CHRONIC DYSFUNCTION OF ASTROCYTIC INWARDLY RECTIFYING K⁺
CHANNELS SPECIFIC TO THE NEOCORTICAL EPILEPTIC FOCUS AFTER FLUID PERCUSSION INJURY IN THE RAT

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ABSTRACT

Astrocytic inwardly rectifying $K^+$ currents ($I_{\text{KIR}}$) have an important role in extracellular $K^+$ homeostasis, which influences neuronal excitability, and serum extravasation has been linked to impaired $K_{\text{IR}}$-mediated $K^+$ buffering and chronic hyperexcitability. Head injury induces acute impairment in astroglial membrane $I_{\text{KIR}}$ and impaired $K^+$ buffering in the rat hippocampus, but chronic spontaneous seizures appear in the perilesional neocortex -not the hippocampus- in the early weeks to months postinjury. Thus, we examined astrocytic $K_{\text{IR}}$ channel pathophysiology in both neocortex and hippocampus after rostral parasaggital fluid percussion injury (rpFPI). rpFPI induced greater acute serum extravasation and metabolic impairment in the perilesional neocortex than in the underlying hippocampus, and in situ whole-cell recordings showed a greater acute loss of astrocytic $I_{\text{KIR}}$ in neocortex than hippocampus. $I_{\text{KIR}}$ loss persisted through 1 month postinjury only in the neocortical epileptic focus, but fully recovered in the hippocampus that did not generate chronic seizures. Neocortical cell-attached recordings showed no loss or an increase of $I_{\text{KIR}}$ in astrocytic somata. Confocal imaging showed depletion of KIR4.1 immunoreactivity especially in processes -not somata- of neocortical astrocytes, while hippocampal astrocytes appeared normal. In naïve animals intracortical infusion of serum, devoid of coagulation-mediating thrombin activity, reproduces the effects of rpFPI both in vivo and at the cellular level. In vivo serum infusion induces partial seizures similar to those induced by rpFPI, while bath-applied serum, but not dialyzed albumin, rapidly silenced astrocytic $K_{\text{IR}}$ membrane currents in whole-cell and cell-attached patch clamp recordings in situ. Thus, both acute impairment in astrocytic $I_{\text{KIR}}$ and chronic spontaneous seizures typical of rpFPI are
reproduced by serum extravasation, while the chronic impairment in astroglial $I_{KIR}$ is specific to the neocortex that develops the epileptic focus.

INTRODUCTION

The mechanistic etiology of human posttraumatic epilepsy remains elusive. While seizures can result from neuronal or network abnormalities, they can also be precipitated by impaired homeostasis of the extracellular space. Normal neuronal activity results in extrusion of neurotransmitters and $K^+$, whose decreased extracellular clearance depolarizes neuronal membranes and collapses inhibitory electrochemical gradients, producing a potent endogenous proepileptic stimulus (Delgado-Escueta et al. 1999; D’Ambrosio 2004).

Astrocytes play a major role in the homeostasis of the extracellular space, and it has been hypothesized (Pollen and Trachtenberg 1970) that injury-induced astroglial reaction involves changes in membrane properties that impair $K^+$ homeostasis and cause posttraumatic epilepsy (PTE). Astrocytic homeostatic functions rely on proper membrane resistance, potential ($V_m$), electrochemical gradients, and $K^+$ permeability to drive the clearance of neurotransmitters by electrogenic transporters (Brew and Attwell 1987), and $K^+$ by spatial buffering or uptake (Orkand et al. 1966). Astrocytic $K_{IR}$ channels are ideally suited for $V_m$ regulation and passive control of extracellular $K^+$ because 1) their large $K^+$ conductance clamps $V_m$ close to $E_K$, 2) their inward rectification facilitates influx of $K^+$ while offering higher resistance to its efflux (Hagiwara...
and Takahashi 1974; Newman et al. 1984; Karwoski et al. 1989), 3) their pore
c conductance increases with increasing \([K^+]_o\) (Hille 2001), and 4) their buffering activity in
situ complements that of active pumps (D’Ambrosio et al. 2002). Several lines of
evidence support this notion. Pharmacological blockade of glial \(K_{IR}\) channels results in
impaired extracellular \(K^+\) regulation and in neuronal hypersynchrony (Ballanyi et al.
inactivation of the Tsc1 gene results in epilepsy (Uhlmann et al. 2002) and loss of
astrocytic \(K_{IR}\) current \((I_{KIR};\ Jansen et al. 2005)\) in the mouse. \(K_{IR}\) gene knock-out results
in impaired \(K^+\) buffering and dysregulation of neuronal activity in the retina (Kofuji et al.
2000) and brainstem (Neusch et al. 2006), and stress-induced seizures with impaired
buffering of both glutamate and \(K^+\) in the hippocampus (Djukic et al. 2007). RNA
interference of \(K_{IR}\) gene expression in culture diminishes astrocytic uptake of both
glutamate and potassium (Kucheryavykh et al. 2008) and glial \(K_{IR}\) channel genes have
been linked to seizure susceptibility in humans (Buono et al. 2004) and mice (Ferraro et
al. 2004). Therefore, pathologies of astrocytic \(K_{IR}\) channels may play an important role
in human epileptogenesis. Indeed, MRI confirms the coexistence of epileptic foci and
foci of reactive astroglia in humans (Messori et al. 2005), and decreased astrocytic \(I_{KIR}\)
and impaired extracellular \(K^+\) homeostasis have been well documented in human
surgical tissue (Jauch et al. 2002; Schröder et al. 2000; Hinterkeuser et al. 2000;
Bordey and Sontheimer 1998; Bordey and Spencer 2004).

In particular, a role for astrocytic \(K^+\) buffering in posttraumatic epilepsy was
indicated by the decrease in astroglial \(I_{KIR}\) and impaired \(K^+\) regulation observed acutely
after fluid percussion injury in the rat (D’Ambrosio et al., 1999), a model that is
mechanically identical to human contusive closed head injury and shares with it all pathological features that have been investigated (Thompson et al. 2005), including intraparenchymal and subdural hemorrhage which are risk factors for human PTE (Temkin, 2003). Recently, serum extravasation was linked to impaired $K_{\text{IR}}$-mediated $K^+$ buffering and PTE. In vivo exposure of naïve neocortex to blood serum or bovine serum albumin, and blood-brain-barrier (BBB) failure, were both shown to induce chronic hyperexcitability, impaired buffering of $K^+$ and a decrease in astrocytic $K_{\text{IR}4.1}$ mRNA (Seiffert et al., 2004; Ivens et al., 2007; Cacheaux et al., 2009).

FPI induces widespread serum extravasation (Hoshino et al., 1996; Tanno et al., 1992), acute impairment in astroglial $I_{\text{KIR}}$ and $K^+$ buffering in the hippocampus (D’Ambrosio et al., 1999), and chronic spontaneous recurrent partial seizures (CRSPSs) of both neocortical and limbic origin (D’Ambrosio et al., 2004, 2005, 2009), suggesting a role for serum-induced astrocytic pathophysiology in FPI-induced PTE. However, the first epileptic focus to develop after FPI is in the neocortex, while the hippocampus generates seizures only several months later (D’Ambrosio et al., 2005; 2009). To better understand the mechanistic links among head injury, serum extravasation, astroglial pathophysiology and epilepsy, we used PTE induced by rostral parasaggital fluid percussion injury (rpFPI) in the rat. We performed whole-cell and cell-attached patch-clamp recordings from astrocytes in situ before and after the onset of seizures, and used confocal imaging to examine the changes in expression and distribution of one type of $K_{\text{IR}}$ channel. In addition, we delivered blood serum to naïve brain slices and astrocytes in situ while blocking neuronal and synaptic activity, and to the naïve neocortex in vivo. We demonstrate that head injury induces a pro-epileptic pathology of
astrocytic $K_{IR}$ channels that persist specifically in the cells’ processes in the perilesional epileptic focus, but recovers in the hippocampus, and that astroglial $I_{KIR}$ impairment and spontaneous seizures similar to those induced by FPI are reproduced by applications of blood serum, but not dialyzed albumin, to naïve astrocytes in situ or to the naïve cerebral cortex in vivo.

**MATERIALS AND METHODS**

All procedures were approved by the University of Washington Institutional Animal Care and Use Committee. All reagents were purchased from Sigma (St. Louis, MO) unless otherwise noted. All data are expressed as mean±SEM unless otherwise specified.

*Rostral parasagittal fluid percussion injury:* has been described in detail previously (D’Ambrosio et al. 2004, 2005). Briefly, male Sprague-Dawley rats (post natal days 30-35; Charles Rivers, Hollister, CA) were anesthetized with 4% halothane, intubated and mechanically ventilated on 1.5% halothane and 30% $O_2$ and air. Core temperature was maintained at 37°C with a heat pad. A 3 mm burr hole was drilled 2 mm posterior to Bregma and 3 mm from the midline over the right convexity. FPI was delivered to the intact dura at 3.25±0.1 atm, as measured by a pressure transducer (Measurement Specialities, Hampton, VA). Injured animals had righting times >10 minutes. Acute (≤1 week) mortality rate was ~10%. 
**ECoG Recording:** A random subset of animals used for patch-clamp recording at 1 month post-rpFPI (7/21) was selected for 24 hrs of video-ECoG recording (D’Ambrosio et al. 2004, 2005). Briefly, 7 animals were anesthetized as per FPI and a montage of 5 epidural electrodes was implanted 5-7 days prior to video-ECoG acquisition. A reference electrode was placed midline on the frontal bone and 2 electrodes per parietal bone were placed at coordinates bregma 0 mm and -6.5 mm, 4 mm from the midline. Video-ECoG recordings were obtained, as previously described (D’Ambrosio et al. 2004, 2005), 1-4 days prior to sacrifice for patch clamp study. All 7 animals presented chronic seizures consistent with our previous work. Sixteen additional animals underwent video-ECoG recordings after intraparenchymal injection of artificial cerebrospinal fluid (aCSF) or diluted rat serum (DRS). These animals received the same 5-electrode montage as those used for patch clamp analysis, but the tip of a fine glass micropipette (tapering from ~30 μm to ~10 μm in ~700 μm) was lowered ~500 μm beneath the dura though a burr hole into the frontal parietal neocortex at stereotaxic coordinates bregma -2 mm, lateral 3 mm. Recordings (24h) were acquired weekly from week 1 to week 8 after implantation.

