

Article

Gender-Associated Differences in the Regulation of Potassium Channels in Astrocytes of Type 2 Diabetic Mice

Luis A. Rojas-Colón ¹ , David E. Rivera-Aponte ^{2,3}, Jadier Colón-Vázquez ² , Arelys A. Angueira-Laureano ¹, Miled A. Maisonet-Nieves ^{1,4}, Misty J. Eaton ², Yanitza Hernández ², Christian J. Malpica-Nieves ^{5,6,*}, Serguei N. Skatchkov ^{2,5} and Miguel P. Méndez-González ^{1,2,7,*} 

¹ Department of Natural Sciences, University of Puerto Rico, Aguadilla, PR 00603, USA; luis.rojas-colon@nih.gov (L.A.R.-C.); arelys.angueira@upr.edu (A.A.A.-L.); mamaisonetnieves@crimson.ua.edu (M.A.M.-N.)

² Department of Biochemistry, Universidad Central del Caribe, Bayamon, PR 00956, USA; david.rivera@uccaribe.edu (D.E.R.-A.); 419jcolon@uccaribe.edu (J.C.-V.); misty.eaton@uccaribe.edu (M.J.E.); yanitza.hernandez@uccaribe.edu (Y.H.); sergei.skatchkov@uccaribe.edu (S.N.S.)

³ Department of Biology, University of Puerto Rico, Bayamon, PR 00959, USA

⁴ Department of Biology, University of Alabama, Tuscaloosa, AL 35487, USA

⁵ Departments of Physiology, Universidad Central del Caribe, Bayamon, PR 00956, USA

⁶ School of Chiropractic, Universidad Central del Caribe, Bayamon, PR 00956, USA

⁷ Department of Sciences and Technology, Antillean Adventist University, Mayagüez, PR 00680, USA

* Correspondence: christian.malpica@uccaribe.edu (C.J.M.-N.); miguel.mendez3@upr.edu (M.P.M.-G.); Tel.: +1-787-798-3001 (ext. 2057) (C.J.M.-N.); +1-787-546-3656 (M.P.M.-G.)

Highlights

What are the main findings?

- Kir4.1 channels are downregulated in diabetes in both sexes.
- Diabetic db/db male mice have a larger impact on their Kir4.1 channel than females.

What are the implications of the main findings?

- Kir4.1 channels should be the focus of anti-diabetic treatments.
- Since Kir4.1 may be partially recovered by aminoguanidine as was recently shown, the polyamine strategy needs to be investigated further.

Abstract

Hyperglycemia is linked to a higher risk of diabetes, epilepsy, and seizures, which contribute to increased mortality. Previous research has shown that hyperglycemia impairs the function of the polyamine-dependent Kir4.1 channels in cultured astrocytes, and a similar effect was observed in male astrocytes from diabetic (db/db) mice. However, whether there are sex differences in this effect remains unclear. This study aims to compare Kir4.1 channel function in 10–12 week old brains of female db/db mice with that in males and non-diabetic heterozygous (db/+). We performed mRNA analyses, Western blotting (WB), glucose level measurements, and patch-clamp recordings from hippocampal astrocytes in brain slices to assess Kir4.1 channel activity in db/db and db/+ mice of both sexes. Our results showed increased glucose levels in diabetic db/db mice, especially in males, along with (1) reduced synthesis of Kir4.1 channel protein, (2) reduced membrane potential, (3) decreased Kir currents, and (4) compromised potassium uptake. Female diabetic astrocytes exhibited significantly lower barium-blocked Kir4.1 currents compared to diabetic males. Additionally, barium-insensitive currents (leakage currents) were upregulated in db/db mice, likely as a compensatory response to hyperglycemia. In conclusion, diabetes impairs astrocyte function by downregulating Kir4.1 channels, with a more pronounced



Academic Editor: Menachem Hanani

Received: 19 December 2025

Revised: 30 January 2026

Accepted: 4 February 2026

Published: 11 February 2026

Copyright: © 2026 by the authors.

Licensee MDPI, Basel, Switzerland.

This article is an open access article distributed under the terms and conditions of the [Creative Commons Attribution \(CC BY\)](https://creativecommons.org/licenses/by/4.0/) license.

effect in male diabetic mice. This impairment may increase seizure risk by affecting the ability of astrocytes to maintain extracellular ion balance.

Keywords: diabetes; mouse db/db and db/+ models; astrocytes; Kir4.1 channels; sex differences; potassium uptake; membrane potential; membrane currents

1. Introduction

The global prevalence of type 2 diabetes mellitus is rising worldwide, making it one of the most common debilitating health conditions [1]. Diabetes development is directly associated with neurodegeneration, which speeds up aging and leads to various central nervous system disorders, including diabetic peripheral neuropathy [2], retinopathy [3–6], and cognitive decline [7]. Notably, astrocytes, oligodendrocytes, Müller retinal glial cells, satellite glial cells (SGC), and microglia exhibit reactivity, dysfunction, and shrinkage in both animal models and humans affected by diabetes [2–7].

Astrocytes are vital guardians of brain homeostasis, closely linked to diseases and disorders of central and peripheral nervous systems (CNS, PNS) [4,8,9]. While neurons maintain conserved functions and morphology, astrocytes display remarkable diversity across different species: (i) the ratio of glia to neurons (RGN) increases from simple-brain animals to humans [10], (ii) growing complexity of astrocyte features [11–13], (iii) the emergence of new functions [8,9,14], and (iv) gene diversity [15]. The RGN is one of the highest in humans [10,16], and there are about 12 astroglial cells for every neuron in the brainstem and approximately 3.5 in the human cortex [17] showing that astrocytes are specialized cells in the CNS [18–22].

Diabetes significantly impacts glial cell function [3], leading to neurodegeneration [2], epilepsy [23], and seizures associated with further hypoglycemia or hyperglycemia [24]. Furthermore, 25% of diabetics experience seizures with an unknown mechanism [25]. Glial cells possess a limited number of principal channel types involved in potassium buffering, such as polyamine-dependent inwardly rectifying potassium channel 4.1 (Kir4.1) channels [4,8,18–21,26,27]; leak channels like the acid-sensitive two-pore domain (2P) TASK, TREK, and TWIK channels [28,29]; and BK, Kv, and Ka channels [4]. Since potassium Kir4.1 channels are expressed simply in glial cells, not by neurons [4,30–33], and because Kir4.1 channels are key potassium channels responsible for K-buffering [8,19,21], glutamate transport [21,22], and brain pathology [8,33,34], it is essential to explore gender differences in Kir4.1 activity under hyperglycemic conditions.

Hyperglycemia downregulates astrocytic Kir4.1 channels [35], and a diabetic mouse model (db/db mice) exhibits epileptogenic pattern, with the frequency of action potentials in neurons about nine times higher compared to non-diabetic male mice [36]. Since Kir4.1 channels regulate extracellular potassium level, a lack of Kir4.1 expression will lead to hyperexcitability in neurons. On the other hand, there are other potassium channels, such as the 2P domain channels, that can substitute Kir4.1 function partially [28]. Therefore, since diabetic hyperglycemia is associated with increased acidosis [37], it activates acid-sensitive astrocytic 2P-domain channels (TASK, TREK) which cannot be neglected [28,35,37–41]. While TREK-1 is mostly expressed in neurons [38,42], TREK-2 is found to be functional in astrocytes [43,44]. Acidosis was accompanied with synchronous overexpression of TREK-2 and downregulation of Kir4.1 channels [44]. Therefore, two types of K-channels work in synergy: when Kir channels were inhibited, the 2P-domain channels were activated in glia [4,28]. Therefore, the recruitment of 2P-domain channels is a pathway that can serve as an alternative mechanism of neuroprotection [4,40]. Hyperglycemia induces

downregulation of Kir4.1 channels, leading to astrocytic dysfunction [35,45] and increasing the risk of developing epilepsy and seizures, all of which contribute to higher rates of illness and death; however, gender differences were not investigated [36].

Glucose homeostasis in type 2 diabetes is regulated by testosterone [46]. In males, testosterone naturally boosts glucose-stimulated insulin secretion and lowers inflammation, supporting beta cell health. Conversely, in females, increased testosterone leads to insulin hypersecretion, mitochondrial dysfunction, and oxidative stress [46]. Intriguingly, (i) males have more complex astrocytes than females, (ii) higher numbers of astrocytes in the murine hypothalamus and hippocampus compared to females, and (iii) male hippocampal astrocytes show lower levels of the reactivity marker GFAP+ than females [47]. Stress-induced hyperactivity of orexin neurons in males versus hypoactivity in females results from differences in astrocyte glucocorticoid receptor expression and lactate exchange between sexes [48].

Therefore, we hypothesized that hyperglycemia differentially impairs astrocytic Kir4.1 channel function in male and female diabetic mice models. The present study aims to assess potential sex differences in the function of Kir4.1 in astrocytes. We used the type 2 diabetic mouse model (db/db), which develops diabetes due to reduced leptin receptor expression caused by a homozygous point mutation in the leptin receptor encoding gene [49]. This study investigates whether a similar decrease in Kir4.1 channel function occurs in the brains of female mice with type 2 diabetes as was found in males [36]. We analyzed hippocampal brain slices from homozygous mice (diabetic db/db mice) compared with heterozygous (non-diabetic db/+ (control) mice). Our findings show that: (i) hyperglycemia is higher in db/db males than in db/db females, (ii) protein levels of Kir4.1 subunits in both sexes are reduced, (iii) Kir4.1 channel currents, and (iv) K⁺-uptakes are strongly decreased in hippocampal astrocytes of diabetic db/db female that differ from db/db male mice. Additionally, we identify notable differences between female and male leakage currents (2P-domain channels) in db/db astrocytes.

2. Materials and Methods

2.1. Animals

Homozygous (db/db) diabetic and heterozygous (db/+) non-diabetic female and male mice were obtained from Jackson Laboratories (Bar Harbor, ME, USA). Mice had access to water and food ad libitum and were kept on a 12 h light/dark cycle. All experiments were approved by the Institutional Animal Care and Use Committee (IACUC) at the Universidad Central del Caribe. protocol number #051-2021-18-01-CSC, (renewed 051-2023-31-91-PHA and approved 28 September 2023), adhering to the National Institutes of Health guidelines for the humane treatment of laboratory animals.

All electrophysiological, molecular, biochemical, and imaging datasets presented in this manuscript were newly generated specifically for the current study. No quantitative results, figures, or raw data from our previously published studies were reused. References to prior publications are provided solely to contextualize the findings and to highlight consistency between independent experiments, not to indicate reuse of previously published datasets.

2.2. Blood Glucose Measurement

For further experiments, we used mice of both sexes of 10–12 weeks old because Kir4.1 channel functional maturation is established after 28 postnatal days [8]. For performing blood glucose measurements, mice were fasted for 5 h with access only to water. Fasting blood glucose levels were measured using a glucose meter and test strips from the blood samples, which were obtained from the tail vein following established methodology [36].

2.3. Real-Time Quantitative PCR Analysis

Hippocampal RNA was isolated using the RNeasy Mini Kit (Qiagen, Valencia, CA, USA, cat# 74104,) from db/db (diabetic homozygous) and non-diabetic heterozygous (db/+) female and male mice following established methodology [36]. From the total RNA, 0.5 µg was used for cDNA synthesis following the RT² First Strand Kit (Qiagen, Valencia, CA, USA, cat # 330401) protocol. RT-qPCR was used to determine the mRNA expression using a custom RT² ProfilerTM PCR Array (Qiagen, Valencia, CA, USA, cat # CLAM49636). The numbers in parenthesis represent the internal Qiagen gene detection codes for the following genes: Kir4.1 (*Kcnj10*; PPM04090A), and the Transferrin receptor (HKG) (*TRFC*; PPM03499C). Quantitative polymerase chain reaction (qPCR) was carried out using the CFX Connect real-time PCR detection system (Bio-Rad, Hercules, CA, USA, cat. # 1855200). Each 96-well plate contained two samples run in triplicate. The threshold cycle (CT) value was obtained using CFX Maestro software, version 2.3 (Bio-Rad; Hercules, CA, USA), with the same baseline carried over between runs as recommended by the protocol. CT values were analyzed using the $2^{-\Delta\Delta CT}$ method according to the manufacturer's manual. HKG was used as the reference gene for data normalization. Quality controls used in the array were the reverse transcription control (RTC) and the positive PCR control (PPC).

