NEURAL REGENERATION RESEARCH
www.nrronline.org

K^+ channel-mediated retarded maturation of interneurons and its role in neurodevelopmental disorders

Kaizhen Li, Daria Savitska, Olga Garaschuk*

De novo mutations in genes encoding K^+ channels are implicated in many severe neurodevelopmental disorders. Specifically, mutations in KCNA2, encoding the Shaker-type voltage-gated K^+ channel Kv1.2, and KCNJ2, encoding the inwardly rectifying K^+ channel Kir2.1, associate with focal and generalized epilepsies, brain atrophy, autism, ataxia and hereditary spastic paraplegia (Syrbe et al., 2015; Masnada et al., 2017; Cheng et al., 2021). Complicated forms of the disease often include other neurological manifestations, such as cognitive impairment/intellectual disability, aggressiveness, irritability, dysarthria, cerebellar atrophy, polyneuropathy, or amyotrophy (Helbig et al., 2016; Masnada et al., 2017). Strikingly, the gain-of-function mutations of Kv1.2 channels, which are supposed to promote neuronal repolarization and termination of neuronal firing, caused more severe symptoms in terms of epilepsy, ataxia, and intellectual disability than the loss-of-function mutations, which are supposed to promote neuronal hyperactivity (Syrbe et al., 2015; Allen et al., 2020). Likewise, gain-of-function mutations in a Kir2.1 channel were shown to be associated with autism spectrum disorder (Cheng et al., 2021). Moreover, a recent study has shown that Kir2.1 is highly expressed in medulloblastoma, one of the most common childhood malignant brain tumors (Wang et al., 2022). In these cells, Kir2.1 promoted tumor cell invasion, metastasis, as well as epithelial-mesenchymal transitions, and higher levels of Kir2.1 expression were associated with the significantly shorter lifespan of the patients.

The overexpression of Kv1.2/Kir2.1 K^+ channels, which in humans and mice are present in both excitatory and inhibitory neurons (https://www.proteinatlas.org; http://mousebrain.org), mimics their gain of function. Using the mouse olfactory bulb as a model system, we have recently shown that overexpression of Kv1.2 or Kir2.1 channels in the two different types of adult-born GABAergic interneurons (juxtaglomerular and granule cells) dramatically impairs their migration, differentiation, morphogenesis (**Figure 1A–D**) and survival as well as their ability to integrate into the existent neuronal circuitry (Li et al., 2023). The *in vivo* two-photon imaging of the juxtaglomerular neurons and the RNA sequencing data suggest that this developmental retardation was caused by a reduced Ca^{2+} entry via voltage-gated Ca^{2+} channels and the NMDA receptor channels, reduced cytosolic fluctuations of the intracellular free Ca^{2+} concentration, reduced activation of the Ca^{2+}/calmodulin kinase pathway and phosphorylation of CREB as well as a specific downregulation of the CREB-mediated gene expression (see Figures 5–7 in Li et al., 2023). At the mRNA level, a concomitant decrease in the expression of anti-apoptotic and an increase in the expression of pro-apoptotic genes suggested a plausible molecular mechanism for a decreased survival rate of adult-born interneurons.

In contrast to the dramatic changes in neuronal development described above, our experimental paradigm had a surprisingly small effect on the membrane properties of adult-born cells, including their input resistance, the threshold for firing action potentials, or the action potential amplitude and duration. Moreover, the overexpression of the non-conducting dominant-negative mutant of the Kir2.1 channel had little effect on dendritic morphogenesis (Li et al., 2023), thus supporting the notion that the membrane excitability per se is not a key determinant of interneuronal maturation. Interestingly, a recent study analyzing a loss-of-function mutation in another voltage-gated potassium channel (Kv2.1 encoded by the *KCNB1* gene) lends support to this idea by showing that Kv2.1 channels form macromolecular complexes with integrins, and these complexes can regulate migration, proliferation and survival of cortical excitatory (pyramidal) neurons via metabotropic pathways (see Figure S7 in Bortolami et al., 2023).

