**K$_{\text{V7}}$/KCNQ Channels Are Functionally Expressed in Oligodendrocyte Progenitor Cells**

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**Abstract**

**Background:** K$_{\text{V7}}$/KCNQ channels are widely expressed in neurons and they have multiple important functions, including control of excitability, spike afterpotentials, adaptation, and theta resonance. Mutations in KCNQ genes have been demonstrated to associate with human neurological pathologies. However, little is known about whether K$_{\text{V7}}$/KCNQ channels are expressed in oligodendrocyte lineage cells (OLCs) and what their functions in OLCs.

**Methods and Findings:** In this study, we characterized K$_{\text{V7}}$/KCNQ channels expression in rat primary cultured OLCs by RT-PCR, immunostaining and electrophysiology. KCNQ2-5 mRNAs existed in all three developmental stages of rat primary cultured OLCs. K$_{\text{V7}}$/KCNQ proteins were also detected in oligodendrocyte progenitor cells (OPCs), early developmental stages of OLCs) of rat primary cultures and cortex slices. Voltage-clamp recording revealed that the $I_{\text{Kop}}$ antagonist XE991 significantly reduced K$_{\text{V7}}$/KCNQ channel current ($I_{\text{K(Q)}}$) in OPCs but not in differentiated oligodendrocytes. In addition, inhibition of K$_{\text{V7}}$/KCNQ channels promoted OPCs motility in vitro.

**Conclusions:** These findings showed that K$_{\text{V7}}$/KCNQ channels were functionally expressed in rat primary cultured OLCs and might play an important role in OPCs functioning in physiological or pathological conditions.

**Introduction**

The KCNQ gene family encodes five voltage-gated delayed rectifier K$^+$ channels K$_{\text{V7.1-5}}$, and four of these K$_{\text{V7.2-5}}$ are expressed in the nervous system [1,2]. There they form subunits of voltage-gated K$^+$ channel originally termed the ‘M-channel’ and the current called M current, which has been demonstrated to assist in stabilizing the membrane potential in the presence of depolarizing currents and contributing to the resting potential of neurons [3,4]. In CNS, K$_{\text{V7}}$ channels form through homo- or heteromeric assembly of K$_{\text{V7.2}}$ to K$_{\text{V7.5}}$ subunits. So far, homomeric compositions are shown for K$_{\text{V7.2}}$-5 subunits; heteromeric compositions are represented by K$_{\text{V7.2}}$+3, K$_{\text{V7.3+4}}$ and K$_{\text{V7.3+5}}$ channels [2]. In most neurons native K$_{\text{V7}}$ channels are composed of K$_{\text{V7.2}}$ and K$_{\text{V7.3}}$ subunits [5] or sometimes of homomeric K$_{\text{V7.2}}$ subunits [6,7], although probably with a contribution by K$_{\text{V7.5}}$ subunits in some neurons [8]; K$_{\text{V7.4}}$ subunits are predominantly expressed in the auditory and vestibular systems, but also probably contribute to K$_{\text{V7}}$ channels in central dopaminergic neurons [9]. Recent evidences suggest that K$_{\text{V7}}$ channels have profound effects on neuronal excitability [10–15]. Inhibition of channel activity, by either a blocking drug such as linopirdine (DuP 996) [16] or 10, 10-bis(4-pyridinyl-methyl)-9(10H)-anthracenone (XE991), or expression of a dominant-negative K$_{\text{V7.2}}$ construct, strongly enhances repetitive firing and even effects postnatal brain development [17]. Their mutations have been associated with human neurological pathologies including auditory diseases [1,2]. Mutations in either K$_{\text{V7.2}}$ or K$_{\text{V7.3}}$ lead to benign familial neonatal seizures [18] as do mutations in K$_{\text{V7.5}}$ [19,20]. In addition, mutations in K$_{\text{V7.4}}$ are associated with progressive hearing loss [21–23].