**Brain slice preparation:** At 22-26 hrs (“1 day”) or 28-35 days (“1 month”) post-injury, rpFPI- and age-matched naïve rats were anesthetized with halothane and transcardially perfused for 90s with an ice-cold heparin-supplemented (4U/ml) cutting solution containing 206 mM sucrose, 3.1 mM KCl, 2 mM MgCl₂, 26 mM NaHCO₃, 1 mM CaCl₂, 1.25 mM KH₂PO₄, 10 mM glucose, 1 mM kynurenic acid (KYNA). Rats were decapitated, and their brains rapidly removed into an oxygenated iced cutting solution
lacking heparin. Brains were immersed in ice-cold oxygenated cutting solution and 350 μm coronal slices were cut with a Vibratome (Ted Pella Inc., Redding, CA). Acute slices were transferred to a 35 °C 95% O₂/5% CO₂-bubbled holding solution containing aCSF composed of (in mM) 120 NaCl, 3.1 KCl, 2 MgCl₂, 26 NaHCO₃, 2 CaCl₂, 1.25 KH₂PO₄, 10 glucose, and 1 KYNA. It has been shown that glial channel activity can be affected by the spontaneous activity of neighboring neurons (Marrero et al., 1989). This possible confound was minimized by inhibiting neuronal and synaptic activity with KYNA as previously described (D’Ambrosio et al., 1999, 2002). A subset of experiments (Fig. 1) were performed in the presence of 17.5 μM tamoxifen, which has been reported to diminish in situ gap junctional coupling in cardiac astrocytes (Verrecchia and Herve, 1997). While the uncoupling efficacy of tamoxifen has not been verified in astrocytes, it has been reported to have no effect on astrocytic KIR currents (Smitherman and Sontheimer 2001) and it exhibited no effects on KIR currents in our pilot experiments. Slices were incubated at 35 °C for 30 min, after which the bath was allowed to cool to 25 °C and for 1 hour before use.

Patch-clamp recordings: Slices were gently transferred to a submersion recording chamber constantly perfused with a modified oxygenated aCSF composed of (in mM) 120 NaCl, 4.35 KCl, 1 MgCl₂, 26 NaHCO₃, 2 CaCl₂, 10 glucose, and 1 KYNA at a rate of 1-2 ml/min. All electrophysiological recordings were performed at 35°C unless noted otherwise. Glial cells were visually selected for recordings at 800X magnification with a Nikon E600FN microscope equipped with infrared DIC optics and 40x immersion objective. Whole-cell and cell-attached patch-clamp recordings were obtained using an Axopatch 200B (Molecular Devices, Sunnyvale, CA). Patch pipettes had resistance of
4.5-6 MΩ and were filled with either 5mg/ml biocytin or 1mg/ml of rhodamine dextrane, for cell type confirmation, and (in mM): 140 K-Gluconate, 1 MgCl₂, 2 Na₂-ATP, 0.3 NaGTP, 10 HEPES and 0.5 EGTA, adjusted to a final pH of 7.2 with NaOH. The theoretical whole-cell K⁺ reversal potential (E_K) was −92 mV. For whole-cell voltage-clamp recordings, series resistance (R_s) was compensated at ~75% (lag time, 10-15 μs) and monitored during the experiment. Recordings were filtered at 2-5 KHz, digitized at 5-20 KHz (Digidata 1322A, Molecular Devices), and acquired on a Pentium 4 computer with Clampex 9 (Molecular Devices). Cell membrane capacitance (C_m) and input resistance (R_in) were measured in voltage clamp (VC) using ±5 mV steps (125ms) from the holding potential of −70mV. C_m was determined by integrating the capacitive current for the time of the capacitive transient and R_in was estimated from the steady-state current response. Reported currents are not leak-subtracted. To ensure the VC was adequate to measure changes in slow non-regenerative currents like I_KIR in situ, whole-cell recordings were only considered if compensated R_s was ≤7% of R_in (R_s was 6.0±0.2% of R_in; n=101). Thus, recordings from tens of coupled cells (low R_in) or with high R_s, that both tend to result in poor VC, were not used. In addition, we employed quasi-steady state ramps to probe I_KIR, and we provide independent analysis of in situ astrocytes confirmed to be isolated as demonstrated by morphological analysis of biocytin-filled cells and membrane capacitance consistent with isolated cells (D’Ambrosio et al. 1998). To minimize the effects of possible run down, a uniform recording protocol was used in each set of experiments, and its execution time was consistent among recordings. Electrophysiological phenotypes of cells were classified on the basis of whole-cell current responses to 750-ms upward voltage ramps from -
170mV to +100mV. Inward currents were analyzed using 750-ms downward ramps from 0mV to -180mV, and series of 18 voltage steps (-10mV; 200ms) from a potential of 0mV. $I_{KIR}$ was measured during downward voltage ramps, as the difference between the whole-cell currents before and after blockade with bath-applied 40 μM Ba$^{2+}$. Current density ($\delta I$) was calculated by dividing membrane current by the membrane capacitance. Channel conductance and open probability (Po) were studied in cell-attached patch-clamp configuration in situ, using the same pipette solution employed for whole-cell voltage-clamp experiments. Cell type was confirmed by subsequent break-in for whole-cell recordings and biocytin filling. Because this work was not focused on the study of possible subconductance states of the K$\text{IR}$ channels, we estimated conductance and Po of the channels from Gaussian fits to normalized all-points histograms of the current response to series of applied voltage commands ($V_{com}$) ranging +70-0mV after visual examination for quality of the traces. Single channel conductance is reported as slope conductance (+70mV-0mV) to account for a variable unknown cell $V_m$. In order to pharmacologically determine the class of channels in the cell-attached patch, the pipette solution was exchanged for an otherwise identical solution containing 100 μM BaCl$_2$ using a 2PK+ pipette perfusion kit (ALA Scientific Instruments, Westbury, NY). In these experiments, a mean patch current was obtained by integrating the current over each sweep and dividing by the sweep duration, in order to facilitate comparison among patches containing variable numbers of channels. The effect of Ba$^{2+}$ was evaluated in series of 50 s sweeps at $V_{com}=+70$ mV (over the course of ~15min) by comparing the mean patch currents obtained for the 4 sweeps immediately preceding the onset of blockade (control) with the last 4 sweeps obtained
during Ba$^{2+}$-blockade. Open probabilities were estimated for patches containing a single channel, no more than two non-identical channels or up to 4 channels of uniform conductance. Channel conductance was estimated for these patches and for patches containing larger numbers of channels with approximately equal conductance such that the several peaks in all points histograms were well resolved. Finally, while a count of stable conductances could be estimated for a wider set of patches, one third of the patches were unsuitable for analysis due to the presence of a mixture of channels with differing conductances. Patch-clamp data were analyzed with Clampfit 9 (Axon Instruments), and data were graphed and plotted with Origin 5.0 (MicroCal, Northampton, MA).