Considering common molecular pathways shared by the adult and neonatal neurogenesis (Spitzer, 2006; Bando et al., 2014), we tested whether findings, similar to that obtained by (Li et al., 2023) in adult-born GABAergic cells of the olfactory bulb, also hold true for neonatal cortical interneurons. Indeed, GABAergic cortical interneurons, overexpressing Kv1.2 channels by means of *in utero* viral transduction, had a significantly reduced (i) dendritic complexity and (ii) a total dendritic branch length, as well as a significantly smaller number of dendritic (iii) branches, (iv) branch points and (v) endings (**Figure 1E–L**). Together, these data identify the retarded morphogenesis, synaptic wiring, and survival of GABAergic interneurons as a robust consequence of the increased K^+ channel function and as a possible cause of neural network hyperactivity, seizure susceptibility, brain atrophy, and ataxia in carriers of Kv1.2/Kir2.1 gain-of-function variants.

Based on these new and unexpected findings we propose the retarded interneuron development as a key mechanism underlying the aforementioned developmental pathologies. These pathologies are likely further exacerbated by increased apoptosis of the interneuronal population (Li et al., 2023), which can be either promoted by the enhanced transmembrane K^+ efflux (Shah and Aizenman, 2014) or represent a consequence of dysfunctional ongoing Ca^{2+} signaling (Spitzer, 2006) in these cells. The heightened apoptosis, in turn, likely causes neuroinflammation by excessively activating microglia, the immune cells of the brain, thus providing a mechanistic connection between the Kv1.2/Kir2.1 channel dysfunction and autism spectrum disorder. Interestingly, autism was recently associated with excitatory to inhibitory imbalance, caused by the aberrant synaptic pruning by microglia and resulting in increased levels of excitatory synaptic inputs and impaired social behavior (Xiong et al., 2023). Further pathways by which the activated microglia can increase or modulate the excitation/inhibition ratio include (i) impaired glutamate uptake, (ii) heightened release of excitatory neurotransmitters (e.g., glutamate, D-serine or ATP), and (iii) potentiation of the gliotransmitter release from astrocytes (Xiong et al., 2023). Microglia-mediated pruning of GABAergic synapses might also contribute to the excitatory to inhibitory imbalance, but it remains unclear whether and when such pruning becomes dysfunctional.

In conclusion, our perspective offers a different mechanistic view on the repeatedly experimentally documented association between the gain-of-function of Kv1.2/Kir2.1 potassium channels and severe neurodevelopmental disorders like epilepsy, autism, ataxia, and intellectual disability. We draw the reader's attention to the retarded growth, morphogenesis, wiring, and survival of local GABAergic interneurons as well as accompanying microglia-mediated neuroinflammation as important mechanistic causes of these diseases. This hypothesis is new to the neurodevelopmental field, which in the case of Kv1.2/Kir2.1 channel dysfunction as well as in the case of developmental and epileptic encephalopathies, is still thinking in the neurocentric categories of impaired neuronal firing (Masnada et al., 2017; Allen et al., 2020).

We thank E. Zirdum, A. Weible, and K. Schöntag (Institute of Physiology, Department of Neurophysiology, Eberhard Karls University of Tübingen) for technical assistance and X. Su (Institute of Physiology, Department of Neurophysiology, Eberhard Karls University of Tübingen) for his help with reconstructing neuronal morphology.

This work was supported by the German Research Foundation DFG grant GA 654/13-2 to OG.

Kaizhen Li, Daria Savitska, Olga Garaschuk*
Laboratory of Systems Neuroscience, Department of Physiology, University of Bern, Bern, Switzerland (Li K)