Oligodendrocytes are generated from oligodendroglial progenitor cells (OPCs) which proliferate in the subventricular zone and migrate to formative white matter regions, where they further proliferate, differentiate, and form myelin sheaths around axons [24,25]. Migration of OPCs is an essential step not only during the early stage of oligodendrocyte lineage cells (OLCs) development but also in some demyelination pathological conditions such as Multiple Sclerosis (MS) and other variety of CNS injuries [26–28]. Several ion channels have been identified recently in OLCs to participate in regulation of OPCs migration including the early stage of oligodendrocyte lineage cells (OLCs) development but also in some demyelination pathological conditions such as Multiple Sclerosis (MS) and other variety of CNS injuries [26–28]. Several ion channels have been identified recently in OLCs to participate in regulation of OPCs migration including the early stage of oligodendrocyte lineage cells (OLCs) development but also in some demyelination pathological conditions such as Multiple Sclerosis (MS) and other variety of CNS injuries [26–28]. Several ion channels have been identified recently in OLCs to participate in regulation of OPCs migration including the early stage of oligodendrocyte lineage cells (OLCs) development but also in some demyelination pathological conditions such as Multiple Sclerosis (MS) and other variety of CNS injuries [26–28]. Several ion channels have been identified recently in OLCs to participate in regulation of OPCs migration including the early stage of oligodendrocyte lineage cells (OLCs) development but also in some demyelination pathological conditions such as Multiple Sclerosis (MS) and other variety of CNS injuries [26–28]. Several ion channels have been identified recently in OLCs to participate in regulation of OPCs migration including the early stage of oligodendrocyte lineage cells (OLCs) development but also in some demyelination pathological conditions such as Multiple Sclerosis (MS) and other variety of CNS injuries [26–28]. Several ion channels have been identified recently in OLCs to participate in regulation of OPCs migration including the early stage of oligodendrocyte lineage cells (OLCs) development but also in some demyelination pathological conditions such as Multiple Sclerosis (MS) and other variety of CNS injuries [26–28].
epithelial cells [36]. Kv1.10.1 is involved in adhesion and viability of CHO cells [37]. Kv11.1 participates in tumor cells invasion [30] and inhibition of Kv1.3 suppresses the motility and activation of effector memory T (Tem) cells [39]. OLCs express all six members of the delayed rectifier Shaker family K\(^+\) channels, Kv1.1–Kv1.6 [40–45], inwardly rectifying K\(^+\) (Kir) channels Kir2.1, Kir1.1 and Kir4.1 [46,47] and Kv3.1[29]. However, whether OLCs functionally express KV7 channels is still unknown. In this paper, we studied the expression and function of Kv7 channels in OLCs.

**Results**

The mRNAs of Kv7.2–5/ KCNQ2-5 were detected in rat primary cultured OLCs.

Immunocytochemical markers allow for the distinction of three consecutive phenotypically defined stages of OLCs development in vitro: the bipolar GFAP\(^2\)A2B5\(^+\) NG2\(^+\) OPCs, multipolar O4\(^+\)GalC\(^2\) IOs, and complex process bearing MBP\(^+\)GalC\(^2\) MOs [48,49]. In the present study, we got highly pure GFAP\(^2\)A2B5\(^+\) NG2\(^+\) OPC cultures (98.8\(^\pm\)0.2%, assessed by immunocytochemical staining) (Fig.1 A, B). In differentiation medium, OPCs developed into O4\(^+\) IOs and MBP\(^+\) MOs (Fig.1 C, D). As KCNQ1 was not detected in neural system [1,2], we examined the mRNAs of KCNQ2-5 in cultured OLCs by RT-PCR. We found that KCNQ2-5 mRNA were all present in cultured OPCs (Fig.2A left). KCNQ5 was undetectable in IOs (Fig.2C left). In MOs, only KCNQ4 was detectable very weakly (Fig.2D left). No positive line was present in negative control which implied that the mRNA was not contaminated with genome DNA (Fig.2A, C, D right).