Albumin preparation and dialysis: Dialyzed and undialyzed albumin solutions were prepared using 99% pure bovine serum albumin (BSA, Sigma, cat.# A3059), and always prepared at the same time and stored in the refrigerator for the same time before use. Undialyzed BSA (0.4 mM) in aCSF was maintained at 4 degrees for 48 hrs before use. Dialyzed albumine was obtained from a solution of 30g of BSA in 150ml of ultrafiltered water (pH=7). This was placed in dialysis membranes (25kD molecular-weight cutoff; Spectra/Por 7, Spectrum Laboratories, Rancho Dominguez, CA), and dialyzed at 4°C degrees first against 4L of ultrafiltered water (pH=7) for 24 hrs, and then against 4L of aCSF (bath solution) for an additional 24 hrs. Final BSA concentration was determined by evaporatating of the resulting solution and weighing the dehydrated crystals. Dialyzed BSA was then diluted to 26 mg/ml (0.4 mM) with aCSF.
Serum application: to determine the cellular effects of serum in brain slices, rats were decapitated under deep halothane anesthesia and rapidly exsanguinated. Heterologous blood was obtained from siblings. Autologous blood was collected in limited quantities from the punctured heart of the rat providing brain slices for electrophysiology. Whole blood was allowed to coagulate, diluted with an equal volume of oxygenated aCSF with KYNA 1mM and without tamoxifen, spun at 3000 rpm for 10 min at 4°C and the supernatant serum collected, resulting in ~3-fold dilution of blood serum. DRS (autologous or heterologous) was bubbled with 95% O₂/ 5% CO₂ and loaded into a syringe pump. An agar bridge prepared from 1% agarose in aCSF was employed for these experiments, and no change was observed in pipette potential during DRS application. Serum exposure experiments were conducted at room temperature. Both complex and passive astrocytes were recorded for this experiment, but it is unlikely that NG2⁺ macroglia, representing just 5-8% of total glia in normal tissue (Dawson et al., 2003), contributed significantly to these data. For the in-vivo experiments on the epileptogenesis induced by serum exposure, 300 μl of blood was obtained from the tail vein of the same animal, allowed to coagulate, and was then diluted 3- or 6-fold with aCSF with total K⁺ of 2 mM (1.75mM KCl). DRS was then spun at 3000 rpm for 10 min at 4°C and the supernatant serum collected. The autologous DRS was bubbled with 95% O₂/ 5% CO₂ and loaded into a primed subcutaneous osmotic minipump (Durect, Cupertino, CA). Animals received primed minipumps loaded with either low-potassium aCSF or DRS and were infused for 6-7 days at 0.25μl/hour through a glass micropipette in the animals’ headsets.
Histochemistry: For visualization of biocytin-filled glia, slices were fixed overnight in 4% paraformaldehyde in phosphate buffered saline (pH 7.4; 4°C). They were then washed (3x 10 min) in 0.1 M sodium phosphate (pH 7.5; PB), quenched for 30 min in PB containing 1% H$_2$O$_2$ and 20% methanol, and washed 3 more times (10 min) in PB. After permeabilization in 0.3% Triton X-100 in PB for 2 hrs, sections were incubated overnight at room temperature with the Vectastain ABC reagent (PK-4000 or PK-6700; Vector Laboratories, Burlingame, CA) diluted 1:50 in PB. Slices were then washed extensively (3 x 10 min, 6 x 1 h, and overnight) in PBT, equilibrated with Tris-buffered (0.01M; pH 7.5) saline (TBS; 3 x 10 min), and developed with 0.01% H$_2$O$_2$, 0.5 mg/ml diaminobenzidine (DAB) and 0.02% ammonium nickel(II) sulfate in TBS. Stained sections were mounted on gelatin coated slides, dehydrated through graded ethanol, cleared in xylene, and coverslipped in Permount. For localization of serum extravasation, rats were transcardially perfused with 50 ml ice-cold PBS and 200 ml 4% paraformaldehyde/4% sucrose under deep halothane anesthesia 5-6 hrs after rpFPI. Brains were removed and post-fixed in 4% paraformaldehyde/4% sucrose for 12h at 4°C and then transferred to a solution of 30% sucrose in PB. Following equilibration with the sucrose solution, brains were rapidly frozen in dry ice/isopentane and 30 µm sections were prepared using a sliding microtome. Sections were washed in PB (3x10min), quenched in 1% H$_2$O$_2$ and further rinsed in 0.1M PB (2x20min). Slices were incubated for 60min at room temperature in a blocking solution containing 0.3% Triton X-100, and 5% normal goat serum in PB, and then overnight at 4°C with Axell horseradish peroxidase conjugated goat anti-rat albumin antibody (1:1250; Accurate Chemical and Scientific Inc., Westbury, NY) in blocking solution. Following rinses in
blocking solution (2x20min) and PB (2x20min), sections were incubated with 0.0125% DAB for 10 minutes, and developed with 0.0125%DAB/0.0015% H₂O₂ in PB. Stained sections were rinsed 3x10min in PB and mounted, dehydrated, cleared and coverslipped.

Densitometry: Densitometric assessment of albumin extravasation in hippocampus and neocortex was carried out using coronal sections collected from rats sacrificed 6h after rpFPI (Bregma -2 mm to -4 mm) and stained as described for albumin. Control and FPI sections were stained together and the reaction was stopped before saturation. Photomicrographs (24-bit RGB) were acquired using a Nikon Optiphot-2 microscope equipped with a 2x objective and a Spot IIe camera. Images were acquired under uniform lighting conditions using identical exposure times and were white balanced and flatfield corrected. These images were converted to 8-bit gray-scale prior to analysis using ImageJ (http://rsb.info.nih.gov/ij/). For each section, hippocampal regions of interest (ROI) were drawn to include the entire region bounded by but excluding the pyramidal and granule cell layers, carefully omitting obvious high and low transmittance artifacts. Neocortical ROIs of similar (±5%) area were drawn to sample continuous neocortical regions of high immunoreactivity in the perilesional cortex, also omitting high and low transmittance artifacts. The ranges of pixel values obtained from hippocampal and neocortical ROIs defined in this manner did not differ in either control (62±11 vs 58±9; p=0.50, Wilcoxon) or in injured (109±16 vs 100±13; p=0.55, Wilcoxon) tissue. Immunoreactivities are reported for each rat as the area-weighted median hippocampal or neocortical optical density (http://rsbweb.nih.gov/ij/docs/examples/calibration)
determined from sections taken at Bregma -2mm, -3mm and -4mm.

HPLC quantification of adenosine nucleotides: Tissue content of ATP and ADP was measured in naïve rats at 30-35 (1 day control) or 58-65 days of age (1 month control) and at 12 hr, 1 day, 3 days and 1 month post-FPI from rats injured at 30-35 days of age. Tissue was obtained from frontal-parietal neocortex and hippocampus beneath the rpFPI site (Bregma -0.5 mm to-3.5 mm; 0-6 mm lateral). Injury, perfusion and brain slice preparation were as per electrophysiological experiments, except that the ipsilateral frontal parietal neocortex and hippocampus were dissected from each coronal slice for separate analysis. After 1hr incubation in a 95%O₂/5%CO₂-bubbled holding solution, hippocampal or neocortical sections were frozen in liquid nitrogen, crushed, and then homogenized in 1.8ml 70 °C 0.5 mM EGTA in 60% methanol. Homogenates were centrifuged at 10,000g for 10 minutes at 5°C. Pellets were stored at -80°C for subsequent protein assay and supernatants were filtered through a 0.22 μm pore-size filter, and immediately used for HPLC determination of nucleotide content. ATP and ADP were determined in 200 μl samples of tissue homogenate supernatant by anion-exchange HPLC at 254 nm (Kratos SF773 detector). Samples were injected onto an APS-2 Hypersil column (250 x 4.6 mm) with guard column (10 x 4 mm, 5 μm) (ThermoElectron) held at 35°C with a sleeve column heater (Restek, Bellefonte, PA) and eluted isocratically with 5 mM KH₂PO₄ (pH 4.15) for 5 minutes, followed by a linear gradient to 750 mM KH₂PO₄ (pH 4.15) at a constant flow rate of 1.5 ml/min. Absorbance chromatograms were digitally acquired and analyzed using Baseline 810 Chromatography Workstation v 3.3 (Waters, Milford, MA). Nucleotide levels were
determined by comparison of sample peak areas to concentration standards that were run throughout each experiment. Tissue pellets were solubilized in 0.5 M NaOH (Fischer Scientific, Pittsburgh, PA)/0.1% sodium dodecylsulfate (Fluka, Buchs, Switzerland), and protein was determined using a Coomassie Plus assay kit (Pierce, Rockford, IL). Nucleotide concentrations were expressed as nmol/mg protein.

Confocal microscopy: Rats were euthanized using isofluorane, followed by transcardial perfusion with 50 ml of phosphate buffered saline (PBS) at pH 7.4, and then perfused with 150 mL fixation buffer (4% formalin, 4% sucrose in PBS). Brains were fixed overnight in the same fixation buffer and then cryoprotected in 30% sucrose/PBS. Frozen coronal brain sections (20 um), were collected in the region of injury ranging from bregma 0 to bregma -3.5. Thawed, air dried sections were post-fixed with acetone, rinsed in PBS, and blocked in PBS containing 5% goat serum and 1% bovine serum albumin. Kir4.1 was immunodetected with a polyclonal rabbit antisera (Alomone Lab, Jerusalem, Israel) previously characterized as immuno-specific for Kir4.1 on the basis of positive labeling in normal, but not Kir4.1 knockout mouse brain (Djukic, et al 2007). Glial fibrillary acid protein (GFAP) was detected using mouse monoclonal anti-GFAP (Covance, Emeryville, CA). Primary antibodies were detected using goat anti-rabbit Alexa-488 and goat anti-mouse Alexa-568 secondary antibodies (Molecular Probes/Invitrogen, Carlsbad, CA). Sections were then treated with Vectashield anti-fade mounting medium (Vector Laboratories, Burlingame, CA). Single or double-labeled laser confocal microscopy was performed using a Leica DMR/TCS-SP (Leica Microsystems, Bannockburn, IL). For each experiment, injured and control tissue sections were immunostained and images acquired under identical conditions. All image processing
and formatting (Photoshop, Adobe Systems, San Jose, CA) was limited to linear brightness and contrast adjustments that were performed identically on experimental and control images.

**Assays of serum thrombin activity:** Thrombin/prothrombin activity was determined in samples of DRS by the clinical laboratory at Harborview Medical Center (Seattle, WA) using an assay based on the ability of a serum sample to restore clotting in prothrombin-deficient plasma. The sample to be analyzed was combined with prothrombin-deficient plasma (Precision Biologic Inc, Dartmouth, Nova Scotia, Canada) and the clotting cascade initiated by addition of rabbit brain thromboplastin (Diagnostica Stago Inc., Parsippany, NJ). Clotting time was determined using a STAR analyzer (Diagnostica Stago Inc., Parsippany, NJ), and enzymatic activity reported as % normal plasma activity.

**Statistical Analyses:** Non-parametric statistical tests were employed when feasible and the Mann-Whitney test was used unless noted otherwise. Parametric tests were conducted on data transformed as needed to satisfy their distributional requirements. ANOVA-based comparison of differently scaled whole-cell (real valued) and cell attached (both real and integer valued) data required data transformation. For this purpose the classes of data to be compared (whole cell and cell attached) were separately aggregated (control and FPI), ranked (ties each assigned the appropriate mean rank), and each rank divided by the aggregate standard deviation of the ranks. These normalized ranks were used for analysis. Maple 9 (Maplesoft, Waterloo, Ontario,
Canada) was used to compute Fisher’s exact probabilities. All other statistics were computed with SPSS 12.0 (SPSS Inc., Chicago, IL).

