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<tr>
<td>8:00-12:50 AM</td>
<td>Picking up guests from the International airport and hotel accommodations</td>
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<tr>
<td>1:30 – 1:50 PM</td>
<td>Registration</td>
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<tr>
<td>1:50 PM - 2:00 PM</td>
<td><strong>Welcome and Overview</strong>&lt;br&gt;&lt;br&gt;<strong>Serguei Skatchkov, Ph.D.</strong>&lt;br&gt;Distinguished Research Professor&lt;br&gt;Departments of Biochemistry and Physiology&lt;br&gt;Universidad Central del Caribe, Puerto Rico, USA</td>
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<tr>
<td><strong>SESSION I</strong></td>
<td><strong>Moderator – Yuriy Kucheryavykh, Ph.D</strong></td>
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<td>2:00 PM – 2:20 PM</td>
<td><strong>Oligodendrocytes: the axon’s corn belt</strong>&lt;br&gt;- The role of oligodendroglial NMDA receptors –&lt;br&gt;&lt;br&gt;<strong>Frank Kirchhoff, Ph.D.</strong>&lt;br&gt;Professor&lt;br&gt;Department of Molecular Physiology, Institute of Physiology&lt;br&gt;University of Saarland, Germany, EU</td>
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<td>2:30 PM – 2:50 PM</td>
<td><strong>Evolution of Astroglial and Neuronal Disruption in Stroke and TBI Revealed by in vivo Two-photon Imaging</strong>&lt;br&gt;&lt;br&gt;<strong>Sergei Kirov, Ph.D.</strong>&lt;br&gt;Associate Professor,&lt;br&gt;Department of Neurosurgery, Medical College of Georgia,&lt;br&gt;Georgia Health Sciences University, Georgia, USA</td>
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<td>3:00 PM - 3:20 PM</td>
<td>BREAK</td>
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<td><strong>SESSION II</strong></td>
<td><strong>Moderator – Mikhail Inyushin, Ph.D</strong></td>
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<td>3:20 PM – 3:40 PM</td>
<td><strong>Physiopathology of vesicle dynamics in astrocytes</strong>&lt;br&gt;&lt;br&gt;<strong>Robert Zorec, Ph.D.</strong>&lt;br&gt;Professor&lt;br&gt;Head National Research Programme “Cell Physiology”&lt;br&gt;Institute of Pathophysiology&lt;br&gt;University of Ljubljana, Slovenia, EU</td>
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<td>3:50 PM – 4:00 PM</td>
<td>Microglia promote glioma cell migration and dispersal by activating the PLCγ1 and Pyk2 signaling cascade</td>
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<td>Kimberleve Rolon-Reyes</td>
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<td>Cell and Molecular Biology Program, School of Medicine</td>
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<td>Universidad Central del Caribe, Bayamon, Puerto Rico, USA</td>
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<td>4:05 PM – 4:25 PM</td>
<td>Regulation of cell-cell communication and voltage gating of connexin36 GJ channels by biologically active compounds</td>
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<td>Feliksas Bukauskas, Ph.D.</td>
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<td>Professor</td>
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<td>Dominick P. Purpura Department of Neuroscience</td>
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<td>Albert Einstein College of Medicine, New York, USA</td>
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<td>4:35 PM – 4:45 PM</td>
<td>Intracellular polyamines enhance astrocytic coupling</td>
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<td>Jan Benedikt, Ph.D.</td>
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<td>Postdoctoral Fellow</td>
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<td>4:50 PM – 5:20 PM</td>
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<td>SESSION III: Moderator – Lilia Kucheryavykh, Ph.D</td>
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<td>5:20 PM - 5:40 PM</td>
<td>By sensing vigilance states astrocytes modulate extracellular purines</td>
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<td>Philip Haydon, Ph.D.</td>
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<td>Annetta and Gustav Grisard Professor of Neuroscience</td>
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<td>Department of Neuroscience, School of Medicine</td>
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<td>Tufts University, Boston</td>
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<td>5:50 PM – 6:00 PM</td>
<td>Closing Remarks</td>
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<td>Time</td>
<td>Session IV</td>
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<td>8:00 AM - 8:30 AM</td>
<td>Picking up guests from the hotels</td>
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<tr>
<td>8:30 AM - 4:00 PM</td>
<td>Discussion on collaborative projects and future joint plans</td>
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<tr>
<td>4:30 PM – 5:00 PM</td>
<td>Delivery of guests to airport</td>
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Seminar Title: 
Oligodendrocytes: the axon’s corn belt
- The role of oligodendroglial NMDA receptors