Localization of Kv7.2-5/ KCNQ2-5 in OLCs

The expression of Kv7.2-5 in cultured OLCs was further confirmed by immunostaining. The antibodies of anti-NG2, anti-O4 and anti-MBP were used to identify OPCs, IOs, and MOs in cultures respectively. The staining for Kv7.2-5 was on the soma and processes of OPCs. The immunofluorescence signals were positive in both the cytoplasm and the cell membrane of OPCs (Fig.3A–D). With the maturation of OLCs, the immunofluorescence signals of Kv7.2-5 became weaker and were restricted to the cell bodies in IOs, and MOs. (Fig.3E–H, I–L). Immunohistochemistry was also performed to verify the expression of Kv7.2-5 proteins in OPCs in vivo. In cortex, Kv7.2, 3 or 5 were detected to localize on a part of NG2\(^+\) OPCs (26\(^\pm\)9%, 27.1\(^\pm\)11% and 30.5\(^\pm\)7% respectively) (Fig.4A–C, a1–a3; d1–d3; g1–g3), while other part of NG2\(^+\) OPCs did not express Kv7.2, 3 or 5 (Fig.4A–C, b1–b3; e1–e3; h1–h3). We also found that some cells, which expressed Kv7.2, 3 or 5, were not NG2 positive (Fig.4A–C, c1–c3; f1–f3; i1–i3). Those cells may be neurons or other glia cells. All NG2\(^+\) cells were Kv7.4 negative (Fig.4D, j1–j3).

**Kv7/KCNQ Channel currents (IK(Q)) in OLCs**

In order to determine the Kv7 channels are functionally expressed in OLCs, we recorded 61 cultured OLCs with whole-cell patch clamp recording to evaluate the electrophysiological property of the currents. In an attempt to isolate the Kv7 channel currents from other voltage-gated K\(^+\) currents, the membrane potential was held at a relatively depolarized potential (\(-20\) mV) to activate Kv7 channels [3,50] and to inactivate many of the other K\(^+\) channels that activate in this membrane potential region [51–53]. The membrane potential was then stepped down to more hyperpolarized potentials (\(-60\) mV, in 10 mV decrements) for

![Figure 1. Morphological and immunostaining characterization of OLCs in rat primary cultures.](https://www.plosone.org/content/video/sd/21792/21792/21792/S1/Figure1.jpg)
The amplitude of the $I_{K\theta}$ was measured as the difference between the instantaneous current at the onset of hyperpolarization and the steady-state current at the end of voltage command [3]. Fig.5A shows the current–voltage relationship of $I_{K\theta}$ from 17 OPCs. The mean $I_{K\theta}$ amplitude was voltage dependent and the maximal $I_{K\theta}$ amplitude (61.16±6.32 pA) was measured at −40 mV. The deactivation time constant of $I_{K\theta}$ was determined by fitting the current curves measured at each voltage with a single exponential function. Fig.5C shows the mean deactivation time constant of $I_{K\theta}$ as a function of voltage ($n=17$). The mean $I_{K\theta}$ deactivation time constant was 292.94±26.79 ms (−30 mV), 246.94±24.97 ms (−40 mV), 152.85±17.59 ms (−50 mV) and 131.89±14.18 ms (−60 mV), indicating that it was voltage dependent. Note that the deactivation time constant was a linear function of voltage (correlation coefficient $r=0.93$) and was shorter at more negative membrane potentials which means at these potentials, $I_{K\theta}$ was deactivated faster. We also recorded $I_{K\theta}$ in IOs and MOs and the inward deactivation relaxation currents were almost not existed. (Fig.6C, D). Consequently, in this study, the characterizations of $I_{K\theta}$ mainly were obtained from OPCs.