RESULTS

Whole-cell astrocytic $K_{IR}$ current is lost after head injury

Previous work using epidural grid ECoG demonstrated the perilesional neocortex to generate CRSPSs from 2-4 weeks onward (D’Ambrosio et al., 2004, 2005, 2009), while depth-electrode recordings showed the hippocampus to be silent in the early months postinjury (D’Ambrosio et al. 2005). Thus, these regions were specifically targeted for investigation of the role of astrocytes in epileptogenesis.

We examined the electrophysiology of astrocytes in the perilesional neocortex and underlying hippocampus in slices obtained 1 day and 1 month following rpFPI and in age-matched controls. All glial cells targeted for patch clamp had clearly visible rounded or oval cell bodies ~10-12 μm in diameter. Neurons were readily identifiable on the basis of spontaneous EPSCs or IPSCs, depolarization-induced action potentials, and by the morphological properties of the biocytin-filled cells. Glial cells lacked EPSCs or IPSCs, did not produce action potentials following depolarization, and presented consistent morphology at the microscopic analysis of biocytin-filled cells. Nine out of 317 glial cells patched for determination of the effect of rpFPI on astrocytic whole-cell
$K_{IR}$ current density ($\delta l_{KIR}$) were identified as microglia and excluded from subsequent analysis. In agreement with previous reports for intact microglia (Boucsein et al. 2000; Abraham et al. 2001; Soltys et al. 2005; Bordey and Spencer 2003), these cells were all depolarized ($V_m=-26\pm5\ mV$, $n=9$), exhibited significantly higher $R_{IN}$ ($840\pm300\ \Omega$; $p<0.001$) and lower $C_m$ ($17\pm6\ \text{pF}$ $p<0.001$), than confirmed astrocytes ($n=81$; $R_{IN}=76\pm5\ \Omega$, $C_m=29\pm2\ \text{pF}$) that met our inclusion criteria for $\delta l_{KIR}$ determination, and displayed morphological features of either activated or ramified microglia. Remaining glial cells were identified as macroglia. They all exhibited $V_m<-50\ mV$ (Boucsein et al. 2000), and the three electrophysiological profiles (linear, inwardly rectifying and complex) previously described in the hippocampus (D’Ambrosio et al. 1998). Oligodendrocytes most commonly displayed a complex electrophysiological profile, and could be identified on the basis of their polygonal cell bodies and comparatively fine, straight and directed processes bearing periodic “cup-like” swellings (D’Ambrosio et al. 1998), and were excluded from further analysis. Astrocytes employed in this study were characterized by hyperpolarized membranes, fibrous or protoplasmic morphologies often with some processes extending toward blood vessels, and with varying degrees of cell-to-cell coupling (D’Ambrosio et al. 1998).

Neocortical astrocytes were significantly depolarized (-68±1 mV; $n=9$) with respect to uninjured control (-72±0.5mV; $n=10$; $p=0.01$) at 1 day post-injury, but no differences were detected at 1 month (Table 1). The effect of rpFPI on whole-cell $\delta l_{KIR}$ (Fig. 1) was assessed by its sensitivity to external barium (DiFrancesco et al. 1984). We examined the effect of rpFPI on $I_{KIR}$ currents 1 day and 1 month postinjury using 1)}
whole-cell recordings that satisfied stringent quality control of the VC, and 2) the subset of these whole-cell recordings in which astrocytes were confirmed isolated.

Astrocytes with complex electrophysiology were rarer in the neocortex than non–complex ones, resulting in too few complex cells with confirmed astrocytic morphology to allow separate statistical comparisons of the effect of rpFPI on δl_{KIR}. Thus, the astrocytes used for this experiment were characterized by non-complex electrophysiological profile, and NG2+ macroglia were excluded from analysis on the basis of their complex electrophysiological profile.

Whole-cell recordings controlled for quality of VC (n=80; Fig. 1B) showed that, one day after injury, neocortical astrocytic δl_{KIR} decreased ~65% from 11.1±1.2pA/pF in control (n=10) to 2.7±1.2pA/pF in FPI (n=8; p=0.002), while CA3 astrocytic δl_{KIR} decreased ~40% from 12.4±2.7pA/pF in control (n=11) to 5.2±0.4pA/pF in FPI (n=9; p=0.017). The rpFPI-induced loss in astrocytic δl_{KIR} was more severe in the neocortex than in the hippocampus (p=0.012). One month after rpFPI neocortical astrocytic δl_{KIR} was 5.9±1.5pA/pF (n=9), significantly below the age-matched control value of 12.9±2.2pA/pF (n=8; p=0.012). In contrast, 1 month after rpFPI, CA3 astrocytic δl_{KIR} fully recovered to 17.6±3.2pA/pF (n=13; p=0.83) vs control levels of 12.8±2.7pA/pF (n=12). These findings were confirmed by separate analysis of the subset of whole-cell recordings in situ in which astrocytes were confirmed to be isolated by morphological analysis of biocytin filled cells (n=60; Fig. 1F-I). One day after injury, δl_{KIR} measured at -140mV in isolated neocortical astrocytes decreased from 10.7±1pA/pF in control (n=7) to 3.3±1.7pA/pF in FPI (n=6; p=0.015), while δl_{KIR} in isolated CA3 astrocytes decreased from 12±2pA/pF in control (n=7) to 5.5±0.7 in FPI (n=6; p=0.003). One month after
rpFPI δ_l_{KIR} in neocortical astrocytes was 5.2±1.7pA/pF (n=8), significantly below the age-matched control value of 12.6±2.1pA/pF (n=6; p=0.014). In contrast, 1 month after rpFPI, CA3 astrocytic δ_l_{KIR} fully recovered to 17.5±3.5pA/pF (n=11; p=0.47) vs control levels of 13.9±3.5pA/pF (n=9).

Somatic astrocytic K_{IR} current increases chronically after head injury

The injury-induced loss in whole-cell I_{KIR} could result from a change in number, conductance or open probability (P_o) of K_{IR} channels. We examined each of these parameters in neocortical astrocytes in situ by cell-attached patch-clamp recordings (Fig. 2). These can only be obtained from the somata due to the small diameter of the astrocytic processes. K_{IR} channels were identified based on four defining features. First, E_{REV}, extrapolated from IV plots of single channel currents and assuming astroglial V_m=-70mV, was -22 mV (Fig. 2D), in perfect agreement with the theoretical E_K computed for 35°C and 66mM intracellular K^+, the [K^+] measured directly in neocortical macroglia in situ (Ballanyi et al. 1987). Second, P_o of individual channels increased with depolarizing voltage commands (Fig. 2A, F). Third, the observed range of conductances (30-70 pS; Fig. 2E) was compatible with the expected heterogeneous expression of astrocytic K_{IR} channels in situ. Fourth, channels displayed sensitivity to extracellular Ba^{2+} (100 μM) delivered through the patch pipette (Fig. 2B-C). The average patch current (V_{com} +70mV) decreased significantly from 5.4±3.0 pA in control to 0.32±0.14 pA in Ba^{2+} (n=4; p=0.004; paired t-test after logarithmic transformation). rpFPI induced no detectable change in the channel E_{REV} (Fig. 2D) or conductance (Fig. 2E) of the currents carried
through 19 control and 27 rpFPI K\textsubscript{IR} channels. Mean conductance was 52.3±2.1 pS (n=13) for controls and 52.2±1.2 pS (n=19) for rpFPI at 1 day, and 59.2±3.0 pS (n=6) for controls and 57.1±2.2 pS (n=9) for rpFPI at 1 month post-injury. \(P_0\) of the same channels displayed identical magnitude and voltage dependence in control and FPI channels (Fig. 2A, F). Therefore, neither \(E_{\text{REV}}\), conductance nor \(P_0\) of somatic cell-attached channels could account for the loss in whole-cell \(\delta I_{\text{KIR}}\) 1 day after rpFPI.

The number of active K\textsubscript{IR} channels in the patch was estimated by the number of discrete conductance states that could be resolved in all-points histograms. Patches from controls age-matched to the 1 day and 1 month time points contained 2.6±0.7 (n=13) and 2.4±0.7 (n=11) active channels, respectively. There was no change in number of channels 1 day after injury (2.4±0.6 channels/patch; n=23; p=0.9), and the increase to 4.1±0.7 (n=16; p=0.027) at 1 month post-injury was significant when compared to a pooled control (n=47) incorporating the other groups (Fig. 2G). We also examined the effect of rpFPI on somatic cell-attached currents (Fig. 2I; \(V_{\text{com}}=+70\text{mV}\)), which were nearly identical in FPI (n=27) vs controls (n=46) at 1 day post-injury, and significantly elevated at 1 month (p=0.04). After completing cell-attached recordings, patches were ruptured for the acquisition of whole-cell downward inward currents.