SDS-PAGE, Western Blot, and Analysis

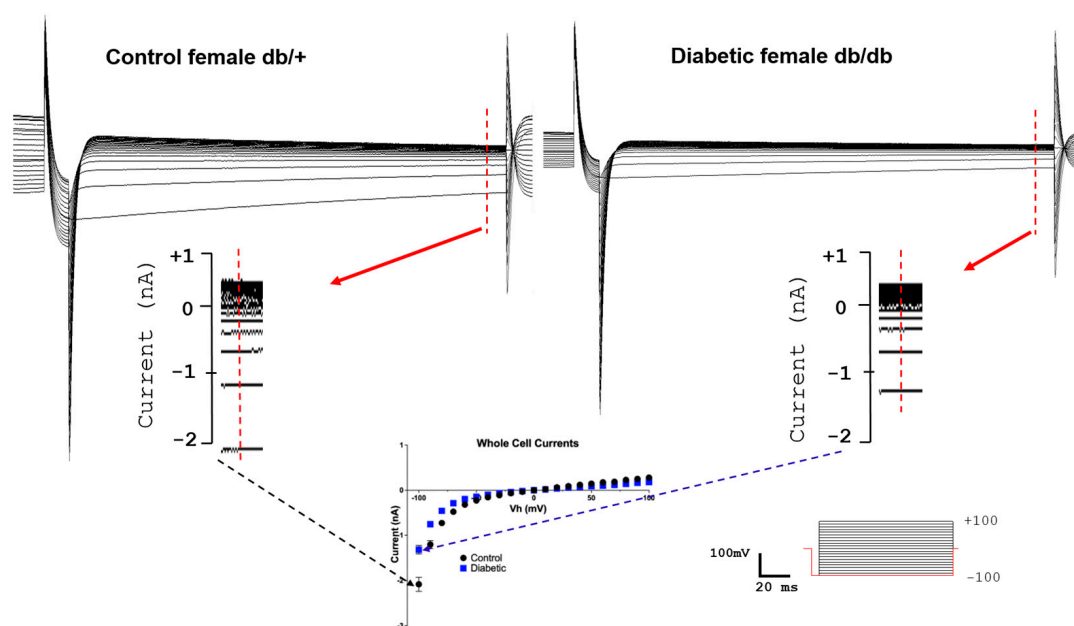
The hippocampus was isolated from the brains of control (db/+) and diabetic (db/db) mice and immediately placed in ice-cold RIPA buffer containing 150 mM NaCl, 1% Triton X-100, 0.1% SDS, and Tris-HCl (1.5 M, pH 8.8), supplemented with 1.0 mM phenylmethylsulfonyl fluoride (PMSF) and a protease inhibitors cocktail (bestatin, aprotinin, pepstatin, and leupeptin). Protein concentrations were determined using the Bradford Protein Assay (BioRad), followed by a 1:3 dilution in urea buffer (4% SDS, 8 M urea, 0.015% bromophenol blue, 5% β-mercaptoethanol, 20 mM EDTA, 62 mM Tris-HCl, stabilized at pH 6.8), and the resulting concentration was 10 µg protein/µL. After resolution on 4–15% polyacrylamide gradient gels, the samples were transferred onto membranes which were then immunoblotted using polyclonal antibody against Kir4.1 (1:2000, Alomone Labs, Jerusalem, Israel; catalog # AGP—035). The signals were detected using chemiluminescence methodology (SuperSignal[®] West Dura Extended Duration Substrate; Pierce, Rockford, IL, USA). The intensity of the signal was measured with a gel documentation system (ChemiDoc, BioRad, Hercules, CA, USA). It has been shown that Kir4.1 can exist in two forms of monomer: unglycosylated and glycosylated, corresponding to molecular weights of 37 kDa and 43 kDa, respectively [36,50]. We calculated the total monomer consisting of both the unglycosylated and glycosylated proteins. The chemiluminescence signal intensity was corrected for minor differences using the protein content after densitometric analysis of the India ink-stained membrane [51].

2.4. Patch-Clamp Electrophysiology in Astrocytes from Hippocampal Slices

Hippocampal brain slices were obtained from adult female and male diabetic (db/db) and genetic control (db/+) mice of 90–110 postnatal days following established methodology [36,52]. Oxygenated artificial cerebrospinal fluid (ACSF) was used for brain slice incubation containing: 127 mM NaCl; 1.25 mM NaH₂PO₄; 25 mM NaHCO₃; 2.5 mM KCl; 1 mM MgCl₂; 2 mM CaCl₂; and 25 mM D-glucose at 34 °C for 1 h before patch-clamp recording. The ACSF solution was bubbled with mixture of 95% O₂ and 5% CO₂ to achieve gas saturation and to stabilize pH for 7.4. Recordings were performed at room temperature.

Selection of Astrocytes and Patch-Clamp Recording

Astrocytes were selected in the CA1 area of the hippocampal stratum radiatum and were identified by their rounded and small cell bodies having characteristically tiny and irregular processes. Astrocytes were arbitrarily selected (cell processes attached to or near blood vessels) for patch-clamp electrophysiology. The cells were carefully chosen by their (i) strongly hyperpolarizing membrane potential with (ii) an I/V curve showing inward rectification (Scheme 1). This was necessary to focus on Kir4.1 channel behavior because Kir4.1 is the inwardly rectifying channel and is a focus of current research. Selected inwardly rectifying astrocytes were used from the depth of the brain slice (~50–200 micrometer from slice surface) to eliminate recording from injured cells that resided on both surfaces of the cutting slice edges. By clamping astrocytes in stratum radiatum, we avoided recording from satellite oligodendrocytes [53] and their precursors [54] that were found in proximity to pyramidal cells.



Scheme 1. Description of a voltage-step protocol combined with patch-clamp recording techniques to study steady-state currents in astrocytes within hippocampal brain slices. This method allows for the analysis of current–voltage (I/V) relationships in hippocampal astrocytes. We recorded representative whole-cell currents from astrocytes located in the stratum radiatum of the CA1 hippocampal region. Examples for female db/+ (left) and db/db (right) mice are presented. The analysis of the current–voltage (I–V) relationship was conducted using twenty 100 ms and 10 mV voltage steps that ranged from −100 mV to +100 mV. High frequency filters were not used. Current amplitudes were measured within a 90 ms time window, corresponding to the steady-state phase after a spermine block of Kir channel’s outward currents had been achieved (indicated by red vertical dotted lines). The currents were evoked using the voltage protocol illustrated at the bottom right side of the figure. Currents obtained during this steady state were averaged for each voltage step, and these data were used to construct the I–V curves presented in Figures 3–5. The lower panel displays a representative I–V relationship generated from the averaged steady-state currents, allowing for a functional comparison between control and diabetic conditions.

It is noteworthy that theoretical junction potentials between intracellular and extracellular solution membrane potentials were not corrected. Cell currents were measured using the common patch-clamp technique for whole-cell recording with a borosilicate glass pipettes (I.D. 1.0 mm, O.D. 1.5 mm; World Precision Instruments, Sarasota, FL, USA), pulled in four steps using a P-97 puller (Sutter Instruments, Novato, CA, USA) to achieve a resistance of 6–9 MΩ. The intracellular solution (ICS) comprised: 138 mM KCl, 2 mM KOH,

1 mM CaCl_2 , 1 mM MgCl_2 , 1 mM spermine HCl, 10 mM EGTA, 10 mM HEPES, and 3 mM Na_2ATP , with pH adjusted to 7.2. The ICS has an osmolarity of about 284 ± 3 mOsm/L. After cell penetration, we achieved an access resistance of 10–18 M Ω , compensated by at least 75%. We determined the astrocyte membrane potentials immediately upon reaching whole-cell mode and held them under voltage clamp. The holding potential (V_h) was equal to the resting membrane potential (V_m). After a 5 min stabilization in ACSF, we applied a voltage-step protocol with 100 ms steps ranging from -100 mV to $+100$ mV to analyze whole-cell currents and the current–voltage (I/V) relationship (detailed in Scheme 1).

The astrocytes were superfused ACSF and stimuli were applied using voltage-step protocol (Scheme 1). Then the ACSF containing a selective Kir channel blocker barium ($100 \mu\text{M}$) [4,55–58] was applied for 10 min and we then used the same voltage-step protocol to record currents in the presence of barium. To obtain Kir currents sensitive to barium, we subtracted the whole-cell current in the presence of barium from that in its absence. Using whole-cell voltage-clamp techniques, we recorded from astrocytes in the stratum radiatum of hippocampal slices from (db/db) diabetic and (db/+) control female and male mice. We measured inward K^+ currents when switching the external solution from 2.5 mM K^+ to 10 mM K^+ , both with and without barium [52,56]. Kir-dependent currents were determined by subtracting the currents recorded in ACSF with barium from those in pure ACSF. Only one cell was recorded per slice during barium perfusion.

Kir currents were differently reduced in CA1 hippocampal astrocytes from diabetic female and male mice (Figures 3–5). Between voltage steps, the holding potential (V_h) was kept equal to the resting membrane potential (V_m) in 2.5 mM $[\text{K}^+]_o$ ACSF ($V_h = V_m$). This helps to keep an equilibrium of cytoplasm ionic content. I/V curves were calculated according to the methods described above in Scheme 1. Scheme 1 shows I/V curves of female astrocytes from db/+ and db/db mice in response to twenty steps of 10 mV and 100 ms duration of between -100 mV and $+100$ mV from the holding potential (V_h). Results from both sexes are presented in the Results section (Figures 3–5).

2.5. Statistical Analysis

Using the Shapiro–Francia estimator, allowing us to confirm the distribution of our data, we performed a normality diagnostic test. To identify any outliers, we applied the Grubbs test, ensuring robust results. We utilized an independent sample's *t*-test to compare the electrophysiological data from astrocyte resting membrane potentials and currents. To further validate our findings, we assessed variance homoscedasticity using the Levene test. The estimated marginal means and their standard errors are provided for each experimental factor, contributing to a clearer interpretation of the results. We established a significance level (α) of ≤ 0.05 , while accepting *p*-values > 0.05 for normality and homoscedasticity tests. All analyses were performed with IBM SPSS version 23.0 (IBM-SPSS, Chicago, IL, USA), facilitating a comprehensive evaluation of the data.

2.6. Quantification and Normalization

Western blot (WB) signals were quantified and normalized using Image Lab-BioRad version 6.1. WB quantification was followed by a Fisher LSD test for comparison. A value of $p < 0.05$ was accepted as significant. The WB data files were evaluated with the help of Graph Pad Prism V 10. 4. 1.

3. Results

3.1. Quantification of Glucose Concentrations in the Blood of Diabetic and Control Mice

The previous literature has reported reduced Kir4.1 expression and function in male db/db mice [36]. These prior findings provide context only and were not included as data

in the present study. Here, we independently obtained new datasets from both sexes to directly evaluate potential sex differences.

First, we measured glucose levels in control db/+ non-diabetic mice and compared them with diabetic female and male (db/db) mice. The average fasting blood glucose levels recorded were (i) 119 ± 13 mg/dL $n = 11$ (mean $\pm$ SEM) for genetic control (db/+) female mice versus 133 ± 11 mg/dL $n = 10$ for control (db/+) male mice, while (ii) 338 ± 38 mg/dL $n = 11$ for diabetic (db/db) female mice and 417 ± 53 mg/dL $n = 10$ for diabetic (db/db) male mice. Control db/+ males and females show negligible differences in glucose levels (119 mg/dL (6.7 mM) versus ~ 133 mg/dL (7.4 mM)); however, in both sexes, glucose levels increase significantly from controls (about 2.84 times in db/db female and 3.14 times in db/db males), with male db/db mice reaching nearly 23.2 mM compared to 18.8 mM in db/db female mice. Previously, it was found that the levels of glucose in fasting blood were 138 ± 23 mg/dL for control and 547 ± 30 mg/dL for db/db male mice (mean $\pm$ SEM; $n = 3$), which reproduces the data well [36]. This suggests that higher glucose levels may strongly influence Kir channel function. Indeed, cultured cortex astrocytes grown in high glucose (25 mM) exhibited an approximately 70% reduction in Kir 4.1 mRNA and about 50% reduction in protein expression compared to those grown in 5 mM [35].

3.2. mRNA Level Comparison Between db/db and db/+ Mice of Different Sexes

Second, since prior studies reported reductions in mRNA levels in male db/db mice [36], we re-evaluated both male and female mice using newly collected tissue to enable direct comparison under identical experimental conditions. Here we measured (i) the mRNA level by RT-PCR in db/db females (Figure 1A) and (ii) protein levels of Kir4.1 in the hippocampus of (db/+) genetic control and diabetic (db/db) female and male mice by Western blot (Figure 1(B1)). We compared non-diabetic female mice versus diabetic (db/db) female, and we found that hippocampal astrocytic Kir4.1 mRNA from diabetic (db/db) female mice was significantly downregulated to $61.7 \pm 4.1\%$; ($n = 3$) when compared to astrocytes from female (db/+) genetic controls (Figure 1A). Earlier reports described approximately 70% reductions in Kir4.1 mRNA in male db/db mice [36]. In the present study, our newly collected male dataset also demonstrated reduced expression of Kir4.1 protein levels under the same experimental conditions in diabetic females (see below).

3.3. Kir4.1 Protein Levels Between db/db and db/+ Mice of Different Sexes

Third, upon assessing hippocampal astrocyte Kir4.1 protein expression, our results revealed a 43 kDa label of glycosylated Kir4.1 subunit monomer as previously justified [50]. Protein levels of (db/db) diabetic female mice were 59.79% significantly lower in the hippocampal region when compared to astrocytes from female (db/+) genetic controls (Figure 1(B1)). Then we compared the Kir4.1 protein level's changes between sexes. A new set of experiments to compare Kir4.1 protein levels in male and female diabetic mice compared to their genetic controls was done. In detail, the Kir4.1 potassium channel protein level was dropped down to a range of 56–59% of the control females, that is significantly downregulated in diabetic female mice ($p = <0.000001$, 95% C.I [33.11 to 53.97], $n = 6$) (Figure 1(B2), right panel). The male's Kir4.1 protein level was declined to 42.7%, significantly lower than the control male ($p = <0.000001$, 95% C.I [46.87 to 67.73], $n = 6$) (Figure 1(B2), left panel). The difference between male's and female's diabetic Kir4.1 level was 13.4% ($p = <0.0404$, 95% C.I [−26.63 to −0.8855], $n = 6$ for each sex) (Figure 1(B2)). Since Kir4.1 is a major marker of glial cells in the retina, CNS and PNS [4,8,9,18–21,30,32,50,56], these data represent a decrease in Kir4.1 channel expression in glia.