XE991 has been shown to be a potent and selective inhibitor for $M$ current ($I_M$) in native neurons and currents from artificial expressed $K_v7$ channels [5] and has little impact on $K_v2.1$ [54]. In our experiments, $I_{K\theta}$ was monitored with a 1-s-long hyperpolarizing voltage stepped from a holding potential of −20 to −40 mV. XE991 (10 μM) reduced about 46.1±6.3% $I_{K\theta}$ in OPCs. Higher concentration of XE991 (30 μM) inhibited $I_{K\theta}$ more than a half (by 67.4±5.6%) (Fig.6 A, B). Fig.6F shows the pooled concentration–response curve which plots the mean percentage inhibition of $I_{K\theta}$ amplitude versus the log concentration of XE991 from 54 OPCs. The mean inhibition of $I_{K\theta}$ by XE991 was 19.5±3.1% (at 1 μM), 33.3±5.7% (at 3 μM) and 80.1±4.2% (at 100 μM). The mean data was fitted with the Hill equation (see METHODS). The IC50 for XE991 was 13.3 μM and the power term n (Hill slope), which is related to the steepness of curve was 0.63. The goodness of fit $R^2$ was 0.99. In contrast, the currents in IOs and MOs were very insensitive to XE991 (Fig.6 C, D).

Previous studies reported that different $K_v7$ channel proteins have different sensitivities to TEA [55;56;5], and therefore it was of interest to examine the TEA sensitivity of $I_{K\theta}$ in OPCs. The voltage protocol used to measure $I_{K\theta}$ is the same as XE991 on $I_{K\theta}$ recorded in OLCs. We then applied TEA at concentrations ranging from 0.3 mM to 30 mM. TEA caused a concentration dependent reduction in $I_{K\theta}$ in OPCs. Fig.6F shows the pooled concentration–response curve which plots the mean percentage inhibition of $I_{K\theta}$ amplitude versus the log concentration of TEA from 20 OPCs. The mean inhibition of $I_{K\theta}$ by TEA was 33.3% (0.3 mM), 54.7% (1 mM), 72.1% (3 mM), 87% (10 mM) and 92.8% (30 mM). Application of 30 mM TEA completely abolished the current. The mean data was fitted with the Hill equation (see METHODS). The IC50 for TEA was 0.84 mM and the power term n (Hill slope) was 0.7. The goodness of fit $R^2$ was 0.99.

The inhibition of $K_v7$/$KCNQ$ channels promotes OPCs motility in vitro

Besides the electrophysiological properties of the $K_v7$ channels, we also investigated the effect of these channels on OPCs migration, which is important for myelin development. We
Figure 3. Immunofluorescence localization of KV7.2-5/KCNQ2-5 subunits on the OLCs in rat primary cultures. (A–D) Co-localization of immunostaining for KV7.2-5 subunits (green) and OPCs (NG2-positive, red) is displayed in the merged image. Higher magnification of the boxed area in A, B, C, D was shown in a1, a2; b1,b2; c1,c2; d1,d2. (E–H) The expression of KV7.2-5 subunits (green) on IOs (O4-positive, red). Higher magnification of the boxed area in E, F, G, H was shown in e1, e2; f1,f2; g1,g2; h1,h2. (I–L) The expression of KV7.2-5 subunits (green) on MOs (MBP-positive, red). Higher magnification of the boxed area in I, J, K, L was shown in i1, i2; j1,j2; k1,k2; l1,l2. Scar bar = 100 μm.

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Figure 4. Immunofluorescence localization of KV7.2-5/KCNQ2-5 on NG2-positive cells (green) of the rat brain slices. (A–C) Co-localization of KV7.2, 3, 5 and OPCs is displayed in the merged image. Higher magnification of the boxed area in A, B and C was shown in a1–a3; b1–b3;c1–c3;d1–d3;e1–e3;f1–f3;g1–g3;h1–h3 and i1–i3. (D) KV7.4 was not detected on OPCs. j1–j3: higher magnification of the boxed area in D. The nuclei were stained with Hoechst (blue). (E) Schematic diagram of the brain coronal and the box represented A–D areas. Scar bar = 100 μm.

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measured the mobility of OPCs cultured in a Boyden chamber. After 8 h incubation with 1 μM, 3 μM or 10 μM XE991 in the lower wells of the chemotaxis chambers, the number of migrated OPCs was significantly increased compared with the control group (Fig. 7A, C), suggesting that inhibition of Kv7/KCNQ channels promotes OPCs migration. We also tested the effect of another blocker TEA on OPCs migration. As shown in Figure 7B and D, in the presence of TEA (1 mM, 3 mM or 10 mM) the number of OPCs migrating through the transwell was significantly increased.