While whole-cell measurements \textit{in situ} indicated a global loss in \(I_{\text{KIR}}\) in isolated neocortical astrocyte at both intervals after rpFPI, cell-attached measurements indicated that both the count of somatic K\textsubscript{IR} channels and the amplitude of somatic inward currents were unchanged at 1 day and \textit{increased} at 1 month post-injury. This discrepancy was investigated using 2-way ANOVA focusing on the interaction between treatment (control vs rpFPI) and technique (whole-cell vs cell-attached). ANOVA
detected significant measurement x treatment interactions at both 1 day (F=5.37; 1 df; p=0.025) and 1 month (F=11.1; 1 df; p=0.002) post-FPI for whole-cell \( I_{KIR} \) (Fig. 1) vs cell-attached \( K_{IR} \) channel count (Fig. 2H). In a subset of cells that yielded both cell-attached and whole-cell currents, a separate two-way ANOVA detected significant measurement x treatment interaction (F=9.8; 1 df; p=0.003) at 1 month (n=27), but not at 1 day post-FPI (n=26), for whole-cell inward current vs cell-attached patch current. Thus both the inward currents and the number of somatic \( K_{IR} \) channels measured in the soma in cell-attached patches vary independently of the corresponding inward currents and \( I_{KIR} \) measured in whole-cell. Since whole-cell \( I_{KIR} \) cannot decrease without a decrease in \( I_{KIR} \) in either soma or processes, and we observed either no change or an increase in \( I_{KIR} \) in the soma, these data point to rpFPI-induced loss of astrocytic \( I_{KIR} \) predominantly in the astrocytes' processes.

Confocal imaging indicates loss of KIR 4.1 in astrocytic processes.

While many KIR channels could potentially contribute the observed injury-induced changes in astroglial electrophysiology, numerous studies have shown the inwardly rectifying K\(^+\) channel, K\(_{IR}4.1\), to be prominent in astrocytic processes and to play a role in astrocytic K\(^+\) buffering. We therefore examined the astrocytic expression of K\(_{IR}4.1\) in the ipsilateral hippocampi and neocortices of rpFPI rats 1 month after injury and age-matched naives. In the hippocampus, no differences in K\(_{IR}4.1\)-staining were found in injured vs naïve tissue one month post-injury (Fig. 3 J,M). The stratum pyramidale is rich in astrocytic processes that wrap pyramidal neuron somata, which are
devoid of K_{IR4.1}-immunoreactivity. In the naïve neocortex, K_{IR4.1}-immunoreactivity colocalized with GFAP in most cases (Fig. 3A-C), and was apparent in both processes and somata of cells with morphological characteristics of astrocytes. In the perilesional neocortices of injured rats, K_{IR4.1}-immunoreactivity remained evident in the somata of astrocytes and substantially diminished beyond the most proximal processes (Fig. 3D-F).

The contrasting distributions of K_{IR4.1}-immunoreactivity in sections from age-matched naïve and 1 month post-FPI rats permitted the reliable identification of the perilesional neocortex on the basis of blind evaluation K_{IR4.1}-immunofluorescence alone (Fig. 3I). For the blind analysis, one investigator randomized 16 coronal sections (8 naïve, 8 FPI). Another investigator, who was blinded to the identity of the tissue, mounted them on the fluorescent microscope at 20x magnification, and selected a field within the perilesional frontal neocortex. A third investigator, who was blind to the identity of the tissue and was not allowed to explore beyond the frontal perilesional cortex, evaluated the field. After all fields were evaluated, the blind was broken and 15 of 16 sections were correctly identified (p<0.0003, exact binomial probability).

Thus, in accord with the electrophysiological data, confocal imaging studies confirmed a striking and consistent mislocalization of K_{IR4.1} in neocortical, but not hippocampal, astrocytes one month after head-injury.

Serum induces acute shut-down of astrocytic KIR currents in situ

Serum extravasation is a reliable feature of FPI (Hoshino et al., 1996; Tanno et
and exposure of the brain parenchyma to serum has been shown to result in chronic neuronal hyperexcitability and diminished astroglial $K^+$ homeostasis (Seiffert et al. 2004; Ivens et al. 2007). To determine whether FPI-induced acute astrocytic $I_{KIR}$ loss is due to an effect of serum, we examined the effects of 3-fold DRS in whole-cell with neuronal and synaptic activity suppressed by $1\mu$M TTX and $1mM$ KYNA. Astrocytic inward currents were monitored during upward voltage ramps (-170mV to +100mV) repeated every 20 seconds during DRS exposure, and by voltage steps (-170mV to -0mV) obtained before and during serum exposure. While untreated astrocytes ($n=4$) presented stable inward currents over the course of 12 min (97±2% of initial values), these were reliably reduced during heterologous ($n=8$) or autologous ($n=3$) 3-fold DRS application (Fig. 4E). In four cells surviving 10 min of DRS exposure, inward currents were reduced to 84±1% ($p=0.002$, paired t-test) of their initial values. Therefore, DRS exposure reproduces in naïve tissue the astrocytic $K_{IR}$ impairment observed 1 day after rpFPI.

In order to investigate possible contributors to the effect of serum, we employed similar VC protocols in astrocytes in situ during perfusion with aCSF supplemented with either whole or dialyzed BSA at physiological concentration (0.4mM). Whole BSA ($n=11$) induced a progressive and rapid loss in astrocytic inward currents (75±8% of initial values at 16 minutes). Conversely, astrocytes treated with dialyzed BSA ($n=8$), displayed stable inward currents over the course of 15 minutes ($p<0.05$ compare to undialyzed BSA; Mann Whitney). Subsequent administration of whole BSA rapidly induced loss in inward currents (Fig. 4H). Therefore, the effect of BSA on astrocytic
inward currents is attributable to a soluble contaminant with molecular weight <25kD that copurifies with albumin.

Because thrombin has been implicated in posttraumatic epilepsy, thrombin was assayed in our DRS preparations. It was undetectable in all DRS preparations tested (n=6), as expected for a derivative of clotted blood.

To understand whether the acute loss of $I_{KIR}$ results from a direct interaction of serum components with KIR channels, or requires cytoplasmic signal transduction, we studied neocortical astrocytes in naïve brain slices during bath application of 3-fold DRS with synaptic activity suppressed by 1mM KYNA. We first examined the effect of 3-fold DRS on single $K_{IR}$ channels in cell-attached recordings ($V_{com} =+70mV$) from neocortical astrocytic somata in situ, such that the extracellular face of the channels was inaccessible to bath-applied serum. We measured the peak amplitude (maximized when one conductance state dominates) of all points histograms of the current sweeps to measure channel function and compare serum effects on patches bearing varying numbers of channels. DRS invariably resulted in a progressive leftward shift in the all-point histograms, consistent with diminishing patch conductance (n=7; Fig. 4C). By 15 min of DRS exposure, peak amplitude increased to 2300±700 samples (n=5; p=0.043, Wilcoxon) vs control values of 1500±400 samples. In patches bearing single $K_{IR}$ channels (n=2), activity ceased abruptly after a period of DRS exposure (Fig. 4B).

These data indicate that serum-induced loss of astroglial whole-cell $I_{KIR}$ is likely mediated via cytoplasmic signalling.
Serum is sufficient to induce FPI-like spontaneous seizures in vivo

To determine whether serum may play a role in FPI-induced epilepsy, we performed video-ECoG monitoring in 16 naïve rats, following continuous intracortical infusion of autologous DRS (n=9) or aCSF (n=7) into the frontal parietal neocortex. Serum was injected intracranially through a glass micropipette (tip diameter ~10 μm) connected to a subcutaneous osmotic minipump delivering either aCSF or diluted serum for 6 days, a temporal window consistent with opening of the BBB after FPI (Hoshino et al. 1996). Intraparenchymal albumin was visualized immunohistochemically to determine the extent of serum infusion and verify that the fine glass micropipette did not induce blood extravasation (Fig. 5A). Animals were recorded from week 1 to week 8 after the treatment. We observed seizures (Fig. 5B) electrographically identical to focal (grade 1) and focal-onset spreading (grade 2) seizures induced by rpFPI (D'Ambrosio et al., 2004, 2005, 2009). DRS-induced seizures were first detected by the epidural electrode closer to the delivery site. In epileptic animals seizure frequency ranged 1-10 events per day. The cumulative probability that rats developed epilepsy, as defined by the occurrence of at least two seizures, increased over time after treatment, and was dose dependent reaching 50% and 80% for animals receiving serum diluted 6 and 3 fold, respectively (Fig. 5C). No animal injected with aCSF developed any seizure activity over the 8 weeks of monitoring.
Neocortex, not hippocampus, receives the greater damage and serum extravasation after FPI

Injury-induced serum extravasation was mapped using albumin immunohistochemistry. At 5-6 hr post-rpFPI, prominent albumin immunoreactivity was evident in the ipsilateral and medial-contralateral neocortex and hippocampus (Fig. 5D), matching the mapping of chronic neocortical hyperexcitability and of reactive astrocytosis two months after rpFPI (D'Ambrosio et al. 2004). Densitometric analysis in 5 control and 5 rpFPI rats demonstrated injury-induced increase in optical density by 136% in the neocortex (p=0.009) and 67% in the hippocampus (p=0.009). The increase in optical density in the neocortex was ~50% greater than in the hippocampus (p<0.05; Fig. 5E). We also assessed tissue energetics in frontal-parietal neocortical and hippocampal sections from beneath the rpFPI site by HPLC (Fig. 5F). Compared to age-matched controls neocortical ATP levels were significantly reduced by 22% and 11% at 12 hr (p<0.005) and 24 hr (p<0.05) post-injury, respectively, while ADP displayed a significant 120% elevation at 12 hr post-injury (p<0.05). Conversely, hippocampal ATP levels were decrease by just 12% at 12 hr post-injury and no significant changes in ADP were detected. Therefore, the acute disruption of energy metabolism was more severe in the neocortex.
This is the first comparison of the acute vs chronic effects of head injury on astroglial electrophysiology in a developing epileptic focus, and the findings are unexpected. After acute astroglial \( I_{KIR} \) impairment in both neocortex and hippocampus, this impairment recovered fully in hippocampal astrocytes by 1 month postinjury, but persisted in astrocytes in the perilesional neocortical epileptic focus. Also, this is the first study to show that a major loss of astrocytic \( K^+ \) current persists in the cells' processes, not their somata, which points to mechanisms of channel mislocalization. The data also support and expand on the previous finding that serum extravasation induces chronic hyperexcitability and impaired astroglial \( K^+ \) buffering (Seiffert et al., 2004; Ivens et al., 2007), by showing that DRS infusion, in brain tissue ex vivo and naïve animals \textit{in vivo} reproduces, in a dose-dependent fashion, both the acute electrophysiological changes in astrocytic \( I_{KIR} \) one day after rpFPI, and the induction of CRSPSs similar to those induced by rpFPI.