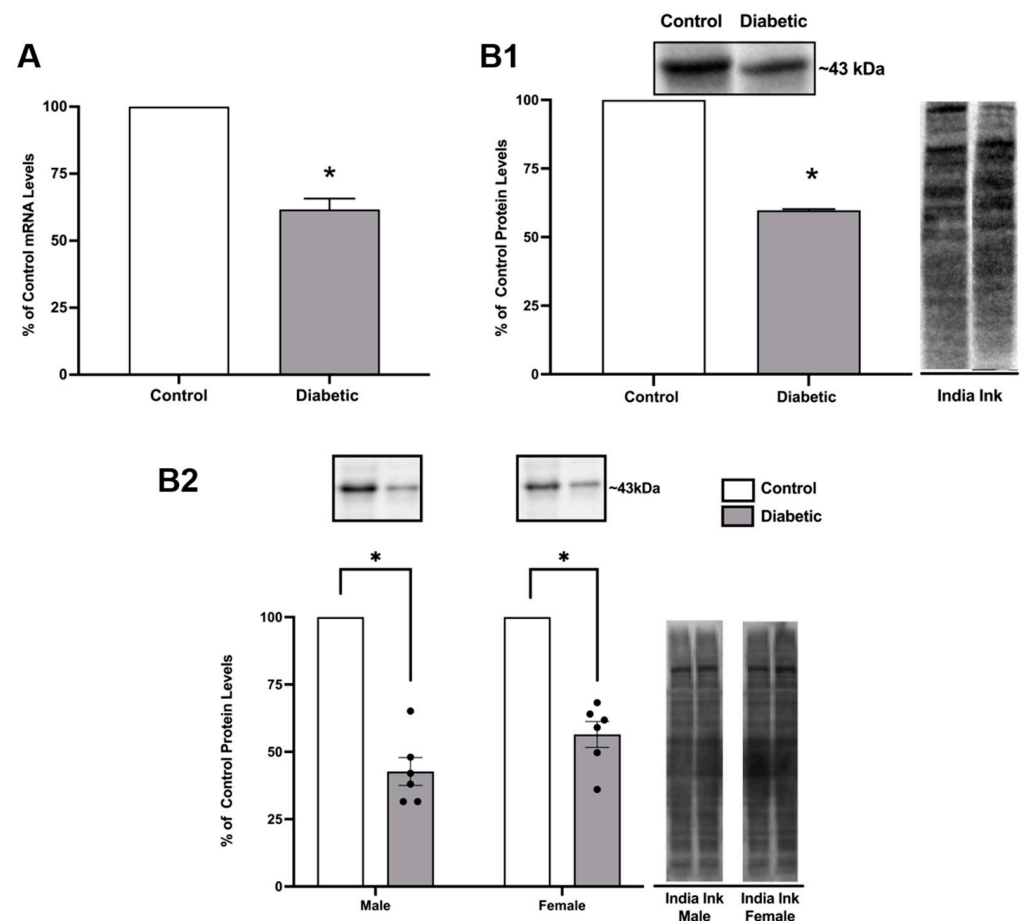


Figure 1. Kir4.1 mRNA and protein levels in the hippocampal region of brains from diabetic (db/db) female mice (Diabetic), as compared with db/+ heterozygote female mice (non-diabetic control). (A) Kir4.1 mRNA from diabetic female mice was significantly downregulated ($61.7 \pm 4.1\%$ SEM, $n = 3$) as compared to db/+ female control. Transferrin receptor HKG 2 (TRFC) was used as the reference gene for the $2^{-\Delta\Delta C_t}$ calculations. (B1) Kir4.1 potassium channel protein levels were significantly downregulated in diabetic female mice ($59.8 \pm 0.76\%$ SEM, $n = 3$) as compared to control female. Data are expressed as % of control non-diabetic mice. (B2) New set of experiments to compare Kir4.1 protein levels in male and female diabetic mice compared to their genetic controls. In detail, Kir4.1 potassium channel protein level was dropped down to 56% from the control animals, significantly downregulated in diabetic female mice ($p < 0.000001$, 95% C.I [33.11 to 53.97], $n = 6$). Kir4.1 protein levels in diabetic males declined to 42.7%, significantly lower from the control ($p < 0.000001$, 95% C.I [46.87 to 67.73], $n = 6$). The difference between male's and female's diabetic Kir4.1 level was 13.4% ($p = < 0.0404$, 95% C.I [−26.63 to −0.8855], $n = 6$ for each sex). The India ink stain was used as loading control. Data analyzed using GraphPad Prism V9 software. Asterisks represent significant differences.

3.4. Comparison of Astrocytic Membrane Potentials Between db/db and db/+ Mice of Different Sexes

Using electrophysiology, we examined the functional Kir4.1 electrical properties in astrocytes from the stratum radiatum of diabetic (db/db) and (db/+) genetic control female and male mice. Furthermore, we aimed to assess any sex differences in Kir4.1 channel functionality. Astrocytes show a highly negative membrane potential (strongly hyperpolarized) due to the presence of actively functional Kir4.1 channels in cells (Figure 2, Vh in mV). Therefore, we first measured astrocytic membrane potentials from CA1 hippocampal astrocytes in ex vivo slices obtained from diabetic (db/db) compared to (db/+) genetic control female and male animals. Hippocampal astrocytes from diabetic (db/db) female mice were significantly more depolarized (-73.9 ± 1.4 mV; $n = 67$ from 11 mice) in comparison with

their non-diabetic (db/+) genetic controls (-82.0 ± 1.3 mV; $n = 52$ from 10 mice) (Figure 2, Females). Similarly, astrocytes from diabetic (db/db) male mice were significantly more depolarized (-69.4 ± 1.9 mV; $n = 46$ from 9 mice) if compared to (db/+) genetic control (-83.3 ± 1.4 mV; $n = 56$ from 9 mice) (Figure 2, Males). This difference was reflected in the averaged membrane potentials (V_h) for astrocytes from diabetic (db/db) and genetic control (db/+) mice, which in the average were -73.9 mV versus -82.0 mV for females and -69.4 mV versus -83.3 mV for males, respectively. The diabetic (db/db) mice cell population was strongly depolarized (about 9 mV between female groups and 14 mV in the male groups). This can be seen in the following scatter plot (Figure 2). The asterisks in Figure 2 indicate significant differences from the control groups within the same sex ($p < 0.05$; Two-way ANOVA, followed by Tukey's post hoc test).

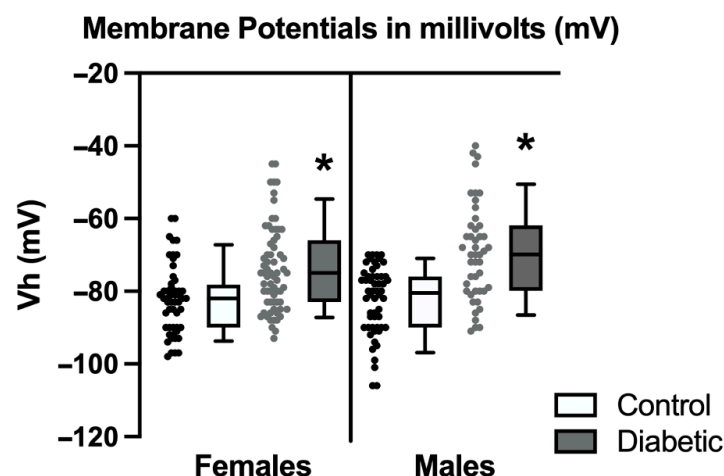


Figure 2. Astrocyte membrane potentials (V_h) in hippocampal CA1 slices of control mice compared to diabetic mice of different sexes. Black and grey dots represent individual membrane potentials, while white and black boxes show the averaged membrane potentials from control, db/+, and diabetic, db/db, mice respectively. Left panel represents female astrocytes in CA1 area of stratum radiatum from control (-82 ± 1.3 mV; $n = 52$ from 10 mice) and diabetic mice (-73.9 ± 1.4 mV; $n = 67$ from 11 mice). Right panel represents control male mice (-83.3 ± 1.4 mV; $n = 56$ from 9 mice-) and diabetic mice (-69.4 ± 1.9 mV; $n = 46$ from 9 mice). Asterisks (*) indicate significant difference from the control group within the same sex ($p < 0.05$; Two-way ANOVA, followed by Tukey's post hoc test). Note: we kept each astrocyte after cell opening and during patch-clamp recording at the V_h equal the resting membrane potential.

3.5. Recording of Ionic Currents Using Full-Scale I/V and Comparison Between db/db and db/+ Mice of Different Sexes

To assess sex differences in the contribution of Kir channels to the total whole-cell astrocytic currents, brain slices were initially perfused with standard ACSF, and astrocytes were voltage-clamped at resting the native potential of the membrane (V_m), such that V_m equaled the holding potential (V_h). Under such conditions, the initial membrane currents were zero, which helps to avoid unwanted ionic flow. Under these conditions, 100 ms voltage steps (by the increment of 10 mV from -100 mV to $+100$ mV) were applied relative to the holding potential. Such stimulation evoked currents that were diminished in those recorded from (db/+) genetic controls of both sexes (Figures 3–5).

A greater barium inhibitory effect was observed in diabetic male astrocytes than in female mice (Figure 3(A3)), as clearly summarized in Figure 3C. To isolate the currents contributed by expression of the functional Kir channel, the Ba^{2+} -sensitive currents were subtracted A1 from A2. Barium-insensitive currents represent a leakage residual current by basically 2P-domain channels [28]. A subtraction of the currents recorded in the perfusion containing Ba^{2+} (A2) from the entire whole-cell currents (A1) gives important information (Figure 3). The average

currents at -100 mV in ACSF are presented in Figure 3B. The average Ba^{2+} -sensitive currents measured at -100 mV from diabetic, db/db, vs. control mice of both sexes are shown in Figure 3C. Figure 3(A3) shows a difference between males and females. For example, female db/db mice astrocytic currents (-0.5 ± 0.1 nA; $n = 24$ cells from six mice) were markedly smaller than the currents obtained from db/+, genetic controls (-1.2 ± 0.15 nA; $n = 13$ cells, six mice). Similarly, Ba^{2+} -sensitive inward currents from diabetic (db/db) male mice (-0.5 ± 0.1 nA; $n = 22$ cells, six mice) were significantly smaller than those obtained from db/+ genetic control male mice (-1.9 ± 0.2 nA; $n = 13$ cells from six mice). These results are summarized as a percentage of the current blockage by Ba^{2+} in Figure 3C.

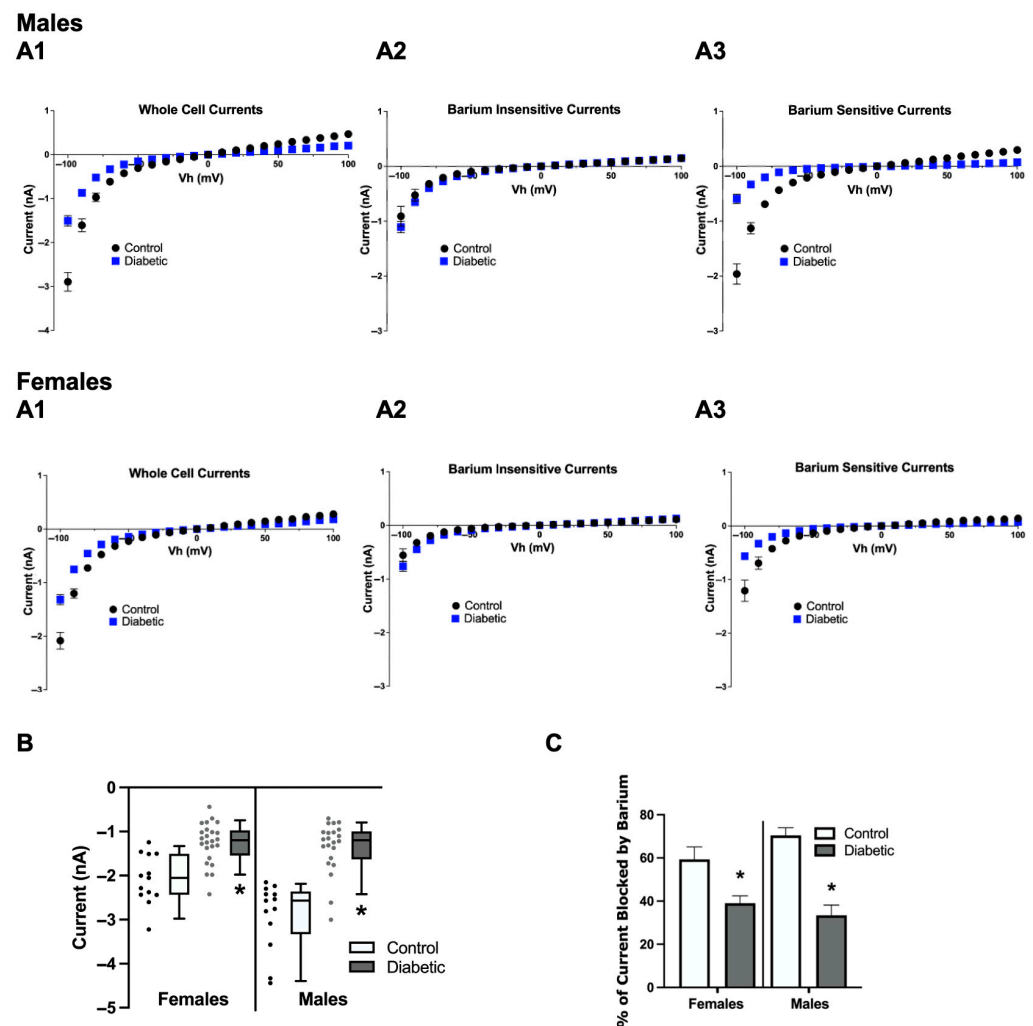


Figure 3. Differentially reduced Kir currents from diabetic female and male mice in CA1 hippocampal astrocytes. Astrocytes were clamped at holding potential (V_h) which was equal to the resting membrane potential (V_m) in 2.5 mM $[\text{K}^+]_o$ containing artificial cerebrospinal fluid (ACSF), $V_h = V_m$. I/V curves are calculated accordingly the methods described above in Scheme 1 (Methods, Section 2.4). I/V curves are shown in response to a voltage-step protocol (100 ms voltage-steps to potentials between -100 mV and $+100$ mV from the holding potential (V_h)). Results from both sexes are presented. (A1) Panels show recorded whole-cell currents in response to a voltage step protocol from astrocytes in hippocampal brain slices obtained from control male mice (black circles) or diabetic (blue squares) male mice (**upper panel**) and females (**lower panel**). (A2) Whole-cell patch-clamp currents recorded in the presence of a blocker of Kir channels (extracellular 100 μM Ba^{2+}) from hippocampal astrocytes in brain slices obtained from control or diabetic male mice (**upper panel**) and females (**lower panel**). This panel represents residual cell currents after barium application. (A3) Ba^{2+} -sensitive currents in male (**upper panel**) and female (**lower panel**) mice. The panel represents

the contribution of Kir channels to the whole-cell currents. The currents were obtained by subtraction of (A1) and (A2) data since (A1) graphs in male and female show total currents in astrocytes while (A2) graphs show the residual barium-insensitive currents after block of Kir4.1 channels by barium. Therefore, (A3) is showing that female mice have almost half the number of barium-insensitive currents (2P-domain-like channels) than males; (A3) graphs show Kir currents in astrocytes from control or diabetic mice that are Ba^{2+} -sensitive (see for details Figures 4 and 5). (B) Inward currents at -100 mV in astrocytes of control and diabetic female and male mice. Total potassium currents in astrocytes are much more reduced in db/db males than in females. (C) Percentage of current blocked by $100 \mu\text{M}$ Ba^{2+} in astrocytes. Data from control and diabetic mice are presented for comparison. Barium-sensitive Kir4.1 currents are much more reduced in db/db males than db/db females when compared with their controls, db/+. Data were acquired from 5 db/+ (genetic control) and 5 db/db (diabetic) female and male mice. Data (B,C) are shown as mean $\pm$ SEM, and asterisks (*) specify significant differences ($p < 0.05$; Two-way ANOVA, Tukey post hoc multiple comparisons).

