F142-149.

Tomlinson, S. E., Tan, S. V., Kullmann, D. M., Griggs, R. C., Burke, D., Hanna, M. G., & Bostock, H. (2010). Nerve excitability studies characterize Kv1.1 fast potassium channel dysfunction in patients with episodic ataxia type 1. *Brain, 133*, 3530-3540.

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.

Trimmer, J. S., & Rhodes, K. J. (2004). Localization of voltage-gated ion channels in mammalian brain. *Annu Rev Physiol, 66*, 477-519.

Tytgat, J., Debont, T., Carmeliet, E., & Daenens, P. (1995). The alpha-dendrotoxin footprint on a mammalian potassium channel. *J Biol Chem, 270*, 24776-24781.

Vacher, H., Misonou, H., & Trimmer, J. S. (2007). *Determinants of voltage gated potassium channel distribution in neurons*. Amsterdam ; Boston: Elsevier/Academic Press.

Vacher, H., Mohapatra, D. P., Misonou, H., & Trimmer, J. S. (2007). Regulation of Kv1 channel trafficking by the mamba snake neurotoxin dendrotoxin K. *FASEB J, 21*, 906-914.

Vacher, H., Mohapatra, D. P., & Trimmer, J. S. (2008). Localization and targeting of voltage-dependent ion channels in mammalian central neurons. *Physiol Rev, 88*, 1407-1447.

Veh, R. W., Lichtinghagen, R., Sewing, S., Wunder, F., Grumbach, I. M., & Pongs, O. (1995). Immunohistochemical localization of five members of the Kv1 channel subunits: contrasting subcellular locations and neuron-specific co-localizations in rat brain. *Eur J Neurosci, 7*, 2189-2205.

Wang, F. C., Parcej, D. N., & Dolly, J. O. (1999). alpha subunit compositions of Kv1.1-containing K+ channel subtypes fractionated from rat brain using dendrotoxins. *Eur J Biochem, 263*, 230-237.

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.

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, 14*, 4588-4599.

Wolfart, J., & Laker, D. (2015). Homeostasis or channelopathy? Acquired cell type-specific ion channel changes in temporal lobe epilepsy and their antiepileptic potential. *Front Physiol, 6*, 168.

Yellen, G. (2002). The voltage-gated potassium channels and their relatives. *Nature, 419*, 35-42.

Zerr, P., Adelman, J. P., & Maylie, J. (1998a). Characterization of three episodic ataxia mutations in the human Kv1.1 potassium channel. *FEBS Lett, 431*, 461-464.

Zerr, P., Adelman, J. P., & Maylie, J. (1998b). Episodic ataxia mutations in Kv1.1 alter potassium channel function by dominant negative effects or haploinsufficiency. *J Neurosci, 18*, 2842-2848.

Zhu, J., Alsaber, R., Zhao, J., Ribeiro-Hurley, E., & Thornhill, W. B. (2012). Characterization of the Kv1.1 I262T and S342I mutations associated with episodic ataxia 1 with distinct phenotypes. *Arch Biochem Biophys, 524*, 99-105.

Zhu, J., Gomez, B., Watanabe, I., & Thornhill, W. B. (2005). Amino acids in the pore region of Kv1 potassium channels dictate cell-surface protein levels: a possible trafficking code in the Kv1 subfamily. *Biochem J, 388*, 355-362.

Zhu, J., Watanabe, I., Gomez, B., & Thornhill, W. B. (2001). Determinants involved in Kv1 potassium channel folding in the endoplasmic reticulum, glycosylation in the Golgi, and cell surface expression. *J Biol Chem, 276*, 39419-39427.

Zhu, J., Watanabe, I., Gomez, B., & Thornhill, W. B. (2003). Trafficking of Kv1.4 potassium channels: interdependence of a pore region determinant and a cytoplasmic C-terminal VXXSL determinant in regulating cell-surface trafficking. *Biochem J, 375*, 761-768.

Zuberi, S. M., Eunson, L. H., Spauschus, A., De Silva, R., Tolmie, J., Wood, N. W., McWilliam, R. C., Stephenson, J. B., Kullmann, D. M., & Hanna, M. G. (1999). A novel mutation in the human voltage-gated potassium channel gene (Kv1.1) associates with episodic ataxia type 1 and sometimes with partial epilepsy. *Brain, 122 (Pt 5)*, 817-825.