**Discussion**

**OLCs express Kv7/KCNQ channels**

Neuronal Kv7 channels are constructed from a family of at least four subunits (Kv7.2–5) [1,2,5]. These subunits are expressed widely in the brain and prominently localized in several types of neurons [37,38]. Some studies also suggested that a population of glial cells in the white matter expressed the Kv7.4/5, but they didn’t state clearly the type of glial cells [38,59]. The present study represents the first attempt to identify the Kv7 channel subunits in OLCs. The mRNA of the four genes (KCNQ2-5) was detectable in OPCs. KCNQ3 and KCNQ5 mRNAs were detected strongly, and lesser abundances of mRNAs encoding KCNQ2 and KCNQ4 were observed. KCNQ2-4 mRNAs also existed in IOs with similar expression levels. In MOs, we only find very week sign of KCNQ4 mRNA. These indicated that the transcripts of KCNQ2-5 might be down regulated during the maturation of OLCs. Previous studies found that KCNQ2, 4, 5 genes have alternative splice variants [60-63]. However, the primers used in this study were designed based on regions outside the putative

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**Figure 5. Kv7/KCNQ channel current (I_{K(Q)}) in OPCs of rat primary cultures.** (A) $I_{K(Q)}$ was measured with whole cell patch clamp recording from OPCs. Left insert: Standard $I_{in}$ deactivation voltage protocol used to measure $I_{K(Q)}$. Hyperpolarizing voltage steps were given from a holding potential of $-20$ to $-60$ mV (in 10 mV decrements). Currents recorded are shown below; the dashed line represents the zero current level. Right: Current recorded in response to the voltage step to $-40$ mV. $I_{K(Q)}$ was measured as the inward relaxation current caused by deactivation of $I_{K(Q)}$ during the voltage step; i.e., the difference between the instantaneous current at the beginning and the steady-state current at the end of the voltage step (arrows). (B) Current–voltage relationship for $I_{K(Q)}$ (mean data from 17 OPCs) showing that $I_{K(Q)}$ amplitude was voltage dependent and was largest at $-40$ mV. (C) $I_{K(Q)}$ deactivation time constants were directly related to voltage (mean data from 17 OPCs). Correlation coefficient $r = 0.93$. doi:10.1371/journal.pone.0021792.g005
splice variation position of rat KCNQ2-5 genes. Theoretically, our primers can recognize all of these splice variants but cannot distinguish them in OLCs mRNA preparations. We also examined the presence of KV7.2-5 proteins in cultured OLCs. The signals of four proteins (KV7.2-5) were weakly detected in differentiated OLCs (IOs and MOs), while they were obviously dyed out in OPCs. In agreement with the immunostaining experiments, the inward deactivation relaxation currents almost did not exist in IOs and MOs. It is likely that the mRNAs for KV7.2-4, or only KV7.4 which were detected in IOs and MOs respectively were unable to be translated into enough Kv7 proteins to be detected by immunocytochemistry or electrophysiology method. This developmental regulation may reflect some yet unknown roles played by KV7 channels in early development of OLCs.

However, previous work on the expression of members of the Kv channel family suggested that the precise topographical distribution of Kv channel subunits in cultured cells may not fully reproduce that obtained in situ [64,65]. Similarly, we detected the immunoreactive signal of KV7.2, 3 and 5 proteins, but not KV7.4, in NG2+ OPCs in rat cortex slices. These results agree with the work of Kharkovets et al. [22] that the cortex does not contain KV7.4 channel transcripts.