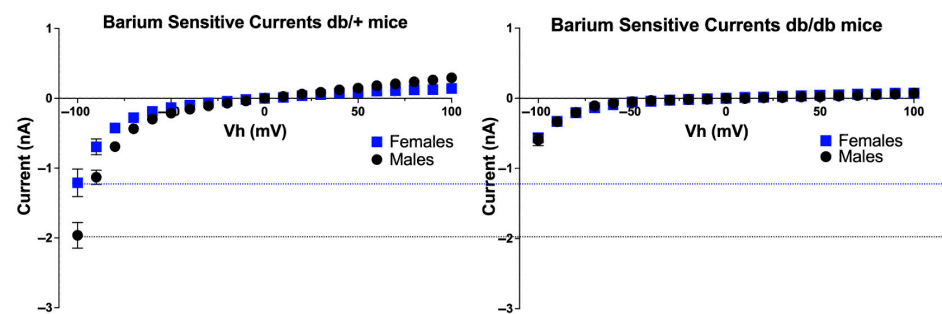


Figure 4. Kir currents isolated from db/+ control astrocytes compared to db/db astrocytes. The Kir currents were isolated from total cell currents by barium application. In control db/+ females, there is a significant difference in Kir current compared to males (**left panel**), whereas in db/db mice, both sexes exhibit equal Kir currents (**right panel**). At the holding potential -100 mV, the average current in db/+ male astrocytes from stratum radiatum CA1 area of hippocampal brain slices was $-1.9 \text{ nA} \pm 0.2 \text{ nA}$, with $n = 12$ from 10 mice, (**left panel**, black dots) and diabetic, db/db males, had $-0.5 \pm 0.1 \text{ nA}$, with $n = 15$ from 11 mice (**right panel**, black dots). Similarly for female control ($-1.2 \pm 0.2 \text{ nA}$; $n = 16$ from 9 mice, **left panel**, blue squares) and diabetic females ($-0.5 \pm 0.1 \text{ nA}$; $n = 17$ from 9 mice, **right panel**, blue squares). The dotted line indicates significant difference from the control group within the same sex ($p < 0.05$; Two-way ANOVA, followed by Tukey's post hoc test).

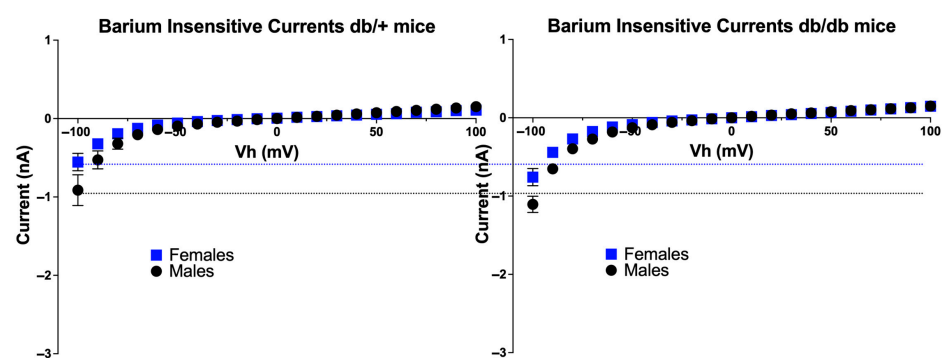


Figure 5. 2P-domain leakage currents isolated from barium-sensitive currents in db/+ control astrocytes compared to db/db astrocytes. Hyperglycemic astrocytes in db/db mice show increased 2P-domain-like currents in both sexes, (**Left panel**): average membrane currents that remained after barium (block of Kir4.1 currents) in db/+ astrocytes from stratum radiatum CA1 area of hippocampal brain slices. The currents at -100 mV holding potential in control db/+ females ($-0.5 \pm 0.1 \text{ nA}$; $n = 16$ from 10 mice) are smaller than in db/+ males ($-0.9 \pm 0.2 \text{ nA}$; $n = 12$ from 10 mice). (**Right panel**): average currents that remained after barium block in astrocytes from diabetic, db/db, females ($-0.8 \pm 0.1 \text{ nA}$; $n = 18$ from 11 mice) are still smaller than in db/db males ($-1.1 \pm 0.1 \text{ nA}$; $n = 13$ from 9 mice), but (i) both sexes demonstrated growing barium-insensitive currents and (ii) the ratio of current growth was about 1.52 times in female db/db astrocytes. This ratio is larger than the ratio in

db/db males (about 1.15 times). Dotted lines indicates significant difference from the control group within the same sex ($p < 0.05$; Two-way ANOVA, followed by Tukey's post hoc test).

Collectively, (i) the decrease in Kir4.1 protein levels, (ii) depolarized membrane potential, (iii) reduced current amplitude, and (iv) diminished response to Ba^{2+} in astrocytes from db/db diabetic mice suggest a significant reduction in functional membrane Kir channel activity compared to astrocytes from db/+ genetic controls, regardless of biological sex. Additionally, astrocytes from male db/db mice appear to exhibit stronger sensitivity to barium than those from diabetic females, as indicated by the smaller percentage of current blocked by Ba^{2+} in females (Figures 3C and 4).

3.6. Recording of K-Uptake (Inward Potassium Ionic Currents) in Brain Slices and Comparison Between db/db and db/+ Mice of Different Sexes

One key role of astrocytes is removing excess potassium from active synaptic areas [18,59]. Using a physiologically relevant protocol [20,21,35,57], we determined the Kir4.1 potassium uptake ability in glial cells. In this study, we examined the potassium uptake capacity of astrocytes in diabetic db/db mice and non-diabetic control (db/+) mice using the same protocol. We held each astrocyte at its resting membrane potential, which results in zero-current, during perfusion and the slices with control ACSF contained 2.5 mM K^+ . Then, inward K^+ currents were measured in response to changing the external solution from 2.5 mM K^+ to 10 mM K^+ (Figure 6A). This was done both with and without 100 μM Ba^{2+} . In females, inward currents caused by increasing external K^+ from 2.5 mM to 10 mM were significantly smaller in hippocampal astrocytes from diabetic (db/db) female mice (-469 ± 49 pA; $n = 11$ cells from six mice) in comparison to those from non-diabetic (db/+) female mice (-836 ± 183 pA; $n = 12$ cells from six mice) (Figure 6A, left panel).

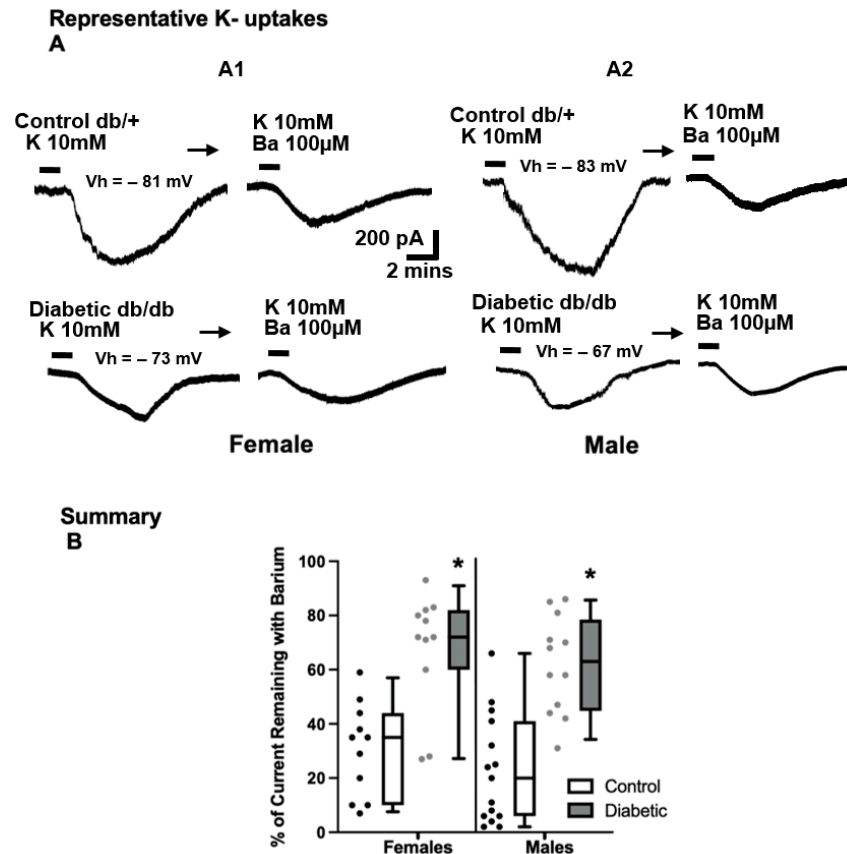


Figure 6. Potassium uptake is impaired in CA1 hippocampal astrocytes from both diabetic male and female mice. (A) Whole-cell currents obtained from astrocytes of (db/+) genetic control and (db/db)

diabetic female (A1) and male (A2) mice. Potassium inward currents were elicited by changing K^+ from 2.5 mM in extracellular solution to 10 mM in the presence or absence of 100 μ M of barium (Ba^{2+}). The cells were clamped at the resting potential in 2.5 mM of extracellular potassium ($V_h = V_m$). The calibration scale is equal for all recorded currents. (B) Summary of the relative barium-insensitive Kir currents measured in hippocampal astrocytes from (db/+) genetic control female ($n = 11$) and male ($n = 15$) and (db/db) diabetic female ($n = 11$) and male ($n = 12$) mouse brain slices. In (B), the data are expressed as percentage (%) of control current that is barium-sensitive, where 100% is the maximal current measured by switching extracellular $[K^+]$ from 2.5 mM to 10 mM. Data were obtained from 5 db/+ female and male genetic controls, and 5 (db/db) female and male diabetic mice. Asterisks (*) indicate significant difference from the non-diabetic control group within their respective sex ($p < 0.05$; Two-way ANOVA, Tukey post hoc multiple comparisons).

Interestingly, in males, astrocytes from diabetic (db/db) male mice showed significantly smaller inward currents (-421 ± 75 pA; $n = 25$ cells, six mice) compared to astrocytes from db/+ genetic control male mice (-800 ± 190 pA; $n = 20$ cells from six mice). Additionally, we observed that Ba^{2+} -sensitive Kir currents were significantly reduced in astrocytes from diabetic (db/db) female mice ($68 \pm 6.5\%$; $n = 11$ from six mice) compared with non-diabetic (db/+) genetic control female mice ($31 \pm 5\%$; $n = 11$ from six mice). Similarly, hippocampal astrocytes from diabetic (db/db) male mice exhibited a significantly lower Ba^{2+} -sensitive Kir current ($62 \pm 5\%$; $n = 14$ from six mice) compared to astrocytes from non-diabetic (db/+) genetic control male mice ($23 \pm 5\%$; $n = 12$ from six mice) (Figure 6B, Males).

4. Discussion

Diabetes causes chronic inflammation in both the central and peripheral nervous systems, mainly affecting glial cells and leading to neurodegeneration. It accelerates aging and increases the risk of age-related neurodegenerative diseases, such as cognitive decline, peripheral neuropathy, and diabetic retinopathy. Neurodegenerative diseases such as Alzheimer's, Huntington's and amyotrophic lateral sclerosis demonstrate a loss of Kir4.1. Restoration of Kir4.1 function ameliorated functional deficits such as K-currents in astrocytes [45], decreased neuron hyperexcitability, and extended survival in animal models correlating with reductions in DNA methylation of the *kcng10* gene [60,61]. Our data revealed several important new findings regarding Kir4.1 potassium currents in the type 2 diabetic db/db mouse model.

The link between diabetes and CNS disorders is an important area of focus in the medical community, though the mechanisms and sex differences remain unclear [62–65]. Diabetes is more common in men [66], highlighting significant sex differences in various seizure conditions [67]. Research shows that increased neuronal hyperexcitability in diabetic db/db male mice is linked to astrocyte function, with db/db neurons being more excitable due to downregulated astrocytic Kir4.1 channels [36]. Additionally, stress-induced hyperexcitability of hypothalamic orexin neurons in males, contrasted with hypoexcitability in females, is solely caused by differences in astrocytic glucocorticoid receptor function and lactate sensitivity between sexes [48]. Regulation differences have been observed when ischemic stroke-related brain injury is partially protected by GLT-1 glutamate transporter activator in males but not in females [22]. Males have more complex astrocytes than females and higher numbers in the murine hypothalamus and hippocampus [47]. However, differences in female astrocyte Kir4.1 channel function compared to males in diabetes have not yet been explored.

In the current study, we evaluated the behavior of astrocytic Kir4.1 channels in both db/db and db/+ mice of both sexes. Using detailed mRNA analysis, Western blotting (WB), and patch-clamp recordings in brain slices, we investigated the functional behavior of Kir4.1 channels in db/db (diabetic homozygous) versus db/+ (non-diabetic heterozygous) mice

of both sexes. Our comprehensive study revealed that, regardless of gender, db/db mice exhibited a significant reduction in both the mRNA expression (Figure 1A) and protein levels of Kir4.1 channels (Figure 1(B1,B2)). This decline was further confirmed by three key observations: (i) a depolarized resting membrane potential in astrocytes (Figure 2), (ii) a marked decrease in barium-sensitive Kir currents (Figures 3A, 4 and 5), and (iii) a diminished capacity for potassium uptake in hippocampal astrocytes across both genders (Figure 6). Notably, our data indicated that diabetic male astrocytes displayed a significantly greater reduction in barium-blocked Kir4.1 currents compared to their female counterparts, highlighting a potential sex-based disparity in channel expression and function (Figures 3–6).