\textit{Acute and chronic effects of head injury}

Whole-cell recordings were obtained \textit{in situ} from neocortical and hippocampal astrocytes in brain slices from rpFPI animals with epilepsy confirmed by video-ECoG, and from age-matched controls. The data showed rpFPI induced an acute large functional impairment of astroglial \( I_{KIR} \) that was greater in the neocortex than in the hippocampus, and persisted only in the neocortical epileptic focus (Fig. 1). Patch clamp
recordings in situ are necessary to assess $I_{KIR}$ in situ, and we took several precautions to ensure the quality of voltage clamp: we excluded extensively coupled astrocytes, characterized by low $R_{IN}$, and all recordings in which compensated $R_s$ was greater than 7% of $R_{IN}$. In addition, we performed parallel analyses of all qualifying recordings (Fig. 1B), and of the subset of astrocytes that were confirmed isolated after biocytin filling (Fig. 1D-I), with similar results. The measured $I_{KIR}$ loss is likely an underestimate of the actual brain pathophysiology in vivo, especially in the neocortex, since severely injured astrocytes do not survive (Hill-Felberg et al. 1999), or are less likely to yield successful patch-clamp recordings.

The large loss of whole-cell $I_{KIR}$ observed both acutely and chronically in uncoupled neocortical astrocytes (Fig. 1F-G) was not observed in cell-attached patches obtained from astrocytic somata 1 day post-injury (Fig. 2). The somatic $I_{KIR}$ and the number of $K_{IR}$ channels recorded in cell-attached patches both actually increased at 1 month post-injury while whole-cell $I_{KIR}$ remained depressed (Fig. 2G-I). Since whole-cell $I_{KIR}$ cannot decrease in the face of unchanged or increased somatic $I_{KIR}$ without a decrease in the remaining cellular compartments, the loss of $I_{KIR}$ observed in whole-cell must occur in the astrocytic processes which, in normal tissue, are reported to be enriched with $K_{IR}$ channels (Newman 1984; Rojas and Orkand 1999; Higashi et al. 2001; Thomzig et al. 2001; Kofuji et al. 2002; Hibino et al. 2004; Neusch et al. 2006).

While the large size (~15μm in diameter) of vitreal endfeet of retinal Muller cells permits direct electrophysiological recordings (Newman, 1984), such recordings are not possible in CNS parenchyma. The processes of astrocytes in situ taper steeply to reach sub-micrometer diameter around neuronal, synaptic and vascular structures where $K_{IR}$
channels are mostly located, which prevents direct patch-clamp recordings from them. However, in agreement with the conclusions of our electrophysiological measurements, confocal imaging of KIR4.1 channel protein showed that the processes of neocortical astrocytes are profoundly depleted of KIR4.1 channels chronically after injury, while their somata are not. These pathological astrocytes provide a reliable basis for the blind identification of the neocortical epileptic focus (Fig. 3 I). Conversely, hippocampal astrocytes, which suffer a milder acute loss in I\textsubscript{KIR} 1 day postinjury, fully recover by 1 month post-injury (Fig. 1B) and their KIR4.1-rich processes surround CA3 pyramidal neurons indistinguishably from control tissue (Fig. 3J-O).

While KIR4.1 channels clearly contribute to the persistent I\textsubscript{KIR} pathology in neocortical astrocytes we observed, the astrocytic I\textsubscript{KIR} we studied \textit{in situ} is likely carried by several channel types, which likely also contribute to injury-induced I\textsubscript{KIR} pathology. The I\textsubscript{KIR} we studied was susceptible to blockade by 40-100 µM extracellular Ba\textsuperscript{2+}, and exhibited inward rectification to a different degree in individual cells, therefore it was likely carried by multiple channels such as K\textsubscript{IR} 2.x, K\textsubscript{IR} 3.x, K\textsubscript{IR} 4.1, heteromeric K\textsubscript{IR} 4.1/5.1, K\textsubscript{IR} 6.x and/or TWIK channels, which all have been immunocytochemically demonstrated to exist on macroglial membranes (Butt and Kalsi, 2006; Lesage et al., 1996; Patel et al., 2000). The varying single channel conductances we observed in cell-attached patches \textit{in situ} (Fig. 2E) also suggests the presence of diverse K\textsubscript{IR} channels types on astrocytic somata. However, they provide no clues as to their identities because the properties of molecularly identified channels have only been examined in expression systems and in cultured astrocytes. While the depletion of K\textsubscript{IR}4.1 immunoreactivity in astrocytic processes is consistent with whole-cell data,
important changes in other channel types cannot be ruled out. Further work will be required to determine which other channel types may be contributing to the rpFPI-induced chronic changes in the level and distribution of $I_{KIR}$.

The observed increase in somatic $I_{KIR}$ at 1 month, but not at 1 day, post-injury (Fig. 2G-I) may represent a compensatory response of the astrocytic somata to chronic pathology of the processes to restore normal cellular $V_m$ and $R_{IN}$, as indeed was observed (Table 1). An increase in $K_{IR}$ channel density in astrocytic somata could partially compensate for failing spatial buffering by promoting the uptake of KCl, but at the cost of collateral pro-epileptic changes. Since uptake into the soma would require diffusion of extracellular $K^+$ from distal sites, the speed of buffering would be reduced (Newman et al. 1984; Karwoski et al. 1989), while KCl accumulation would result in increased water uptake, cellular swelling and shrinkage of the extracellular space (Dietzel et al. 1989; Hochman et al. 1995). It is also possible that the increased somatic expression in $I_{KIR}$ may result from its impaired transport to the processes. Indeed $K_{IR}$ 4.1 is expressed in neocortical astrocytes chronically after injury, but it is predominantly localized in the somata (Fig. 3). A third possibility is that astrocytic processes that wrap blood vessels, and are therefore most exposed to serum after BBB failure, are simply more damaged than somata. Future work is needed to elucidate the specific mechanism that result in the persistent $I_{KIR}$ loss in the processes.

Astrocytic $I_{KIR}$ in posttraumatic epileptogenesis

The $I_{KIR}$ dysfunction persisted only in the perilesional neocortex (Fig. 1) that
developed an epileptic focus (D'Ambrosio et al., 2009), and not in the underlying hippocampus that sustained a milder metabolic injury (Fig. 5F), less serum extravasation (Fig. 5E) and milder acute astrocytic $I_{KIR}$ loss (Fig. 1B), and that does not initiate seizures by one month post-injury (D'Ambrosio et al. 2005). Thus, posttraumatic epileptogenesis may require a more prolonged and/or severe astroglial $K_{IR}$ dysfunction than was induced in the hippocampus by the head injury employed in this study. An alternative hypothesis is that additional mechanisms of epileptogenesis are at work in the perilesional neocortex, and contribute to the development of seizures which, in turn, contribute to the persistence of neocortical astrocytic $I_{KIR}$ loss. These seizures may result from greater blood extravasation, greater astrocytic $I_{KIR}$ loss, thrombin extravasation (Lee et al. 1997; Maggio et al. 2008), extracellular K$^+$ and glutamate accumulation (Katayama et al. 1990) or the many other acute neuronal/synaptic changes described in the posttraumatic brain (D'Ambrosio and Perucca 2004; Cohen et al., 2007). In particular, the observed transient deficit in energy metabolism (Fig. 5F) could exacerbate virtually all other deficits and promote epileptogenesis. While further work will be required to elucidate the mechanisms of consolidation of the astroglial $K_{IR}$ hypofunction in the neocortex, our data suggest that the posttraumatic neocortical and hippocampal epileptogenesis after rpFPI may be mechanistically distinct. Neocortical epileptogenesis likely involves mechanisms unleashed as a direct result of the initiating trauma, including a severe extravasation of serum sufficient to trigger an epileptogenic cascade that includes shut-down of astrocytic KIR channels (Fig. 4), and the development of spontaneous seizures similar to those induced by rpFPI (Fig. 5B,C). Conversely, the later emergence of a hippocampal epileptic focus (D'Ambrosio et al.}
2005), after full recovery from its initial milder injury, may reflect a kindling-like phenomenon due to the relentless activity of the early neocortical focus which propagates seizures to the hippocampus. Further work will be required to test this hypothesis.