The electrophysiological properties of KV7/KCNQ channels in OPCs

Functional Kv7 channels are composed of four homomeric or heteromeric subunits. The sensitivity to XE991 of these homomeric or heteromeric channel currents differs considerably. Homomeric Kv7.2 channels have an IC50 value for XE991 inhibition which is 0.7 μM. The Kv7.2+3 heteromultimer retains the sensitivity of the Kv7.2 homomultimer. However, homomeric Kv7.3 and homomeric Kv7.5 are very insensitive to XE991 with...
estimated IC_{50} values of <50 μM and 65 μM [2]. In our study, XE991 (10 μM) showed a inhibition of current (by 46.1±6.8%) and high concentration (30 μM) displayed over a half inhibition of I_{K(Q)} (by 67.4±5.6%) in OPCs. The IC50 for XE991 was 13.3 μM.

Different K\textsubscript{V7} channel proteins also have different sensitivities to TEA [55;56]. In the present experiments, the I_{K(Q)} relaxations were completely inhibited by TEA with an IC50 of 0.84 mM, which is less than the IC50 for block of artificially expressed homomeric K\textsubscript{V7.4} channels and heteromeric K\textsubscript{V7.2/3} channels currents [5;55], though somewhat higher than the IC50 for block of homomeric K\textsubscript{V7.2} currents. K\textsubscript{V7.2-5} channel proteins were all detected in OPCs, however, their exactly expression level was unknown. The difference of pharmacological sensitivity to XE991 or TEA might due to various expression level and composition of each K\textsubscript{V7} channel subunit in OPCs.

In the present study, the amplitude of I_{K(Q)} in OPCs was found to be voltage dependent, which is similar to I_{M} measured in sympathetic ganglion, hippocampal and dopamine neurons [3,5,8,66,67]. The maximal I_{K(Q)} amplitude in the OPCs was obtained at -40 mV with the deactivation protocol. The deactivation time constant was voltage dependent in OPCs, becoming shorter at more hyperpolarized membrane potentials, as has been observed for native I_{M} currents in neuronal cell types [3,5,10,67]. The time course of I_{K(Q)} deactivation in OPCs was well fitted with a single exponential function and the value of the deactivation time constant was 152.85 ms at -50 mV. The deactivation time constant in OPCs seems to be closest to the fast component of the deactivation time constant in sympathetic neurons, which was reported to be 145 ms at -50 mV [5]. Native I_{M} currents in neurons have a biphasic (double-exponential) time course [5,8,10]. The absence of this slow component of deactivation in our experiments could be attributable to a difference in the types of K\textsubscript{V7} channels underlying I_{K(Q)} in OPCs.

**Function of K\textsubscript{V7}/KCNQ in OPCs**

During development, OLCs express all six members of the delayed rectifier Shaker family K\textsubscript{+} channels, Kv1.1–Kv1.6 [40–45], inwardly rectifying K\textsubscript{+} (Kir) channels Kir2.1, Kir1.1 and Kir4.1 [46,47] and Kv3.1 [29]. In our experiments, we found that K\textsubscript{V7} channels were expressed in OLCs, and downregulated in IOs and MOs. This developmental regulation may reflect some yet unknown roles played by K\textsubscript{V7} channels in early OLCs development.
Migration of OPCs from proliferation zones to their final position is an essential step in the development of the nervous system [26,68,69], yet the physiological mechanisms of OPCs migration are still largely unknown. The idea that the K⁺ channels may be linked to cell migration is supported by several studies [36–39]. Importantly, Kᵥ7.1 potassium channels have been implicated recently in the regulation of migration and invasiveness of stem-like cell types [35]. Our results support the concept that Kᵥ7 channels are important for the regulation of OPCs migration in vitro. In our migration assay, the motility of the OPCs was promoted by the inhibition of Kᵥ7 channels. In fact, in neurons, Kᵥ7 channels can be inhibited by many endogenous factors. For example, stimulation of a variety of Gq11-coupled neurotransmitter receptors, local changes in PiP2 concentration [70–72] and calmodulin [73], etc. In dissociated rat superior cervical sympathetic neurons, purinergic P2Y receptors can couple to G protein thereby modulating Kᵥ7.1 channel [74]. Agresti et al. [32] found that activation of P2Y receptors by ATP can promote OPCs migration. It is likely that ATP released following neuronal activity, astrocyte Ca²⁺ waves or cell lysis [75,76] might inhibit Kᵥ7 channels though P2Y1, and consequently promote the OPCs migration.