Firstly, we found that control male (db/+) mice (Figure 3(A3), right panel, black dots, “barium sensitive currents”) exhibit larger Kir4.1 currents (2 nA) than control female db/+ mice (Figure 3(A3), right panel, black dots) which show 1.2 nA (summarized in Figure 4, left panel). However, in db/db males and females, these currents are equalized and are approximately 0.5 nA (Figure 3, male (A3) and female (A3), right panels, blue squares). Figure 4 clearly summarizes this difference.

Secondly, astrocytes from db/db male mice exhibit about a threefold decrease in Kir4.1 currents compared to controls, while astrocytes from db/db female mice show a less pronounced reduction of approximately 1.8 times (Figure 4). Interestingly, Kir4.1 protein levels do not significantly differ between sexes in the db/+ group. However, there is a notable decrease in Kir4.1 protein in db/db mice, with minimal sex differences. The difference between male’s and female’s diabetic Kir4.1 level was 13.4% ($p = <0.0404$, 95% C.I [−26.63 to −0.8855], $n = 6$ for each sex) (Figure 1). Several studies demonstrate that Kir4.1 protein levels do not reliably predict functional inward-rectifying potassium currents because channel activity depends on tetramerization, trafficking, and proper membrane insertion [68–70]. For example, Kir4.1 can be present but nonfunctional under conditions involving defective assembly [71], impaired localization [72,73], DNA methylation of gene encoding Kir4.1 [61]; or pathological regulatory mechanisms [8,68]. Trafficking-defective mutations in *KCNJ10* further confirm that non-functional Kir4.1 subunits can accumulate inside cells despite normal protein levels [74,75]. Our study shows a greater reduction in Kir4.1 channel functionality in males compared to females in diabetic db/db mice.

Thirdly, the barium-insensitive currents, which usually represent two-pore domain K-channels (2P-domain leakage currents [4,28,40,76] are larger in non-diabetic db/+ male mice compared to their female counterparts (Figures 3 and 5)). In contrast, barium-insensitive currents are upregulated in db/db mice of both sexes (Figure 5). This increase may serve as a compensatory mechanism to restore K-currents following the downregulation of Kir4.1 during hyperglycemia in the type 2 diabetic db/db mouse model. A similar compensatory upregulation was found in ischemic rodents [43,44]. As evident from Figures 3, 5 and 6, there are residual Em- and K-currents after Kir4.1 channel block by barium due to other commonly known K-channels in addition to the Kir4.1 channel in glia, which are barium- and polyamine-insensitive potassium channels, such as two-pore domain (2P) K^+ channels like TASK-1 [28] and astrocytes that exhibit two-pore domain K^+ channels such as TWIK-1, TREK-1 and TREK-2, as well as TASK-3 and TASK-1 in addition to Kir4.1 channels [28,44,77,78]. TASK-1 channels contribute to the Ba^{2+} -insensitive component of K^+ uptake and the resting membrane potential in glia [28]. For example, after the Kir channel block, TASK1 2P channels contribute about 50% to glial hyperpolarized membrane potential and K-transport [21,28,77,79]. Other astrocytic 2P channels, such as TWIK-1 channels, implement negligibly to membrane potential [39,78], but mainly to glutamate/glutamine metabolism coupled with NH^+ uptake [8]. TREK-2 channels in astrocytes are temperature-sensitive and are robustly and rapidly expressed in response to ischemia, rescuing potassium and glutamate clearance [43,44]. In addition, hippocampus astrocytes [80] and retinal

glial cells [81] also express the Kir6.1 pore-forming component called KATP channels [27]; however, these Kir6.1-SUR1 containing channels only work when glial cells are physiologically stressed, and their ATP concentration is near zero [81], while our ICS contains 3 mM ATP.

Hyperglycemic conditions are associated with increased acidosis [37], making the roles of 2P-domain acid-sensitive astrocytic channels, such as TASK and TREK, especially important [28,37,40,41,43]. Although TREK-1 is mainly expressed in neurons [38,42], TREK-2 expression increases along with Kir4.1 downregulation in astrocytes during ischemia-induced acidosis [43,44]. In fact, when Kir channels are blocked, 2P-domain channels remain functional, and db/db mice show increased 2P-domain-like channel currents compared to controls (Figure 5). This may be a compensatory response in astrocytes to partially recover the loss of Kir4.1 K-currents, indicating that 2P-domain channels could provide an alternative protective mechanism. Our findings highlight how diabetes downregulates Kir4.1 channels and leads to astrocytic dysfunction [35], which can be partially reversed by aminoguanidine, an amino group-enriched drug [45]. The role of gender differences in this context remains intriguing. Overall, the interaction of astrocytic potassium (K^+) channels may enhance resilience to transient acidification, suggesting that glial Kir4.1 and K2P channels could be potential targets for treating brain diseases associated with hyperglycemia. Studying which 2P-domain channels are expressed in db/db mice is a large and separate project and falls outside the scope of the current research.

In summary, our findings indicate that diabetes disrupts normal astrocyte function by reducing Kir4.1 channels, with a notably stronger effect seen in male diabetic mice. We propose that this loss of Kir4.1 channel function may increase the risk of seizures by impairing the astrocytes' ability to control extracellular potassium levels, potentially leading to greater neuronal excitability and seizure susceptibility. It has been shown that GFAP immunoreactivity increases in the hippocampus of hyperglycemic streptozotocin-treated mice [82]. Additionally, there is an increase in the number of GFAP-positive Müller cells of db/db mice compared with non-diabetic controls in the retina [83]. Furthermore, it is known that hyperglycemia decreases Kir4.1 potassium channel expression and activity in cultured cortical astrocytes [43] and in retinal Müller glial cells [3]. To date, there are no reports of sex differences in Kir4.1 expression in type 2 diabetic db/db mice.

Functional Kir4.1 channel expression in astrocytes contributes to its hyperpolarized membrane potential. They naturally exist as homomeric or heteromeric channels when combined with Kir5.1 subunits. However, the Kir4.1 homomer predominates in the hippocampus [50,77]. The strong sensitivity of Kir4.1 channels to extracellular K^+ levels enables K^+ entry at hyperpolarized membrane potentials and K^+ exit under conditions of astrocyte depolarization or reduced extracellular K^+ concentration [18,52,57,84]. Both passive and active astrocytic regulation of extracellular K^+ , as well as its role in the initiation and/or persistence of epileptiform activity was shown by several theoretical analyses [41,85,86].

Additionally, research has shown that Kir4.1 channel downregulation disrupts overall ion gradients and hampers uptake of potassium and glutamate by astrocytes [19–22]. Moreover, impaired ion gradients and cell depolarization hinder glutamate transport function, and can cause even transporter reversal, leading to the release of glutamate from a glial cell, which may further influence the excitability of neurons [87,88]. Conversely, the normal balance of estrogen and progesterone may affect glutamatergic transmission in the brain [89,90]. Seizure susceptibility in females could be partly related to imbalanced levels of these hormones. Recent studies suggest that estrogen, particularly the estradiol subtype, can promote anticonvulsant effects through its neuroprotective properties, while elevated progesterone levels in females may lead to hyperexcitability [91–94].

Kir4.1 channel dysfunction can disrupt brain ionic homeostasis and affect neuronal activity. Based on this study's findings, Kir4.1 channel dysfunction in diabetic mice negatively affects neurons regardless of gender. The *kcnj10* gene mutations and variations, which encodes Kir4.1, are identified in seizure-prone human and animal models [52,95,96], that includes severe SeSAME/EAST syndrome [74,97,98]. In seizure-sensitive DBA mice [96], mutated astrocytic Kir currents are reduced, which has been linked to decreased K⁺ uptake and glutamate uptake by these cells [52]. Downregulation of Kir4.1 channels and impaired potassium and glutamate uptakes were shown when glial cells were exposed to hyperglycemic or diabetic conditions, both in cultured glial cells [35] and in streptozotocin-induced diabetes [3]. In this study, we observed that astrocytes from diabetic mice displayed a depolarized resting membrane potential and impaired functional Kir-channel activity in both female and male mice.

Extracellular K⁺ levels increase during the propagation of an action potential [99]. It is well known that astrocytes control extracellular K⁺ level by removing excess K⁺ from synaptic zones and transporting it through the astrocytic syncytium to areas with lower K⁺ levels [8,59]. Insufficient potassium removal can lead to hyperexcitability, convulsions, and neuronal degeneration. Therefore, we assessed astrocytes' ability to uptake excess K⁺ by increasing external K⁺ from 2.5 mM to 10 mM and measuring the resulting inward current. As expected, astrocytes from diabetic mice showed reduced K⁺ uptake compared to non-diabetic mice. In summary, decreased astrocytic membrane potential, reduced Kir channel activity, and compromised K⁺ uptake in db/db mice may affect the ability of astrocytes to maintain extracellular potassium homeostasis, which could, in turn, influence neuronal excitability.

Considering that drugs enriched with aminogroups (polyamine-like), such as metformin administered to db/db mice orally [100] and aminoguanidine given to cultured astrocytes [45], can recover Kir4.1 expression, this highlights a potential role of polyamines in regulating Kir4.1 expression. Notably, glial cells accumulate polyamines, such as putrescine, spermidine, and spermine [101–105], in regions where Kir4.1 channels are co-localized with these polyamines [102,106]. This accumulation of polyamines, which is crucial for life and longevity [107–114], suggests a direct relationship between polyamine accumulation and glial cell function, mediated by normalization of Kir4.1 channel expression [106]. Further study of endogenous polyamine co-localization with Kir4.1 channels in db/db mice needs to be done. Finally, diabetic db/db mice are genetically obese leptin receptor-deficient mice exhibiting features of Type 2 diabetes in humans [115] represent a good model for further investigation. Other areas of CNS and PNS need to be investigated in terms of diabetic dysfunction of Kir4.1 channels in, for example, the respiratory center, spinal cord, and peripheral ganglia, which are enriched with astrocytes and SGCs.

5. Conclusions

We investigated the role of the astrocytic Kir4.1 potassium channel in hippocampal brain slices from both female and male type 2 diabetic db/db mice, comparing them to control db/+ mice. Our findings revealed that the functional activity of the Kir4.1 channel in astrocytes was reduced differently in both genders of db/db mice. Specifically, male db/db mice showed approximately a threefold reduction in Kir4.1 current, while female db/db mice exhibited a smaller reduction, roughly 1.8 times less. Additionally, we observed that db/db mice exhibited larger barium-insensitive currents (2P-domain-type channels) than db/+ controls. This may indicate a compensatory cellular response to hyperglycemia that helps buffer potassium when Kir4.1 channels are reduced. Overall, our data provide insights into a possible Kir4.1 channel-dependent mechanism explaining the high incidence of seizures observed in both females and males with uncontrolled

hyperglycemia. A following study should investigate gender-dependent neuronal behavior in the db/db mice's brains.

Author Contributions: Conceptualization: M.P.M.-G., M.J.E., D.E.R.-A., C.J.M.-N. and S.N.S.; Research plan: M.P.M.-G., L.A.R.-C., D.E.R.-A. and S.N.S.; Western blot data acquisition and analyses: D.E.R.-A., J.C.-V., A.A.A.-L., M.A.M.-N. and M.P.M.-G.; Patch-clamp study: L.A.R.-C., S.N.S. and M.P.M.-G.; Data analysis, acquisition, and graphics: M.P.M.-G., L.A.R.-C., A.A.A.-L., D.E.R.-A., J.C.-V. and S.N.S.; Resources: M.P.M.-G., D.E.R.-A., S.N.S. and UCC-core; Supervision: M.P.M.-G., D.E.R.-A., M.J.E. and S.N.S.; Visualization: M.P.M.-G., Y.H. and S.N.S.; Writing of the initial part of the manuscript: L.A.R.-C., J.C.-V., A.A.A.-L., M.A.M.-N. and D.E.R.-A.; Finalizing the manuscript: M.P.M.-G., C.J.M.-N., D.E.R.-A., Y.H. and S.N.S. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the National Institutes of Health Grants: The National Institute of Neurological Disorders and Stroke (NINDS-R01-NS065201 and 2R15NS116478-02 to S.N.S. and C.J.M.-N.); NIH-NINDS SC2NS124907 (M.P.M.-G., D.E.R.-A. and S.N.S.), American Diabetes Association 1-19-IBS-300 (M.J.E., D.E.R.-A. and S.N.S.), the National Science Foundation STaRTp Grant No. 2122203 (M.P.M.-G.), and the National Institute on Minority Health and Health Disparities (NIMHD-G12 MD007583 for the Institutional core facilities). Also, this work was supported by the US Department of Education (USDE-Title-VPO31S130068) and U54-MD007587. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Institutional Review Board Statement: The animal studies were reviewed and approved by the UCC (Universidad Central del Caribe) Institutional Animal Care and Use Committee at the Universidad Central del Caribe. Protocol number #051-2021-18-01-CSC, (renewed 051-2023-31-91-PHA and approved 28 September 2023). The study was conducted according to the NIH Guide for the Care and Use of Laboratory Animals.

Informed Consent Statement: Not applicable.

Data Availability Statement: The original contributions presented in this study are included in the article. Further inquiries can be directed to the corresponding authors.

Acknowledgments: The authors express their deepest gratitude to the University of Puerto Rico at Aguadilla (UPR-Aguadilla) for its institutional support. We thank Vanessa López-Quiles (UPR-Aguadilla) for her essential role in fund administration, procurement coordination, and overall project support. We also acknowledge the outstanding technical and logistical contributions from collaborators at Universidad Central del Caribe (UCC). In particular, we thank Jennifer Guzmán (UCC) for assistance with fund and order administration, Vicmag Cabrera (UCC) for expert grant management, and Lisette Arroyo (UCC) for administrative support related to research and PhD programs. We extend our sincere appreciation to Betzaida Torres, for her exceptional animal care and invaluable assistance in experimental procedures. We also thank Yuriy Kucheryavykh and Gabriel Sierra for their superior technical expertise and generous provision of materials from the imaging core facilities and CIAS. Special thanks are given to Priscila Sanabria, for exemplary assistance with optical imaging resources. We further acknowledge Wined Ramirez Lopez and Diana Fernández (UCC-Bayamón) for their scientific insights, thoughtful discussions, and dedicated support to our students throughout the project.