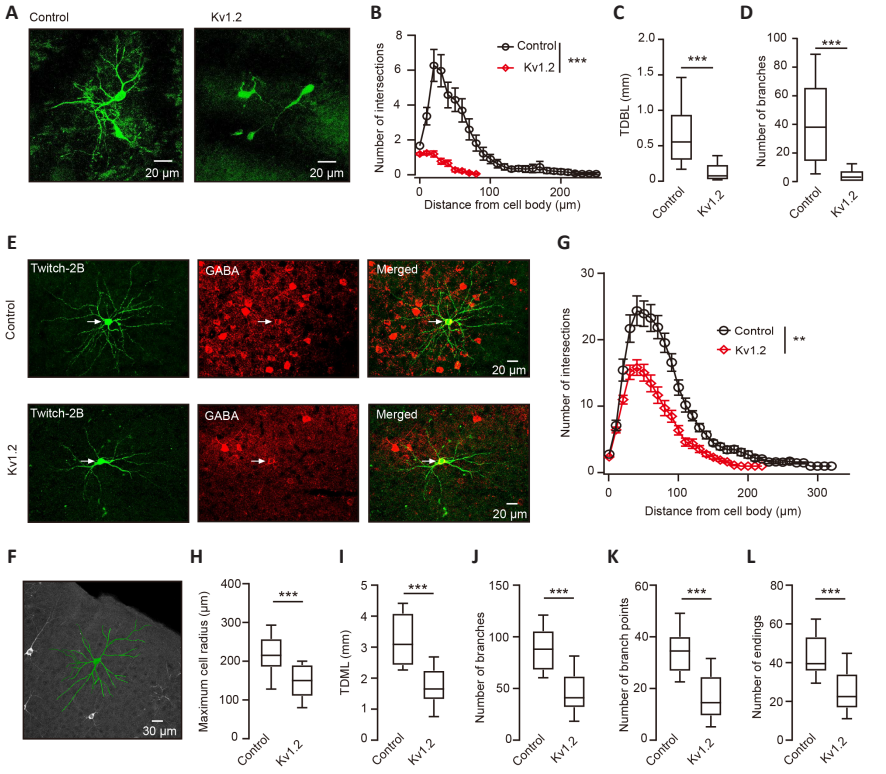

Figure 1 | **Kv1.2 channel overexpression impairs the morphogenesis of interneurons.**
(A) Sample *in vivo* maximum projection images (0-24 µm below the dura) of adult-born GABAergic juxtaglomerular neurons in the olfactory bulb transduced by virus encoding either a green fluorescing Ca^{2+} indicator Twitch-2B (Control) or a Twitch-2B-T2A-Kv1.2 construct (Kv1.2), both expressed under the ubiquitin promoter. (B) Sholl analysis, showing the number of intersections of centered Sholl spheres (here and below 10 µm step size) with the dendritic trees of adult-born juxtaglomerular neurons belonging to either Control or Kv1.2 groups. (C, D) Box plots showing the median (per cell) total dendritic branch length (TDBL, C) and the number of dendritic branches (D) of adult-born juxtaglomerular neurons (*n* = 33/7 and 36/6 cells/mice for control and Kv1.2 groups, respectively). The data shown in A–D belong to the same data set as the one published in Li et al. (2023) and are reproduced under the CC BY 4.0 license. (E) Sample images of cortical neurons transduced in utero either with viruses encoding Twitch-2B (Control) or the Twitch-2B-T2A-Kv1.2 construct (Kv1.2) and labeled in the tissue fixed at DPI 28 with antibodies against GFP (recognizes Twitch-2B, green) and GABA (red). (F) Sample reconstruction of the cell's morphology. (G) Sholl analysis, showing the number of intersections of centered Sholl spheres with the dendritic trees of prenatally born GABAergic cortical interneurons belonging to either control or Kv1.2 groups. (H) Box plot showing the maximum (per cell) cell radius. Here and below *n* = 18/4 and 38/3 cells/mice for control and Kv1.2 groups, respectively. (I–L) Box plots showing the median (per cell) total dendritic branch length (I) and the number of dendritic branches (J), branch points (K), and endings (L) of prenatally born GABAergic cortical interneurons. A generalized linear mixed effect model was used for Sholl analysis statistics and the Mann-Whitney test for box plots. $^{**}P < 0.01$, $^{***}P < 0.001$. (E–L) Unpublished data, sourced from the authors' laboratory. DPI: Days post injection; GABA: γ-aminobutyric acid; GFP: green fluorescent protein; TDBL: total dendritic branch length.