Materials and Methods

Oligodendrocyte lineage cell cultures

The animal experiments were carried out in adherence with the National Institutes of Health Guidelines on the Use of laboratory Animals and were approved by Second Military Medical University Committee on Animal Care (permission SCXK-HU-2007-0003). OLCs were prepared as previously described [77,78] with slight modification. Briefly, cortex was dissected from postnatal day 1–2 Sprague Dawley rats, dissociated in Hanks balanced salt solution containing 0.125% trypsin ( Gibco, Canada) for 20 min, 37°C, suspended in DMEM containing 10% fetal bovine serum (FBS, BIOSOURCE, Brazil), and plated in plastic T75 flasks. After about 10 days in culture, OPCs growing on top of a confluent monolayer of astrocytes were detached by 0.125% trypsin, 0.5 mM CaCl₂, 0.5 mM MgCl₂, 10 mM HEPES, and 20 mM KCl in balanced salt solution containing 0.125% trypsin (Gibco, USA) and TEA (Sigma, USA) was dissolved in water to store at –70°C. All reagents were purchased from Sigma (USA) and TEA (Sigma, USA) at 4°C overnight. Cells were then washed and incubated with fluorescein-conjugated secondary antibodies (Jackson ImmunoResearch Laboratories, Inc.) for 6 hours at room temperature and examined by fluorescence microscopy (Nikon, Japan). For immunohistochemical analysis, animals were deeply anaesthetized with 2% pentobarbital sodium and perfused transcardially with 4% PFA in 0.1 M PBS for 10 minutes. After blocking the non-specific binding with 10% normal goat serum or 1% BSA in 0.1 M PBS, cells were incubated with primary antibodies against A2B5 (Chemicon, USA), GFAP (Sigma, USA), NG2 (Chemicon, USA), O4 (Sigma, USA), MBP (Chemicon,USA), Kᵥ7.2, 3 (Chemicon, USA) , Kᵥ7.4 (Santa Cruz, USA) and Kᵥ7.5 (Millipore, USA) at 4°C overnight. Cells were then washed and incubated with fluorescein-conjugated secondary antibodies (Jackson ImmunoResearch Laboratories, Inc.) for 6 hours at room temperature and examined by fluorescence microscopy (Nikon, Japan). For immunohistochemical analysis, animals were deeply anaesthetized with 2% pentobarbital sodium and perfused transcardially with 4% PFA in 0.1 M PBS, pH 7.4. The brain were subsequently dissected from each animal and post-fixed in the perfusing solution overnight at 4°C. Then, the tissues were cryoprotected in 20% sucrose in PBS for 24–48 h at 4°C. Cryostat sections (10 μm) were cut and mounted onto gelatin-subbed slides and stored at –20°C. For immunostaining, the protocol performed was similar to immunocytochemical analysis.

Electrophysiological recordings

Current recordings were performed in the whole cell configuration of the patch-clamp technique using MultiClamp700A amplifier (Axon, USA). Date were stored in a PC, and analyzed by pClamp8.02 software (Axon Instruments, Sunnyvale, CA, USA). The patch pipettes (6–8 MΩ) were filled with a Narishige puller (PP-83, Japan) and polished using a MF200 Microforge (WPI, USA). The patch pipettes were filled with solution containing 140 mM KCl, 4 mM MgCl₂, 0.1 mM EGTA, 4 mM ATP-2Na, 0.5 mM Na₂-GTP, and 10 mM HEPES (pH 7.4 with KOH). The superfusate solution used to measure I_K(O) contained 150 mM NaCl, 5 mM KCl, 1 mM MgCl₂, 2 mM CaCl₂, 10 mM HEPES, and 10 mM Glucose (pH 7.4 with NaOH). All experiments were done in room temperature. Drugs were applied through OctaFlow System (ALA, USA) to the cell under recording. XE991 (Sigma, USA) and TEA (Sigma, USA) was dissolved in water to store at –20°C, and dilute 1 part to thousand using superfusate solution.