Conflicts of Interest: The authors declare that they have no potential conflict of interest that can be identified as influencing the impartiality of the research presented herein.

Abbreviations

The following abbreviations are used in this manuscript:

2P	Two-pore (domain) potassium channel/leak channel
2- $\Delta\Delta C_t$	Delta-delta cycle threshold method

ACSF	Artificial cerebrospinal fluid
ANOVA	Analysis of variance
Ba ²⁺	Barium ion (Kir channel blocker)
CA1	Cornu Ammonis 1 region of the hippocampus
C.I.	Confidence interval
CNS	Central nervous system
CT	Threshold cycle (qPCR)
DB/DB	Homozygous diabetic mouse carrying the leptin-receptor mutation
DB/+	Heterozygous non-diabetic control mouse
EDTA	Ethylenediaminetetraacetic acid
GFAP	Glial fibrillary acidic protein
HKG	Housekeeping gene
Het	Heterozygous control mouse (db/+)
Homo	Homozygous diabetic mouse (db/db)
IACUC	Institutional Animal Care and Use Committee
I/V	Current–voltage relationship
KCl	Potassium chloride
KCNJ10	Gene encoding Kir4.1
Kir4.1	Inwardly rectifying potassium channel subunit 4.1
mRNA	Messenger ribonucleic acid
nA	Nanoampere
PBS	Phosphate-buffered saline
PFA	Paraformaldehyde
PMSF	Phenylmethylsulfonyl fluoride
PNS	Peripheral nervous system
qPCR	Quantitative polymerase chain reaction
RIPA	Radioimmunoprecipitation assay buffer
RNA	Ribonucleic acid
RT-qPCR	Reverse transcription quantitative PCR
SEM	Standard error of the mean
SGC	Satellite glial cell
SDS-PAGE	Sodium dodecyl sulfate–polyacrylamide gel electrophoresis
TASK-1	Two-pore domain acid-sensitive potassium channel, gene KCNK3
TREK-1	Two-pore domain acid-sensitive potassium channel, gene KCNK2
TREK-2	Two-pore domain acid-sensitive potassium channel, gene KCNK10
Tris-HCl	Tris(hydroxymethyl)aminomethane hydrochloride
TRFC	Transferrin receptor gene (housekeeping gene used in qPCR)
Vh	Holding potential in patch-clamp recordings equal resting membrane potential
WB	Western blot

References

1. World Health Organization Diabetes. Available online: <https://www.who.int/news-room/fact-sheets/detail/diabetes> (accessed on 18 December 2025).
2. Llorián-Salvador, M.; Cabeza-Fernández, S.; Gomez-Sanchez, J.A.; de la Fuente, A.G. Glial Cell Alterations in Diabetes-Induced Neurodegeneration. *Cell Mol. Life Sci.* **2024**, *81*, 47. [[CrossRef](#)] [[PubMed](#)]
3. Pannicke, T.; Iandiev, I.; Wurm, A.; Uckermann, O.; vom Hagen, F.; Reichenbach, A.; Wiedemann, P.; Hammes, H.P.; Bringmann, A. Diabetes Alters Osmotic Swelling Characteristics and Membrane Conductance of Glial Cells in Rat Retina. *Diabetes* **2006**, *55*, 633–639. [[CrossRef](#)] [[PubMed](#)]
4. Bringmann, A.; Pannicke, T.; Grosche, J.; Francke, M.; Wiedemann, P.; Skatchkov, S.N.; Osborne, N.N.; Reichenbach, A. Müller Cells in the Healthy and Diseased Retina. *Prog. Retin. Eye Res.* **2006**, *25*, 397–424. [[CrossRef](#)]
5. Ghaseminejad, F.; Kaplan, L.; Pfaller, A.M.; Hauck, S.M.; Grosche, A. The Role of Müller Cell Glucocorticoid Signaling in Diabetic Retinopathy. *Graefes Arch. Clin. Exp. Ophthalmol.* **2020**, *258*, 221–230. [[CrossRef](#)] [[PubMed](#)]

6. Pfaller, A.M.; Kaplan, L.; Carido, M.; Grassmann, F.; Díaz-Lezama, N.; Ghaseminejad, F.; Wunderlich, K.A.; Glänzer, S.; Bludau, O.; Pannicke, T.; et al. The Glucocorticoid Receptor as a Master Regulator of the Müller Cell Response to Diabetic Conditions in Mice. *J. Neuroinflamm.* **2024**, *21*, 33. [\[CrossRef\]](#)
7. Meng, F.; Fu, J.; Zhang, L.; Guo, M.; Zhuang, P.; Yin, Q.; Zhang, Y. Function and Therapeutic Value of Astrocytes in Diabetic Cognitive Impairment. *Neurochem. Int.* **2023**, *169*, 105591. [\[CrossRef\]](#)
8. Olsen, M.L.; Khakh, B.S.; Skatchkov, S.N.; Zhou, M.; Lee, C.J.; Rouach, N. New Insights on Astrocyte Ion Channels: Critical for Homeostasis and Neuron-Glia Signaling. *J. Neurosci.* **2015**, *35*, 13827–13835. [\[CrossRef\]](#)
9. Skatchkov, S.N.; Antonov, S.M.; Eaton, M.J. Glia and Glial Polyamines. Role in Brain Function in Health and Disease. *Biochem. Mosc. Suppl. Ser. A* **2016**, *10*, 73–98. [\[CrossRef\]](#)
10. Herculano-Houzel, S.; Lent, R. Isotropic Fractionator: A Simple, Rapid Method for the Quantification of Total Cell and Neuron Numbers in the Brain. *J. Neurosci.* **2005**, *25*, 2518–2521. [\[CrossRef\]](#)
11. Oberheim, N.A.; Goldman, S.A.; Nedergaard, M. Heterogeneity of Astrocytic Form and Function. *Methods Mol. Biol.* **2012**, *814*, 23–45. [\[CrossRef\]](#)
12. Oberheim, N.A.; Wang, X.; Goldman, S.; Nedergaard, M. Astrocytic Complexity Distinguishes the Human Brain. *Trends Neurosci.* **2006**, *29*, 547–553. [\[CrossRef\]](#) [\[PubMed\]](#)
13. Holt, M.G. Astrocyte Heterogeneity and Interactions with Local Neural Circuits. *Essays Biochem.* **2023**, *67*, 93–106. [\[CrossRef\]](#) [\[PubMed\]](#)
14. Franze, K.; Grosche, J.; Skatchkov, S.N.; Schinkinger, S.; Foja, C.; Schild, D.; Uckermann, O.; Travis, K.; Reichenbach, A.; Guck, J. Müller Cells Are Living Optical Fibers in the Vertebrate Retina. *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 8287–8292. [\[CrossRef\]](#) [\[PubMed\]](#)
15. Endo, F.; Kasai, A.; Soto, J.S.; Yu, X.; Qu, Z.; Hashimoto, H.; Gradinaru, V.; Kawaguchi, R.; Khakh, B.S. Molecular Basis of Astrocyte Diversity and Morphology across the CNS in Health and Disease. *Science* **2022**, *378*, eadc9020. [\[CrossRef\]](#)
16. Reichenbach, A. Glia:Neuron Index: Review and Hypothesis to Account for Different Values in Various Mammals. *Glia* **1989**, *2*, 71–77. [\[CrossRef\]](#)
17. Lent, R.; Azevedo, F.A.C.; Andrade-Moraes, C.H.; Pinto, A.V.O. How Many Neurons Do You Have? Some Dogmas of Quantitative Neuroscience under Revision. *Eur. J. Neurosci.* **2012**, *35*, 1–9. [\[CrossRef\]](#)
18. Butt, A.M.; Kalsi, A. Inwardly Rectifying Potassium Channels (Kir) in Central Nervous System Glia: A Special Role for Kir4.1 in Glial Functions. *J. Cell. Mol. Med.* **2006**, *10*, 33–44. [\[CrossRef\]](#)
19. Chever, O.; Djukic, B.; McCarthy, K.D.; Amzica, F. Implication of Kir4.1 channel in excess potassium clearance: An in vivo study on anesthetized glial-conditional Kir4.1 knock-out mice. *J. Neurosci.* **2010**, *30*, 15769–15777. [\[CrossRef\]](#)
20. Kofuji, P.; Ceelen, P.; Zahs, K.R.; Surbeck, L.W.; Lester, H.A.; Newman, E.A. Genetic inactivation of an inwardly rectifying potassium channel (Kir4.1 subunit) in mice: Phenotypic impact in retina. *J. Neurosci.* **2000**, *20*, 5733–5740. [\[CrossRef\]](#)
21. Kucheryavykh, Y.V.; Kucheryavykh, L.Y.; Nichols, C.G.; Maldonado, H.M.; Baksi, K.; Reichenbach, A.; Skatchkov, S.N.; Eaton, M.J. Downregulation of Kir4.1 Inward Rectifying Potassium Channel Subunits by RNAi Impairs Potassium Transfer and Glutamate Uptake by Cultured Cortical Astrocytes. *Glia* **2007**, *55*, 274–281. [\[CrossRef\]](#)
22. Tejeda-Bayron, F.A.; Rivera-Aponte, D.E.; Malpica-Nieves, C.J.; Maldonado-Martínez, G.; Maldonado, H.M.; Skatchkov, S.N.; Eaton, M.J. Activation of Glutamate Transporter-1 (GLT-1) Confers Sex-Dependent Neuroprotection in Brain Ischemia. *Brain Sci.* **2021**, *11*, 76. [\[CrossRef\]](#) [\[PubMed\]](#)
23. Falco-Walter, J. Epilepsy-Definition, Classification, Pathophysiology, and Epidemiology. *Semin. Neurol.* **2020**, *40*, 617–623. [\[CrossRef\]](#) [\[PubMed\]](#)
24. Shorvon, S.D. The Etiologic Classification of Epilepsy. *Epilepsia* **2011**, *52*, 1052–1057. [\[CrossRef\]](#) [\[PubMed\]](#)
25. Yun, C.; Xuefeng, W. Association between Seizures and Diabetes Mellitus: A Comprehensive Review of Literature. *Curr. Diabetes Rev.* **2013**, *9*, 350–354. [\[CrossRef\]](#)
26. Oliver, D.; Hahn, H.; Antz, C.; Ruppertsberg, J.P.; Fakler, B. Interaction of permeant and blocking ions in cloned inward-rectifier K⁺ channels. *Biophys. J.* **1998**, *74*, 2318–2326. [\[CrossRef\]](#)
27. Nichols, C.G.; Lee, S.-J. Polyamines and Potassium Channels: A 25-Year Romance. *J. Biol. Chem.* **2018**, *293*, 18779–18788. [\[CrossRef\]](#)
28. Skatchkov, S.N.; Eaton, M.J.; Shuba, Y.M.; Kucheryavykh, Y.V.; Derst, C.; Veh, R.W.; Wurm, A.; Iandiev, I.; Pannicke, T.; Bringmann, A.; et al. Tandem-Pore Domain Potassium Channels Are Functionally Expressed in Retinal (Müller) Glial Cells. *Glia* **2006**, *53*, 266–276. [\[CrossRef\]](#)
29. Zhou, M.; Schools, G.P.; Kimelberg, H.K. Development of GLAST(+) Astrocytes and NG2(+) Glia in Rat Hippocampus CA1: Mature Astrocytes Are Electrophysiologically Passive. *J. Neurophysiol.* **2006**, *95*, 134–143. [\[CrossRef\]](#)
30. Poopalasundaram, S.; Knott, C.; Shamotienko, O.G.; Foran, P.G.; Dolly, J.O.; Ghiani, C.A.; Gallo, V.; Wilkin, G.P. Glial Heterogeneity in Expression of the Inwardly Rectifying K⁺ Channel, Kir4.1, in Adult Rat CNS. *Glia* **2000**, *30*, 362–372. [\[CrossRef\]](#)