Biography:
Frank Kirchhoff is Chair of the Department of Molecular Physiology at the University of Saarland in Homburg, Germany. He studied biochemistry at the University of Hannover, received his PhD degree in neurobiology from the University of Heidelberg and habilitated in biochemistry at the Free University of Berlin. After postdoctoral periods at the University of Heidelberg and the Max-Delbrück-Centrum for Molecular Medicine, Berlin, he started his research group ‘Glial Physiology and Imaging’ at the Max Planck Institute of Experimental Medicine, Department of Neurogenetics in Göttingen in 2000. In 2009, he was appointed as full professor at the University of Saarland. He is Editorial Board Member of GLIA, Journal of Chemical Neuroanatomy and others. His research addresses the molecular and cellular mechanisms of neuron-glia interactions using transgenic mouse models and in vivo-imaging. His lab investigation concerns several main questions: (i) How do neurons and astrocytes interact with each other? (ii) Are Ca2+ signals mediators or only indicators of coordinated network activities? (iii) How do glial transmitter receptors sense and modulate synaptic transmission? (iv) What is the impact for living organisms? (v) How do glial cells respond to injuries within the central nervous system?

For functional analyses they generated several animal models and are continuing to develop new transgenic mouse models (1) with cell-type specific expression of various fluorescent proteins (FPs) and (2) glial-specific inducible gene deletion. Transgenic mouse models with cell-type specific fluorescent protein expression and two-photon laser-scanning microscopy are important tools and techniques to study the dynamic interactions of individual cells and cell types studying neuron-astrocyte crosstalk in situ and in vivo. He used a combination of biochemical and molecular biological methods together with imaging techniques such as two-photon laser-scanning microscopy (2P-LSM) or CCD imaging. He developed a series of transgenic mice with cell-type specific fluorescent protein or inducible cre DNA recombinase expression in various glial cells. These mice appeared as valuable tools to study the structural dynamics of glial cells and the function of glial transmitter receptors in vivo.

Dr. Kirchhoff is the active member of different scientific networks and consortiums for example EduGLIA-Program, NeuroGLIA-consortium and others that select the best research Institutions in Europe and world wide. His work is supported by different foundations including the DFG Research Center Molecular Physiology of the Brain.

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Philip Haydon

Seminar Title:
By sensing vigilance states astrocytes modulate extracellular purines

Biography:
Dr. Philip Haydon is Chair of the Department of Neuroscience and Annetta and Gustav Grisard Professor of Neuroscience at the TUFTS University, School of Medicine, Boston, MA, USA. Dr. Haydon has accomplished his BSc in Physiology, University of Leeds and PhD in Physiology, University of Leeds, England. He has obtained his postdoctoral training at University of Iow and was director of the Signal Transduction Training Group and the Laboratory of Cellular Signaling as well as associate director of the Microanalytical Instrumentation Center. Later, as a member of the Department of Neuroscience at the University of Pennsylvania, Dr. Haydon directed the Center for Dynamic Imaging of Nervous System Function and the Silvio O. Conte Center for Studies of the Tripartite Synapse. His first ground breaking discovery together with Dr. Vladimir Parpura was the finding of neurotransmitter (glutamate) release from glial cells. Currently, Dr. Haydon is extensively and intensively studying regulation of synaptic transmission by glia, synaptogenesis, gliotransmitter release to neuronal network and he is also focusing on neuronal progenitors. Astrocytes act as a third element that wrap around the synaptic structure to control synapse formation and function therefore, the goal of his lab is to identify the mechanisms by which astrocytes regulate synaptic transmission, neuronal circuits and what is their critical role in controlling behavior? His recent studies also focus on the roles of glial cells in disorders of the nervous system. The Department of Neuroscience coordinates neuroscience educational and research programs within the Sackler School of Biomedical Sciences and the Tufts University School of Medicine. Dr. Haydon is active participant of The Neuroscience Program together with more than 30 faculty holding research interests from molecular to systems analysis to understand the form and function of the glio-neuronal system. This graduate program emphasizes multidisciplinary training to prepare students for research careers in the complex field of neuroscience. He is involved synergistically in the development of The Tufts Center for Neuroscience Research (CNR) having funds from the National Institute for Neurological Disorders and Stroke (NINDS) and a support from Tufts University School of Medicine (TUSM) and Tufts Medical Center. The Department of Neuroscience, Medical Center and the CNR promote collaborative interactions among neuroscientists at Tufts University, its affiliated hospitals and with partners abroad providing core research services to Tufts neuroscientists and other investigators. CNR is supported by P30 NS047243 grant and CNR core facilities offer services for biological imaging, biophysics and electrophysiology, computational genomics as well as animal behavior. He is Editorial Board Member of many top national and international journals as one of the world know specialists in tripartite synapse structures where glial cells play a key role.