### Table 1. Primers for PCR.

| Gene   | Accession no. | Primer (5’–3’) | Product size(bp) |
|--------|---------------|----------------|-----------------|
| KCNQ2  | NM133322      | F: GCCTGCTAGTTCGCCTCAT | 172 bp |
|        |               | R: CTTTCCCTGTGAGGTAAGC   |       |
| KCNQ3  | NM031597      | F: CCCTATCATGACCATACATC | 121 bp |
|        |               | R: GCTGAACACCTTGGAGAAC   |       |
| KCNQ4  | XM233477      | F: GACGATTACACTGACGACATT | 110 bp |
|        |               | R: GCCGGCCAAAGAAGAGAT    |       |
| KCNQ5  | XM001071249   | F: GCCTGGCCCTCTTGTTTAAA | 320 bp |
|        |               | R: TTGGGGCGTCGTCGTTCTC   |       |
| GAPDH  | NM017008      | F: TCTGTACATGGCCTGGAGAAAC | 243 bp |
|        |               | R: CACACCCCTGGTTGTTGAAGCAT |       |
|        |               | R: ATTCGATGTGTCCTC       |       |

Specifies forward (F) and reverse (R) primers used for RT-PCR of rat KCNQ channel subunits and GAPDH. doi:10.1371/journal.pone.0021792.t001
Hyperpolarizing voltage steps (1 s duration) were given from a holding potential of −20 to −60 mV (in 10-mV increments).

Graphing and curve fitting of data were performed with Origin 7 software (OriginLab, Northampton, MA). The inward relaxation current, which was attributed to deactivation of IK(Q), was fitted by a single exponential function \(I(t) = Ae^{-t/\tau}\). Where \(A\) is amplitude obtained from the beginning of the fit and \(\tau\) is the decay time constant.

Concentration-response curves for XE991 and TEA were constructed by plotting percentage inhibition of IK(Q) as a function of drug concentration plotted on a log scale. Smooth curves were fit to these data with the Hill equation \(y = y_{\text{max}}x^h/(K^h + x^h)\). Where \(x\) is the concentration, \(y\) is the percentage inhibition, and \(y_{\text{max}}\) is the maximal value of \(y\) (at saturation); in the fitting procedure \(y_{\text{max}}\) was constrained not to exceed 100%. The term \(k\) is the IC50 (the concentration giving half-maximal inhibition) and \(h\) (Hill slope) is the power term related to the slope of the curve.

Boydern chamber migration assay
To measure the motility of OPCs, Boydern chamber migration assay was performed as previously described [79]. In brief, the polyethylene terephthalate filter membranes were coated with poly-L-lysine. The purified OPCs were seeded onto the upper chamber at a density of 2×10^5 cells in 200 μl of culture medium containing 10% FBS per well, and 600 μl DMEM containing 10% FBS were added to lower chamber. When OPCs were adherent (about 40 min later), the DMEM medium containing 10% FBS was added to lower chamber. When OPCs were adherent (about 40 min later), the DMEM medium containing 10% FBS in the upper and lower chamber was replaced with serum-free DMEM supplemented with 30% B104 neuroblastoma conditioned medium, 1% β27, and 1%N2. XE991 or TEA was added to the lower chamber. After incubation for 8 h at 37°C, non-migratory cells on the upper membrane surface were removed with a cotton swab, and migratory cells invading to the underside surface of the membrane were fixed with 4% paraformaldehyde and stained with Coomassie Brilliant Blue. For quantitative assessment, the number of stained cells was counted under microscopy at 12 fields per filter in three independent experiments.

Statistical analysis
Data from at least three independent experiments were all presented as means ± SEM. Statistical significance was evaluated with paired Student’s t-test. Differences were considered significant at p<0.05.

Author Contributions
Conceived and designed the experiments: WW X-FG. Performed the experiments: WW X-FG LX Z-HX. Analyzed the data: WW X-FG. Contributed reagents/materials/analysis tools: WW X-FG. Wrote the paper: WW X-FG CH.

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