31. Vit, J.-P.; Ohara, P.T.; Bhargava, A.; Kelley, K.; Jasmin, L. Silencing the Kir4.1 Potassium Channel Subunit in Satellite Glial Cells of the Rat Trigeminal Ganglion Results in Pain-like Behavior in the Absence of Nerve Injury. *J. Neurosci.* **2008**, *28*, 4161–4171. [\[CrossRef\]](#)
32. Hanani, M.; Spray, D.C. Emerging Importance of Satellite Glia in Nervous System Function and Dysfunction. *Nat. Rev. Neurosci.* **2020**, *21*, 485–498. [\[CrossRef\]](#) [\[PubMed\]](#)
33. Stephan, J.; Haack, N.; Kafitz, K.W.; Durry, S.; Koch, D.; Hochstrate, P.; Seifert, G.; Steinhäuser, C.; Rose, C.R. Kir4.1 channels mediate a depolarization of hippocampal astrocytes under hyperammonemic conditions in situ. *Glia* **2012**, *60*, 965–978. [\[CrossRef\]](#) [\[PubMed\]](#)
34. Lanciotti, A.; Brignone, M.S.; De Nuccio, C.; Sposito, S.; Caprini, E.S.; Belfiore, M.; Nicita, F.; Veroni, C.; Meloni, C.; Carrozzo, R.; et al. Astrocytes differentiated from patient iPSCs model the rare leukodystrophy MLC and uncover disease-linked maturation defects and Kir4.1 channel dysfunction. *Neurobiol. Dis.* **2026**, *218*, 107218. [\[CrossRef\]](#) [\[PubMed\]](#)
35. Rivera-Aponte, D.E.; Mendez-Gonzalez, M.P.; Rivera-Pagan, A.F.; Kucheryavykh, Y.V.; Kucheryavykh, L.Y.; Skatchkov, S.N.; Eaton, M.J. Hyperglycemia Reduces Functional Expression of Astrocytic Kir4.1 Channels and Glial Glutamate Uptake. *Neuroscience* **2015**, *310*, 216–223. [\[CrossRef\]](#)
36. Méndez-González, M.P.; Rivera-Aponte, D.E.; Benedikt, J.; Maldonado-Martínez, G.; Tejeda-Bayron, F.; Skatchkov, S.N.; Eaton, M.J. Downregulation of Astrocytic Kir4.1 Potassium Channels Is Associated with Hippocampal Neuronal Hyperexcitability in Type 2 Diabetic Mice. *Brain Sci.* **2020**, *10*, 72. [\[CrossRef\]](#)
37. Banerjee, A.; Ghatak, S.; Sikdar, S.K. L-Lactate Mediates Neuroprotection against Ischaemia by Increasing TREK1 Channel Expression in Rat Hippocampal Astrocytes in Vitro. *J. Neurochem.* **2016**, *138*, 265–281. [\[CrossRef\]](#)
38. Wang, W.; Kiyoshi, C.M.; Du, Y.; Taylor, A.T.; Sheehan, E.R.; Wu, X.; Zhou, M. TREK-1 Null Impairs Neuronal Excitability, Synaptic Plasticity, and Cognitive Function. *Mol. Neurobiol.* **2020**, *57*, 1332–1346. [\[CrossRef\]](#)
39. Wang, F.; Smith, N.A.; Xu, Q.; Goldman, S.; Peng, W.; Huang, J.H.; Takano, T.; Nedergaard, M. Photolysis of Caged Ca²⁺ but Not Receptor-Mediated Ca²⁺ Signaling Triggers Astrocytic Glutamate Release. *J. Neurosci.* **2013**, *33*, 17404–17412. [\[CrossRef\]](#)
40. Weller, J.; Steinhäuser, C.; Seifert, G. pH-Sensitive K⁺ Currents and Properties of K2P Channels in Murine Hippocampal Astrocytes. *Adv. Protein Chem. Struct. Biol.* **2016**, *103*, 263–294. [\[CrossRef\]](#)
41. Du, M.; Li, J.; Chen, L.; Yu, Y.; Wu, Y. Astrocytic Kir4.1 Channels and Gap Junctions Account for Spontaneous Epileptic Seizure. *PLoS Comput. Biol.* **2018**, *14*, e1005877. [\[CrossRef\]](#)
42. Wang, X.; Levic, S.; Gratton, M.A.; Doyle, K.J.; Yamoah, E.N.; Pegg, A.E. Spermine Synthase Deficiency Leads to Deafness and a Profound Sensitivity to Alpha-Difluoromethylornithine. *J. Biol. Chem.* **2009**, *284*, 930–937. [\[CrossRef\]](#)
43. Rivera-Pagán, A.F.; Rivera-Aponte, D.E.; Melnik-Martínez, K.V.; Zayas-Santiago, A.; Kucheryavykh, L.Y.; Martins, A.H.; Cubano, L.A.; Skatchkov, S.N.; Eaton, M.J. Up-Regulation of TREK-2 Potassium Channels in Cultured Astrocytes Requires De Novo Protein Synthesis: Relevance to Localization of TREK-2 Channels in Astrocytes after Transient Cerebral Ischemia. *PLoS ONE* **2015**, *10*, e0125195. [\[CrossRef\]](#)
44. Kucheryavykh, L.Y.; Kucheryavykh, Y.V.; Inyushin, M.; Shuba, Y.M.; Sanabria, P.; Cubano, L.A.; Skatchkov, S.N.; Eaton, M.J. Ischemia Increases TREK-2 Channel Expression in Astrocytes: Relevance to Glutamate Clearance. *Open Neurosci. J.* **2009**, *3*, 40–47. [\[CrossRef\]](#) [\[PubMed\]](#)
45. Colón-Vázquez, J.; Rosado-Rivera, N.M.; Navedo-Jackson, J.J.; Angueira-Laureano, A.A.; Hernandez-Santiago, Y.; Maldonado-Martínez, G.; Méndez-González, M.P.; Eaton, M.J.; Skatchkov, S.N.; Rivera-Aponte, D.E. Role of Glyoxalase in Astrocytes' Supportive Function Under Hyperglycemic Conditions: Aminoguanidine and Kir4.1 Channel Recovery. *Brain Sci.* **2025**, *15*, 1075. [\[CrossRef\]](#) [\[PubMed\]](#)
46. Xu, W.; Morford, J.; Mauvais-Jarvis, F. Emerging Role of Testosterone in Pancreatic β Cell Function and Insulin Secretion. *J. Endocrinol.* **2019**, *240*, R97–R105. [\[CrossRef\]](#) [\[PubMed\]](#)
47. Gozlan, E.; Lewit-Cohen, Y.; Frenkel, D. Sex Differences in Astrocyte Activity. *Cells* **2024**, *13*, 1724. [\[CrossRef\]](#)
48. Depaauw-Holt, L.R.; Duquenne, M.; Hamane, S.; Peyrard, S.; Rogers, B.; Ireland, C.; Nasu, Y.; Fulton, S.; Bosson, A.; Alquier, T.; et al. A Divergent Astrocytic Response to Stress Alters Activity Patterns via Distinct Mechanisms in Male and Female Mice. *Nat. Commun.* **2025**, *16*, 6372. [\[CrossRef\]](#)
49. Piattini, F.; Le Foll, C.; Kisielow, J.; Rosenwald, E.; Nielsen, P.; Lutz, T.; Schneider, C.; Kopf, M. A Spontaneous Leptin Receptor Point Mutation Causes Obesity and Differentially Affects Leptin Signaling in Hypothalamic Nuclei Resulting in Metabolic Dysfunctions Distinct from Db/Db Mice. *Mol. Metab.* **2019**, *25*, 131–141. [\[CrossRef\]](#)
50. Hibino, H.; Fujita, A.; Iwai, K.; Yamada, M.; Kurachi, Y. Differential Assembly of Inwardly Rectifying K⁺ Channel Subunits, Kir4.1 and Kir5.1, in Brain Astrocytes. *J. Biol. Chem.* **2004**, *279*, 44065–44073. [\[CrossRef\]](#)
51. Fan, H.; Huang, A.; Villegas, C.; Wright, J.A. The R1 Component of Mammalian Ribonucleotide Reductase Has Malignancy-Suppressing Activity as Demonstrated by Gene Transfer Experiments. *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 13181–13186. [\[CrossRef\]](#)

52. Inyushin, M.; Kucheryavykh, L.Y.; Kucheryavykh, Y.V.; Nichols, C.G.; Buono, R.J.; Ferraro, T.N.; Skatchkov, S.N.; Eaton, M.J. Potassium Channel Activity and Glutamate Uptake Are Impaired in Astrocytes of Seizure-Susceptible DBA/2 Mice: Impaired Astrocyte Function in DBA/2 Mice. *Epilepsia* **2010**, *51*, 1707–1713. [\[CrossRef\]](#)
53. Battefeld, A.; Klooster, J.; Kole, M.H.P. Myelinating Satellite Oligodendrocytes Are Integrated in a Glial Syncytium Constraining Neuronal High-Frequency Activity. *Nat. Commun.* **2016**, *7*, 11298. [\[CrossRef\]](#) [\[PubMed\]](#)
54. Maldonado, P.P.; Velez-Fort, M.; Levavasseur, F.; Angulo, M.C. Oligodendrocyte Precursor Cells Are Accurate Sensors of Local K⁺ in Mature Gray Matter. *J. Neurosci.* **2013**, *33*, 2432–2442. [\[CrossRef\]](#) [\[PubMed\]](#)
55. Reichelt, W.; Pannicke, T. Voltage-Dependent K⁺ Currents in Guinea Pig Muller (Glia) Cells Show Different Sensitivities to Blockade by Ba²⁺. *Neurosci. Lett.* **1993**, *155*, 15–18. [\[PubMed\]](#)
56. Olsen, M.L.; Campbell, S.L.; Sontheimer, H. Differential distribution of Kir4.1 in spinal cord astrocytes suggests regional differences in K⁺ homeostasis. *J. Neurophysiol.* **2007**, *98*, 786–793. [\[CrossRef\]](#)
57. Skatchkov, S.N.; Vyklicky, L.; Orkand, R.K. Potassium Currents in Endfeet of Isolated Muller Cells from the Frog Retina. *Glia* **1995**, *15*, 54–64. [\[CrossRef\]](#)
58. Newman, E.A. Inward-rectifying potassium channels in retinal glial (Müller) cells. *J. Neurosci.* **1993**, *13*, 3333–3345. [\[CrossRef\]](#)
59. Chen, K.C.; Nicholson, C. Spatial Buffering of Potassium Ions in Brain Extracellular Space. *Biophys. J.* **2000**, *78*, 2776–2797. [\[CrossRef\]](#)
60. Boni, J.L.; Kahanovitch, U.; Nwaobi, S.E.; Floyd, C.L.; Olsen, M.L. DNA Methylation: A Mechanism for Sustained Alteration of KIR4.1 Expression Following Central Nervous System Insult. *Glia* **2020**, *68*, 1495–1512. [\[CrossRef\]](#)
61. Nwaobi, S.E.; Lin, E.; Peramsetty, S.R.; Olsen, M.L. DNA Methylation Functions as a Critical Regulator of Kir4.1 Expression during CNS Development. *Glia* **2014**, *62*, 411–427. [\[CrossRef\]](#)
62. Verrotti, A.; Scaparrotta, A.; Olivieri, C.; Chiarelli, F. Seizures and Type 1 Diabetes Mellitus: Current State of Knowledge. *Eur. J. Endocrinol.* **2012**, *167*, 749–758. [\[CrossRef\]](#)
63. Kamha, A. Non Ketotic Hyperosmolar Hyperglycemia Presenting as Epilepsia Partialis Continua: An Unusual Presentation of a Common Disorder. *Libyan J. Med.* **2008**, *3*, 111–112. [\[CrossRef\]](#)
64. Kautzky-Willer, A.; Leutner, M.; Harreiter, J. Sex Differences in Type 2 Diabetes. *Diabetologia* **2023**, *66*, 986–1002. [\[CrossRef\]](#) [\[PubMed\]](#)
65. Moien-Afshari, F.; Tellez-Zenteno, J.F. Occipital Seizures Induced by Hyperglycemia: A Case Report and Review of Literature. *Seizure* **2009**, *18*, 382–385. [\[CrossRef\]](#) [\[PubMed\]](#)
66. Mauvais-Jarvis, F. Gender Differences in Glucose Homeostasis and Diabetes. *Physiol. Behav.* **2018**, *187*, 20–23. [\[CrossRef\]](#) [\[PubMed\]](#)
67. Reddy, D.S. Neurosteroids and Their Role in Sex-Specific Epilepsies. *Neurobiol. Dis.* **2014**, *72*, 198–209. [\[CrossRef\]](#)
68. Olsen, M.L.; Sontheimer, H. Mislocalization of Kir Channels in Malignant Glia. *Glia* **2004**, *46*, 63–73. [\[CrossRef\]](#)
69. Gao, F.; Li, F.; Miao, Y.; Dong, L.-D.; Zhang, S.-H.; Wu, J.; Sun, X.-H.; Wang, Z. Group I Metabotropic Glutamate Receptor Agonist DHPG Modulates Kir4.1 Protein and mRNA in Cultured Rat Retinal Müller Cells. *Neurosci. Lett.* **2015**, *588*, 12–17. [\[CrossRef\]](#)
70. Ulbricht, E.; Pannicke, T.; Hollborn, M.; Raap, M.; Goczalik, I.; Iandiev, I.; Härtig, W.; Uhlmann, S.; Wiedemann, P.; Reichenbach, A.; et al. Proliferative Gliosis Causes Mislocation and Inactivation of Inwardly Rectifying K⁺ (Kir) Channels in Rabbit Retinal Glial Cells. *Exp. Eye Res.* **2008**, *86*, 305–313. [\[CrossRef\]](#)
71. Schulte, U.; Müller, C.S.; Fakler, B. Ion Channels and Their Molecular Environments—Glimpses and Insights from Functional Proteomics. *Semin. Cell Dev. Biol.* **2011**, *22*, 132–144. [\[CrossRef\]](#)
72. Kofuji, P.; Newman, E.A. Potassium Buffering in the Central Nervous System. *Neuroscience* **2004**, *129*, 1045–1056. [\[CrossRef\]](#)
73. Neusch, C.; Rozengurt, N.; Jacobs, R.E.; Lester, H.A.; Kofuji, P. Kir4.1 Potassium Channel Subunit Is Crucial for Oligodendrocyte Development and in Vivo Myelination. *J. Neurosci.* **2001**, *21*, 5429–5438. [\[CrossRef\]](#) [\[PubMed\]](#)
74. Bockenbauer, D.; Feather, S.; Stanescu, H.C.; Bandulik, S.; Zdebik, A.A.; Reichold, M.; Tobin, J.; Lieberer, E.; Sterner, C.; Landouere, G.; et al. Epilepsy, Ataxia, Sensorineural Deafness, Tubulopathy, and KCNJ10 Mutations. *N. Engl. J. Med.* **2009**, *360*, 1960–1970. [\[CrossRef\]](#) [\[PubMed\]](#)
75. Reichold, M.; Zdebik, A.A.; Lieberer, E.; Rapedius, M.; Schmidt, K.; Bandulik, S.; Sterner, C.; Tegtmeier, I.; Penton, D.; Baukrowitz, T.; et al. KCNJ10 Gene Mutations Causing EAST Syndrome (Epilepsy, Ataxia, Sensorineural Deafness, and Tubulopathy) Disrupt Channel Function. *Proc. Natl. Acad. Sci. USA* **2010**, *107*, 14490–14495. [\[CrossRef\]](#) [\[PubMed\]](#)
76. Seifert, G.; Henneberger, C.; Steinhäuser, C. Diversity of Astrocyte Potassium Channels: An Update. *Brain Res. Bull.* **2018**, *136*, 26–36. [\[CrossRef\]](#)
77. Seifert, G.; Huttmann, K.; Binder, D.K.; Hartmann, C.; Wyczynski, A.; Neusch, C.; Steinhäuser, C. Analysis of Astroglial K⁺ Channel Expression in the Developing Hippocampus Reveals a Predominant Role of the Kir4.1 Subunit. *J. Neurosci.* **2009**, *29*, 7474–7488. [\[CrossRef\]](#)
78. Zhou, M.; Xu, G.; Xie, M.; Zhang, X.; Schools, G.P.; Ma, L.; Kimelberg, H.K.; Chen, H. TWIK-1 and TREK-1 Are Potassium Channels Contributing Significantly to Astrocyte Passive Conductance in Rat Hippocampal Slices. *J. Neurosci.* **2009**, *29*, 8551–8564. [\[CrossRef\]](#)