Institute of Physiology, Department of Neurophysiology, Eberhard Karls University of Tübingen, Tübingen, Germany (Savitska D, Garaschuk O)
***Correspondence to:** Olga Garaschuk, PhD, olga.garaschuk@uni-tuebingen.de.
https://orcid.org/0000-0001-7400-5654 (Olga Garaschuk)
Date of submission: June 26, 2023
Date of decision: August 26, 2023
Date of acceptance: September 6, 2023
Date of web publication: October 2, 2023

https://doi.org/10.4103/1673-5374.386409
How to cite this article: *Li K, Savitska D, Garaschuk O (2024) K^+ channel-mediated retarded maturation of interneurons and its role in neurodevelopmental disorders. Neural Regen Res 19(7):1403-1404.*

References

Allen NM, Weckhuysen S, Gorman K, King MD, Lerche H (2020) Genetic potassium channel-associated epilepsies: Clinical review of the K(v) family. Eur J Paediatr Neurol 24:105-116.

Bando Y, Hirano T, Tagawa Y (2014) Dysfunction of KCNK potassium channels impairs neuronal migration in the developing mouse cerebral cortex. Cereb Cortex 24:1017-1029.

Bortolami A, Yu W, Forzisi E, Ercan K, Kadakia R, Murugan M, Fedele D, Estevez I, Boison D, Rasin MR, Sesti F (2023) Integrin-KCNB1 potassium channel complexes regulate neocortical neuronal development and are implicated in epilepsy. Cell Death Differ 30:687-701.

Cheng P, Qiu Z, Du Y (2021) Potassium channels and autism spectrum disorder: an overview. Int J Dev Neurosci 81:479-491.

Helbig KL, Hedrich UBS, Shinde DN, Krey I, Teichmann AC, Hentschel J, Schubert J, Chamberlin AC, Huether R, Lu HM, Alcaraz WA, Tang S, Jungbluth C, Dugan SL, Vainionpää L, Karle KN, Synofzik M, Schöls L, Schüle R, Lehesjoki AE, et al. (2016) A recurrent mutation in KCNA2 as a novel cause of hereditary spastic paraplegia and ataxia. Ann Neurol 80:638-642.

Li K, Figarella K, Su X, Kovalchuk Y, Gorzolka J, Neher JJ, Mojtahedi N, Casadei N, Hedrich UBS, Garaschuk O (2023) Endogenous but not sensory-driven activity controls migration, morphogenesis and survival of adult-born juxtaglomerular neurons in the mouse olfactory bulb. Cell Mol Life Sci 80:98.

Masnada S, Hedrich UBS, Gardella E, Schubert J, Kaiwar C, Klee EW, Lanpher BC, Gavrilova RH, Synofzik M, Bast T, Gorman K, King MD, Allen NM, Conroy J, Zeev BB, Tzadok M, Korff C, Dubois F, Ramsey K, Narayanan V, et al. (2017) Clinical spectrum and genotype-phenotype associations of KCNA2-related encephalopathies. Brain 140:2337-2354.

Shah NH, Aizenman E (2014) Voltage-gated potassium channels at the crossroads of neuronal function, ischemic tolerance, and neurodegeneration. Transl Stroke Res 5:38-58.

Spitzer NC (2006) Electrical activity in early neuronal development. Nature 444:707-712.

Syrbe S, Hedrich UBS, Riesch E, Djémié T, Müller S, Møller RS, Maher B, Hernandez-Hernandez L, Synofzik M, Caglayan HS, Arslan M, Serratosa JM, Nothnagel M, May P, Krause R, Löffler H, Detert K, Dorn T, Vogt H, Krämer G, et al. (2015) De novo loss- or gain-of-function mutations in KCNA2 cause epileptic encephalopathy. Nat Genet 47:393-399.

Wang YX, Wu H, Ren Y, Lv S, Ji C, Xiang D, Zhang M, Lu H, Fu W, Liu Q, Yan Z, Ma Q, Miao J, Cai R, Lan X, Wu B, Wang W, Liu Y, Wang DZ, Cao M, et al. (2022) Elevated Kir2.1/nuclear N2ICD defines a highly malignant subtype of non-WNT/SHH medulloblastomas. Signal Transduct Target Ther 7:72.

Xiong Y, Chen J, Li Y (2023) Microglia and astrocytes underlie neuroinflammation and synaptic susceptibility in autism spectrum disorder. Front Neurosci 17:1125428.

C-Editors: Zhao M, Liu WJ, Qiu Y; T-Editor: Jia Y