The consequences of astrocytic I_{KIR} loss for the excitability, function and survival of neurons are pernicious. The resulting astrogial membrane depolarization and decreased permeability to extracellular K^+ disrupt the homeostatic control of the surrounding extracellular space and promote neuronal hypersynchronization, hyperexcitability and seizures. Spatial buffering of extracellular K^+, which requires both \( V_m \) close to \( E_K \) and large membrane K^+ conductance (Orkand et al. 1966; Newman et al. 1984), and clearance of extracellular glutamate, by electrogenic transporters which strongly depend on a hyperpolarized \( V_m \) (Brew and Attwell 1987), are both compromised by the persistent I_{KIR} hypofunction (D’Ambrosio 2004; Olsen and Sontheimer 2008; Djukic et al. 2007). Also, by offering higher resistance to extracellular flow of neuronal and synaptic currents, K_{IR}-depleted astrocytes increase ephaptic depolarization and synchronization of neuronal membranes (Grundfest and Magnes 1951; Dudek et al. 1986; Dietzel et al. 1989). This multiple hit is both ictogenic, in that it lowers the threshold for seizure precipitation, and epileptogenic, in that it can promote the permanent pathophysiological changes in synaptic drive, neuronal excitability and network connectivity observed in acquired epilepsy (Albensi and Janigro, 2003; D’Ambrosio 2004; Zha et al. 2005). Indeed, conditional knock-out of KIR4.1, eliminating just one of the several K_{IR} channels expressed in astrocytes, is sufficient to result in stress-sensitive epilepsy in the mouse (Djukic et al. 2007). Thus the early loss of
Running title: astrocytes in neocortical epileptogenesis

astrocytic \( I_{KIR} \) after rpFPI likely is a very important mechanism contributing to posttraumatic epileptogenesis.

Pro-epileptic effects of FPI are partially reproduced by serum

Recent studies have linked BBB opening and serum extravasation to chronic neocortical hyperexcitability, altered extracellular \( K^+ \) clearance, and decrease in \( K_{IR} \) mRNA (Seiffert et al. 2004; Ivens et al. 2007). Our data support a role for this mechanism in FPI-induced epileptogenesis. The data demonstrate that intraparenchymal infusion of 3-fold and 6-fold diluted autologous serum for 6 days is sufficient to induce CRSPSs that are electrically similar to those induced by rpFPI in the naïve rats in a dose-dependent fashion (Fig. 5A-C). Because serum extravasation is prominent after rpFPI in the incipient epileptic focus (Fig. 5D,E), we conclude that serum extravasation through a leaky BBB is sufficient to initiate the proepileptic loss of astrocytic \( I_{KIR} \) and posttraumatic epileptogenesis. However, both incidence of epilepsy and seizure frequency were much lower than those induced by rpFPI (D'Ambrosio et al., 2004, 2005, 2009). These differences may reflect a more severe and/or widespread extravasation of serum after head injury, or the operation of additional injury-induced epileptogenic mechanisms. Indeed, direct effects of trauma on neuronal and synaptic function have been reported (D'Ambrosio and Perucca, 2004; Cohen et al. 2007) that are likely to act synergistically with the astroglial impairment induced by serum. Furthermore, in addition to the chronic effects of serum extravasation on \( K^+ \) buffering (Seiffert et al., 2004; Ivens et al., 2007), we now show that DRS application induces a
rapid (<15 min) shut-down of membrane $K_{IR}$ channels in neocortical astrocytes *in situ* (Fig. 4E-F), which is expected to happen immediately after posttraumatic BBB failure. The ability of bath-applied DRS to rapidly modulate $K_{IR}$ channels in cell-attached patches (Fig. 4B-D) indicates that serum component(s) do not act by extracellular channel blockade but through the cytoplasm, pointing to receptors and pathways of modulation of $K_{IR}$ channels as possible therapeutic targets.

Blood serum is a complex mixture incorporating many bioactive components, and the components mediating the acute pro-epileptic effects we describe remain to be identified. However, two candidate serum components suggested by the literature, thrombin (Lee et al., 1997; Maggio et al., 2008) and albumin (Seiffert et al., 2004; Ivens et al., 2007), have been ruled out. Thrombin was undetectable in all DRS preparations, as expected for a derivative of clotted blood. Thus, while thrombin may still contribute to posttraumatic epileptogenesis, it does not mediate the loss of astrocytic $I_{KIR}$ and in vivo epileptogenesis we describe after DRS infusion. BSA did induce acute astrocytic $I_{KIR}$ decrease similar to DRS, but this activity was attributable to a dialyzable impurity with molecular weight <25 kD. The dialysis-induced loss of albumin’s activity was not due to time-dependent degradation because no such loss of activity was observed in identically treated, but undialyzed, albumin. A number of apparent actions of albumin on various cells, including astrocytes, have been attributed to incompletely characterized impurities in BSA (Alexander et al., 1998; Bunnemann and Pott, 1993; Kreps et al., 1993; Nadal et al., 1998), and vascular physiologists have found dialysis to be necessary to prevent irreversible vasocostriction (Duling et al., 1981) in a tissue that should not be sensitive to the presence of native albumin. It is worth noting that the
bioactive impurity in commercially prepared BSA need not be identical to the similarly
active component in rat serum, and further work will be required to identify the serum
component(s) responsible for astroglial $I_{KIR}$ reduction and epileptogenesis.

Conclusions

We have delineated a sequence of events linking rpFPI to serum extravasation
and loss of astrocytic $I_{KIR}$, which contributes to epileptogenesis. Exposure of the brain
parenchyma to serum, as occurs in the immediate aftermath of head injury, results in an
acute and rapid loss of astroglial $I_{KIR}$, and in CRSSs that are similar to those induced by
rpFPI. The posttraumatic impairment of astrocytic $I_{KIR}$ predominantly affects the
astrocytic processes, and becomes chronic in the neocortical epileptic focus, but not in
the hippocampus that fully recovers from the milder injury.

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**FIGURE LEGENDS**

**Figure 1:** Astrocytic $K_{IR}$ current loss after rpFPI persists chronically in the neocortex but not in the hippocampus. A) Locations of the astrocytes sampled for *in situ* whole-cell evaluation of $K_{IR}$ current. Patched cells from Bregma -0.5mm to -3.5mm are referred to a single anterior-posterior coordinate. B) Group analysis of whole-cell voltage-clamp recordings satisfying voltage-clamp quality control. Recordings were obtained from neocortical and CA3 astrocytes 1 day and 1 month post-FPI and in age-matched control. $K_{IR}$ current density ($\delta I_{KIR}$) is detected by its sensitivity to $\text{Ba}^{2+}$ (40$\mu$M) and was measured at -140 mV during voltage ramps from 0mV to 170mV. $\text{Ba}^{2+}$-sensitive $\delta I_{KIR}$ is significantly decreased compared to age-matched controls in both neocortex and CA3 one day after injury, but only in the neocortex 1 month later. The acute loss in astrocytic $\delta I_{KIR}$ is more severe in the neocortex than in the hippocampus (Asterisks indicate * p<0.025, ** p<0.0025). C) Camera lucida images of confirmed
isolated astrocytes filled with biocytin during patch clamp recordings in brain slices obtained from FPI animals. Scale bar = 10μm. D-E) Representative whole-cell current-voltage traces obtained with downward voltage ramps in neocortical astrocytes 1 day after rpFPI (E) and in age-matched control (D), before (Cont) and after Ba\(^{2+}\) (40μM) application, demonstrating the acute loss in δ\(_{\text{IKIR}}\). Top left insets in each panel show IV plots of the Ba\(^{2+}\)-sensitive δ\(_{\text{IKIR}}\). F-I) Cumulative IV plots of the Ba\(^{2+}\)-sensitive δ\(_{\text{IKIR}}\) measured during downward voltage ramps in the subset of astrocytes shown in B that were confirmed to be isolated. The δ\(_{\text{IKIR}}\) are plotted at 20 mV intervals from -160mV to -20mV. Ba\(^{2+}\)-sensitive whole-cell δ\(_{\text{IKIR}}\) was depressed one day post-injury in both neocortex (F) and CA3 (H), and remained depressed at 1 month only in neocortex (G), but not in CA3 where it recovered to pre-injury values (I). Asterisks in F-I indicate statistical significance at the p<0.01 level and, for clarity, are shown for voltage range from -160 to -120mV only. Bottom right insets in D, F show the voltage ramp protocol. 

**Figure 2. Neocortical astrocytic somata present no change in K\(_{\text{IR}}\) current at 1 day and a compensatory increase at 1 month after rpFPI.** Cell-attached recordings from astrocytic somata do not show loss of I\(_{\text{KIR}}\), suggesting the I\(_{\text{KIR}}\) loss observed in whole-cell is due the impairment of astrocytes’ processes. A) Channel gating (c=closed) during cell-attached recordings from control and rpFPI neocortical astrocytic somata at V\(_{\text{com}}\) of +70mV and 0mV. B) Segments of consecutive sweeps obtained over 15 min from a cell-attached patch containing one active K\(_{\text{IR}}\) channel before and during Ba\(^{2+}\) (100μM) application through the pipette. Channel opening is potently blocked by Ba\(^{2+}\) as expected for K\(_{\text{IR}}\) channels. C) All-points histograms of K\(_{\text{IR}}\) channel activity during
application of 100µM Ba\textsuperscript{2+} through the pipette to a patch bearing multiple channels. Peaks at t=0 indicate opening of multiple channels before Ba\textsuperscript{2+} application. Peaks are left-shifted and consolidated over time, indicating Ba\textsuperscript{2+}-blockade of all channels on the patch. At t=13 minutes all channels are permanently closed. Y scale in arbitrary units.