79. Djukic, B.; Casper, K.B.; Philpot, B.D.; Chin, L.-S.; McCarthy, K.D. Conditional Knock-Out of Kir4.1 Leads to Glial Membrane Depolarization, Inhibition of Potassium and Glutamate Uptake, and Enhanced Short-Term Synaptic Potentiation. *J. Neurosci.* **2007**, *27*, 11354–11365. [\[CrossRef\]](#)
80. Thomzig, A.; Laube, G.; Prüss, H.; Veh, R.W. Pore-forming subunits of K-ATP channels, Kir6.1 and Kir6.2, display prominent differences in regional and cellular distribution in the rat brain. *J. Comp. Neurol.* **2005**, *484*, 313–330. [\[CrossRef\]](#) [\[PubMed\]](#)
81. Skatchkov, S.N.; Rojas, L.; Eaton, M.J.; Orkand, R.K.; Biedermann, B.; Bringmann, A.; Pannicke, T.; Veh, R.W.; Reichenbach, A. Functional Expression of Kir 6.1/SUR1-K(ATP) Channels in Frog Retinal Müller Glial Cells. *Glia* **2002**, *38*, 256–267. [\[CrossRef\]](#)
82. Wanrooy, B.J.; Kumar, K.P.; Wen, S.W.; Qin, C.X.; Ritchie, R.H.; Wong, C.H.Y. Distinct Contributions of Hyperglycemia and High-Fat Feeding in Metabolic Syndrome-Induced Neuroinflammation. *J. Neuroinflammation* **2018**, *15*, 293. [\[CrossRef\]](#)
83. Bogdanov, P.; Corraliza, L.; Villena, J.A.; Carvalho, A.R.; Garcia-Arumí, J.; Ramos, D.; Ruberte, J.; Simó, R.; Hernández, C. The Db/Db Mouse: A Useful Model for the Study of Diabetic Retinal Neurodegeneration. *PLoS ONE* **2014**, *9*, e97302. [\[CrossRef\]](#)
84. Chvátal, A.; Berger, T.; Vorisek, I.; Orkand, R.K.; Kettenmann, H.; Syková, E. Changes in glial K⁺ currents with decreased extracellular volume in developing rat white matter. *J. Neurosci. Res.* **1997**, *49*, 98–106. [\[CrossRef\]](#)
85. Sibille, J.; Pannasch, U.; Rouach, N. Astroglial Potassium Clearance Contributes to Short-Term Plasticity of Synaptically Evoked Currents at the Tripartite Synapse. *J. Physiol.* **2014**, *592*, 87–102. [\[CrossRef\]](#) [\[PubMed\]](#)
86. Witthoft, A.; Filosa, J.A.; Karniadakis, G.E. Potassium Buffering in the Neurovascular Unit: Models and Sensitivity Analysis. *Biophys. J.* **2013**, *105*, 2046–2054. [\[CrossRef\]](#)
87. Bordey, A.; Sontheimer, H. Modulation of Glutamatergic Transmission by Bergmann Glial Cells in Rat Cerebellum in Situ. *J. Neurophysiol.* **2003**, *89*, 979–988. [\[CrossRef\]](#)
88. Mennerick, S.; Shen, W.; Xu, W.; Benz, A.; Tanaka, K.; Shimamoto, K.; Isenberg, K.E.; Krause, J.E.; Zorumski, C.F. Substrate Turnover by Transporters Curtails Synaptic Glutamate Transients. *J. Neurosci.* **1999**, *19*, 9242–9251. [\[CrossRef\]](#)
89. Del Río, J.P.; Allende, M.I.; Molina, N.; Serrano, F.G.; Molina, S.; Vigil, P. Steroid Hormones and Their Action in Women's Brains: The Importance of Hormonal Balance. *Front. Public Health* **2018**, *6*, 141. [\[CrossRef\]](#)
90. Oberlander, J.G.; Woolley, C.S. 17 β -Estradiol Acutely Potentiates Glutamatergic Synaptic Transmission in the Hippocampus through Distinct Mechanisms in Males and Females. *J. Neurosci.* **2016**, *36*, 2677–2690. [\[CrossRef\]](#)
91. Pottoo, F.H.; Javed, M.N.; Barkat, M.A.; Alam, M.S.; Nowshehri, J.A.; Alshayban, D.M.; Ansari, M.A. Estrogen and Serotonin: Complexity of Interactions and Implications for Epileptic Seizures and Epileptogenesis. *Curr. Neuropharmacol.* **2019**, *17*, 214–231. [\[CrossRef\]](#)
92. Reddy, D.S.; Kim, H.Y.; Rogawski, M.A. Neurosteroid Withdrawal Model of Perimenstrual Catamenial Epilepsy. *Epilepsia* **2001**, *42*, 328–336. [\[CrossRef\]](#) [\[PubMed\]](#)
93. Taubøll, E.; Sveberg, L.; Svalheim, S. Interactions between Hormones and Epilepsy. *Seizure* **2015**, *28*, 3–11. [\[CrossRef\]](#) [\[PubMed\]](#)
94. Velísková, J.; Velíšek, L. Behavioral Characterization and Scoring of Seizures in Rodents. In *Models of Seizures and Epilepsy*; Academic Press: Cambridge, MA, USA, 2017; pp. 111–123.
95. Buono, R.J.; Lohoff, F.W.; Sander, T.; Sperling, M.R.; O'Connor, M.J.; Dlugos, D.J.; Ryan, S.G.; Golden, G.T.; Zhao, H.; Scattergood, T.M.; et al. Association between Variation in the Human KCNJ10 Potassium Ion Channel Gene and Seizure Susceptibility. *Epilepsy Res.* **2004**, *58*, 175–183. [\[CrossRef\]](#) [\[PubMed\]](#)
96. Ferraro, T.N.; Golden, G.T.; Smith, G.G.; Martin, J.F.; Lohoff, F.W.; Gieringer, T.A.; Zamboni, D.; Schwebel, C.L.; Press, D.M.; Kratzer, S.O.; et al. Fine Mapping of a Seizure Susceptibility Locus on Mouse Chromosome 1: Nomination of Kcnj10 as a Causative Gene. *Mamm. Genome* **2004**, *15*, 239–251. [\[CrossRef\]](#)
97. Mir, A.; Chaudhary, M.; Alkhalidi, H.; Alhazmi, R.; Albaradie, R.; Housawi, Y. Epilepsy in Patients with EAST Syndrome Caused by Mutation in the KCNJ10. *Brain Dev.* **2019**, *41*, 706–715. [\[CrossRef\]](#)
98. Scholl, U.I.; Choi, M.; Liu, T.; Ramaekers, V.T.; Hausler, M.G.; Grimmer, J.; Tobe, S.W.; Farhi, A.; Nelson-Williams, C.; Lifton, R.P. Seizures, Sensorineural Deafness, Ataxia, Mental Retardation, and Electrolyte Imbalance (SeSAME Syndrome) Caused by Mutations in KCNJ10. *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 5842–5847. [\[CrossRef\]](#)
99. Heinemann, U.; Lux, H.D. Ceiling of Stimulus Induced Rises in Extracellular Potassium Concentration in the Cerebral Cortex of Cat. *Brain Res.* **1977**, *120*, 231–249. [\[CrossRef\]](#)
100. Alex, A.; Luo, Q.; Mathew, D.; Di, R.; Bhatwadekar, A.D. Metformin Corrects Abnormal Circadian Rhythm and Kir4.1 Channels in Diabetes. *Investig. Ophthalmol. Vis. Sci.* **2020**, *61*, 46. [\[CrossRef\]](#)
101. Laube, G.; Veh, R.W. Astrocytes, Not Neurons, Show Most Prominent Staining for Spermidine/Spermine-like Immunoreactivity in Adult Rat Brain. *Glia* **1997**, *19*, 171–179. [\[CrossRef\]](#)
102. Biedermann, B.; Skatchkov, S.N.; Brunk, I.; Bringmann, A.; Pannicke, T.; Bernstein, H.G.; Faude, F.; Germer, A.; Veh, R.; Reichenbach, A. Spermine/Spermidine Is Expressed by Retinal Glial (Müller) Cells and Controls Distinct K⁺ Channels of Their Membrane. *Glia* **1998**, *23*, 209–220. [\[CrossRef\]](#)
103. Van Veen, S.; Irala, D.; Sakers, K.; Savage, J.; Séjourné, G.; Bindu, D.S.; Ausloos, E.; Dhondt, H.; Schoonvliet, N.; den Haute, C.V.; et al. Astrocytic Polyamine Transport by ATP13A4 Tunes Excitatory Synaptic Transmission. *medRxiv* **2025**. medRxiv:2025.04. [\[CrossRef\]](#)

104. Malpica-Nieves, C.J.; Rivera, Y.; Rivera-Aponte, D.E.; Phanstiel, O.; Veh, R.W.; Eaton, M.J.; Skatchkov, S.N. Uptake of Biotinylated Spermine in Astrocytes: Effect of Cx43 siRNA, HIV-Tat Protein and Polyamine Transport Inhibitor on Polyamine Uptake. *Biomolecules* **2021**, *11*, 1187. [[CrossRef](#)] [[PubMed](#)]
105. Gilad, G.M.; Balakrishnan, K.; Gilad, V.H. The course of putrescine immunocytochemical appearance in neurons, astroglia and microglia in rat brain cultures. *Neurosci. Lett.* **1999**, *268*, 33–36. [[CrossRef](#)] [[PubMed](#)]
106. Skatchkov, S.N.; Eaton, M.J.; Krusek, J.; Veh, R.W.; Biedermann, B.; Bringmann, A.; Pannicke, T.; Orkand, R.K.; Reichenbach, A. Spatial Distribution of Spermine/Spermidine Content and K⁺-Current Rectification in Frog Retinal Glial (Muller) Cells. *Glia* **2000**, *31*, 84–90. [[CrossRef](#)]
107. Eisenberg, T.; Knauer, H.; Schauer, A.; Büttner, S.; Ruckstuhl, C.; Carmona-Gutierrez, D.; Ring, J.; Schroeder, S.; Magnes, C.; Antonacci, L.; et al. Induction of Autophagy by Spermidine Promotes Longevity. *Nat. Cell Biol.* **2009**, *11*, 1305–1314. [[CrossRef](#)]
108. Hofer, S.J.; Daskalaki, I.; Bergmann, M.; Friščić, J.; Zimmermann, A.; Mueller, M.I.; Abdellatif, M.; Nicastro, R.; Masser, S.; Durand, S.; et al. Spermidine Is Essential for Fasting-Mediated Autophagy and Longevity. *Nat. Cell Biol.* **2024**, *26*, 1571–1584. [[CrossRef](#)]
109. Hofer, S.J.; Daskalaki, I.; Abdellatif, M.; Stelzl, U.; Sedej, S.; Tavernarakis, N.; Kroemer, G.; Madeo, F. A surge in endogenous spermidine is essential for rapamycin-induced autophagy and longevity. *Autophagy* **2024**, *20*, 2824–2826. [[CrossRef](#)]
110. Schwarz, C.; Benson, G.S.; Horn, N.; Wurdack, K.; Grittner, U.; Schilling, R.; Märsch, S.; Köbe, T.; Hofer, S.J.; Magnes, C.; et al. Effects of Spermidine Supplementation on Cognition and Biomarkers in Older Adults with Subjective Cognitive Decline: A Randomized Clinical Trial. *JAMA Netw. Open.* **2022**, *5*, e2213875. [[CrossRef](#)]
111. Liang, Y.; Piao, C.; Beuschel, C.B.; Toppe, D.; Kollipara, L.; Bogdanow, B.; Maglione, M.; Lützkendorf, J.; See, J.C.K.; Huang, S.; et al. eIF5A hypusination, boosted by dietary spermidine, protects from premature brain aging and mitochondrial dysfunction. *Cell Rep.* **2021**, *35*, 108941. [[CrossRef](#)]
112. Schroeder, S.; Hofer, S.J.; Zimmermann, A.; Pechlaner, R.; Dambrueck, C.; Pendl, T.; Marcello, G.M.; Pogatschnigg, V.; Bergmann, M.; Müller, M.; et al. Dietary spermidine improves cognitive function. *Cell Rep.* **2021**, *35*, 108985. [[CrossRef](#)]
113. Maglione, M.; Kochlamazashvili, G.; Eisenberg, T.; Rácz, B.; Michael, E.; Toppe, D.; Stumpf, A.; Wirth, A.; Zeug, A.; Müller, F.E.; et al. Spermidine protects from age-related synaptic alterations at hippocampal mossy fiber-CA3 synapses. *Sci. Rep.* **2019**, *9*, 19616. [[CrossRef](#)]
114. Madeo, F.; Carmona-Gutierrez, D.; Kepp, O.; Kroemer, G. Spermidine delays aging in humans. *Aging* **2018**, *10*, 2209–2211. [[CrossRef](#)]
115. Burke, S.J.; Batdorf, H.M.; Burk, D.H.; Noland, R.C.; Eder, A.E.; Boulos, M.S.; Karlstad, M.D.; Collier, J.J. *db/db* Mice Exhibit Features of Human Type 2 Diabetes That Are Not Present in Weight-Matched C57BL/6J Mice Fed a Western Diet. *J. Diabetes Res.* **2017**, *2017*, 8503754. [[CrossRef](#)]

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.