D) Current-voltage plots for control and rpFPI channels recorded in somatic cell-attached patches show the same reversal potential ($V_{\text{com}} \approx \text{-}48\text{mV}$) consistent with a theoretical $E_K$ ($tE_K$) in cell-attached of $\approx \text{-}22\text{mV}$ assuming cell $V_m \approx \text{-}70\text{mV}$, $[K^+]_i = 66\text{mM}$, and $[K^+]_{\text{pipette}} = 140\text{mM}$. E) Slope conductance of cell-attached $K_{IR}$ channels from control and FPI rats one day and one month post-FPI. No injury-induced changes in channel conductance are observed. F) Voltage dependence of $P_o$ in channels from control and 1 day FPI patches. No injury-induced changes in channel $P_o$ are observed. G) Numbers of $K_{IR}$ channels found in cell-attached patches obtained 1 day and 1 month post-injury. No changes are observed 1 day after rpFPI, when the whole-cell $\delta I_{KIR}$ deficit is most severe, while an increase in number of $K_{IR}$ channels is observed 1 month post-injury. Filled asterisk indicates statistical significant difference of rpFPI (1 month) when compared to a pooled control incorporating all other groups ($p=0.027$). Hollow asterisks indicate statistical significance of the two-way ANOVA test shown in H. H) Summary of the two-way ANOVA indicating a statistically significant difference between the effects of rpFPI on whole-cell $\delta I_{KIR}$ (Cell) and on cell-attached $K_{IR}$ channel count (Soma). Cells’ values are normalized to the mean of the control groups. Hollow asterisks indicate statistical significance: 1 day, $p=0.025$; 1 month, $p=0.002$. I) IV plot of mean cell-attached patch currents (corrected for leak current through the gigaseal) recorded during applied potentials from $+70\text{mV}$ to $-70\text{mV}$ in control and FPI at 1 day and 1 month.
postinjury. Currents measured 1 day post-FPI are similar to control, but are increased at 1 month post-FPI. Filled asterisk indicates p<0.05 compared to controls. Hollow asterisk indicates the significant difference (treatment x technique interaction) in the effect of rpFPI on somatic cell-attached vs whole-cell inward currents measured in the same cells (p=0.003). Horizontal dotted arrows (exp. FPI) in E-I indicate the expected changes required to fully account for the whole-cell $K_{\text{IR}}$ deficit after FPI.

Figure 3: FPI induces chronic $K_{\text{IR}4.1}$ mislocalization in cortical, but not hippocampal astrocytes. Confocal microscopic images of $K_{\text{IR}4.1}$ (green) and GFAP (red) immunofluorescence were obtained from regions of neocortex (panels A-H) and CA3 hippocampus (J-O) corresponding to the regions from which astroglial patch-clamp recordings were obtained (see Figure 1A). Controls are shown in panels A-C, G, and J-L. Sections obtained at one month post-FPI are shown in panels D-F, H, and M-O. Panels G, H and all right-most images are merged images showing both $K_{\text{IR}4.1}$ and GFAP immunoreactivity. A-C) Naïve control cortex. $K_{\text{IR}4.1}$ was expressed prominently in GFAP-positive astrocytic processes (arrow-heads), as well as in astrocytic cell bodies. D-F) Perilesional rpFPI cortex 1 month after injury. Compared to controls, $K_{\text{IR}4.1}$ immunoreactivity in injured cortical astrocytes (panels D-F) appears more prominent in swollen-appearing astrocytic cell bodies (arrow-heads), while $K_{\text{IR}4.1}$ immunoreactivity in GFAP-positive processes was markedly reduced. G-H) Lower magnification images of naïve (G) and injured cortex (H) similarly illustrate that FPI was associated with an apparent loss of $K_{\text{IR}4.1}$ immunoreactivity in finely ramifying processes. Panel I shows results of a histological analysis carried out under double-
blind conditions (see results) that confirmed that the patterns of $K_{IR}4.1$ expression in the somata and processes of astrocytes (panels D, F, H) were distinct in naive and perilesional FPI neocortices. $p = \text{exact binomial probability}$. J-O) In contrast to the injury-induced Kir4.1 mislocalization observed in neocortex, the expression pattern of Kir4.1 in the CA3 region of hippocampus of injured rats 30 days post-injury (panels M-O) was comparable to controls (panels J-L). Scale bars: C, F, L, O: 50 $\mu$m; G, H: 100 $\mu$m.

**Figure 4:** Serum, but not dialyzed albumin, induces acute loss of neocortical astrocyte $K_{IR}$ channel activity under conditions of blocked neuronal and synaptic activity. A) Brightest point projection of an astrocyte with complex electrophysiological profile filled *in situ* with rhodamine dextran after cell-attached recordings. B) The activity of a single $K_{IR}$ channel, recorded from a neocortical astrocytic soma in a brain slice, is shown in sweeps obtained at a command potential of +70mV prior to (t=-15 to 0 min) and during (t=0 to 12 min) exposure to 3-fold DRS. Channel activity, stable over the course of 15 minutes prior to DRS application, ceased abruptly after 4 minutes in DRS. C) All-points histograms obtained at a command potential of +70 mV from a somatic cell-attached patch from another neocortical astrocyte, *in situ*, displayed numerous stable conductance states before DRS application, and just one (closed) afterward. The increase in peak amplitude and decrease in the width of the histogram during DRS exposure indicate a progressive shut-down of the channels in the patch. After 12 min of DRS exposure all channel activity has ceased, resulting in an all-points histogram with a single, narrow high-amplitude peak. D) Group analysis of peak amplitudes in all-points histograms obtained in cell-attached recordings after 5-15min of DRS exposure.
normalized to pre-serum control amplitudes. Asterisks indicate p<0.05. E) Group analysis of the effect of exposure of neocortical astrocytes in situ to 3-fold DRS. DRS induces a time-dependent loss of whole-cell $\delta l_{KIR}$ during upward voltage ramps. Plot shows time course of $\delta l_{KIR}$ measured at -140 mV and normalized to pre-treatment values. Slices were treated with aCSF (control) or 3-fold DRS (serum) both with KYNA (1mM) and (TTX 1μM) to prevent epileptiform activity. F-G) Whole-cell current response to descending voltage steps in two in situ astrocytes before (control) and after (serum) ~12 minutes of exposure to DRS. $K_{IR}$ current was lost (effect) in both complex (F) and non-complex (G) astrocytes. Inset shows voltage-clamp commands. H) Group analysis of the effect of exposure to 0.4mM whole BSA (99% purity; filled squares), or 0.4mM dialyzed albumin (gray diamonds), in KYNA (1mM). Whole BSA induces a serum-like time-dependent loss of whole cell $\delta l_{KIR}$ during upward voltage ramps in naïve neocortical astrocytes, that is not reproduced by dialyzed albumin (asterisk indicates p<0.05). Note the positive effect of whole albumin applied to the same cells after the ineffective exposure to dialyzed albumin. Plot shows time course of $\delta l_{KIR}$ measured at -140 mV and normalized to pre-treatment values. Black and gray bars indicate the exposure to whole albumin or dialyzed albumin, respectively. I) Whole-cell current response to descending voltage steps in an in situ astrocyte before (control) and after (albumin) 15 minutes of exposure to 99%-pure albumin in KYNA (1mM). $K_{IR}$ current was lost (effect). Inset shows voltage-clamp protocol. Note the abolished KIR current in the lower panel. L) Whole-cell current recordings during VC protocols identical to that used in G in an in situ astrocyte before (control) and after (dialyzed albumin) 15 minutes of exposure to dialyzed albumin in KYNA (1mM). $K_{IR}$ current was not affected (effect).
Figure 5: Serum infusion *in vivo* induces chronic recurrent spontaneous seizures, and demonstration of a greater severity of injury suffered by the neocortex than the underlying hippocampus. A) Albumin immunoreactivity marks the area affected by 6 days of *in vivo* infusion of either 3-fold autologous DRS or aCSF into the neocortical parenchyma of naïve rats, through a glass micropipette. Infusion of DRS results in prominent albumin immunoreactivity throughout much of the surrounding neocortex and dorsal hippocampus (serum), while there was no detectable albumin extravasation after infusion of aCSF. B) A representative grade 2 focal seizure recorded 2 weeks after infusion of 3-fold DRS. Dotted box delimits the portion of ECoG shown at higher temporal resolution. The numbers next to each ECoG trace indicate the electrode (inset) by which the trace was recorded and the average reference (avg). Inset shows a schematic of the rat skull and 5-electrode epidural ECoG montage (1-5) used to detect serum-induced neocortical seizures. Hollow circle represents the site of insertion of the glass micropipette for intraparenchymal infusion of DRS or aCSF by osmotic minipump (OMP) *in vivo*. C) Time course of epileptogenesis following intracortical infusion of aCSF, 3-fold DRS or 6-fold DRS for 6 days. While seizures were not observed in any aCSF infused animal, those receiving DRS developed epilepsy in a dose-dependent fashion. D) Albumin immunoreactivity (alb-IR) marks extensive blood-brain-barrier compromise and serum extravasation in the neocortex and hippocampus 6hr after rpFPI. *Craniotomy* indicates site of rpFPI delivery. E) Densitometric analysis of the effect of injury on alb-IR in neocortex (Cx) and hippocampus (Hp). The FPI-induced increase in albIR was ~50% greater in neocortex than hippocampus (*p*<0.05, Wilcoxon). C) Changes in tissue ATP and ADP content in the ipsilateral rostral parietal neocortex
Running title: astrocytes in neocortical epileptogenesis

(Cx) and hippocampus (Hp) over time after injury. The neocortex suffers greater
disruption of energy metabolism than the hippocampus. Numbers at the base of each
column indicate group size. Asterisks in B-C indicate statistical significance (* p<0.05,
** p<0.005) compared to age-matched controls.

Table 1: Passive properties of neocortical and hippocampal astrocytes in whole-cell
recordings obtained from injured and control rats 1 day and 1 month after injury.

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Cm (pF)</th>
<th>Vm (mV)</th>
<th>R IN (MΩ)</th>
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<td>Naïve</td>
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<tr>
<td>1 Month CA3</td>
<td>12</td>
<td>13</td>
<td>25±4</td>
<td>23±4</td>
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</tbody>
</table>

C<sub>m</sub>: membrane capacitance; V<sub>m</sub>: membrane potential; R<sub>IN</sub>: input resistance;
Values are mean±S.E.M; *: statistically significant.