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Web: http://www.tufts.edu/central/research/ResearchNews/Researchers/haydon.htm
Robert Zorec

Seminar Title:

**Physiopathology of vesicle dynamics in astrocytes**

Biography:
Robert Zorec is **Professor of Pathophysiology at the University of Ljubljana**, Medical Faculty. He is a member of the Slovenian Academy of Sciences and Arts and the Academia Europaea (Lond.). He was a PhD student at the New Castle-upon-Tyne Medical Faculty, UK and at the University of Ljubljana, Slovenia. He did a post-doc as a Welcome Trust Fellow in Cambridge and started his own laboratory in 1991 at the Institute of Pathophysiology, Medical Faculty in Ljubljana. This lab merged with the labs at the Celica Biomedical Center, Technology Park in 2000 and by establishing the Carl Zeiss Reference Center for Confocal Microscopy (1999 and 2006).

His research interest has been electrophysiology and cell physiology where he developed independently of the Nobel Laureate Erwin Neher in Goettingen the high-resolution membrane capacitance measurements to study elementary properties of exocytosis, involving the fusion of vesicle membrane with the plasma membrane. As model systems he used single pituitary, mast, liver, neurons, plant protoplasts, adipocytes and other cells. His recent interest is vesicle traffic and regulated exocytosis in astrocytes, where the lab developed new optical methods to study subcellular vesicle traffic, single cell metabolism and secondary messenger activation in normal and pathological conditions. In addition to basic research focusing into physiological and pathological problems, the lab is also developing advanced cell-based medicines such as hybridoma cells to treat cancer. The labs operate as GMP facilities and are compliant with ISO/SIST 17025 standards. Dr. Zorec is a member of the Committee for Advanced Medical Products at the European Medicine’s Agency, London.

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Feliksas Bukauskas

Seminar Title:
Regulation of cell-cell communication and voltage gating of connexin36 GJ channels by biologically active compounds

Biography:
Feliksas Bukauskas is a Professor at the Department of Dominick P. Purpura Department of Neuroscience, Albert Einstein College of Medicine. He studied medicine/biophysics at Kaunas University of Medicine (KUM) in Lithuania. He received PhD (1974) and Dr. Habilitatus (1983) degrees in biology/biophysics for studies related to electrophysiology and intercellular communication in the heart. In 1986, for work related to the development of new methods in diagnosis and surgical treatment of cardiac arrhythmias, he and colleagues from KUM and Cardiac Center in Moscow were awarded with the State Prize of the former USSR.

In 1987 he become a professor and in 1988 pro-rector/provost of KUM. From 1990 to 1995 he was working at the University of Bern (Switzerland), from 1995 at the University of Rochester (NY) and from 1997 he is working at the Albert Einstein School of Medicine in New York.

Currently, his major scientific projects are related to intercellular communication under normal and pathological conditions in the nervous system and the conduction system of the heart by focusing to the gating mechanisms of gap junction channels and unapposed hemichannels that are essential for electrical and metabolic communication between cells as well as for paracrine signaling.

For more information Dr. Buakuskas web is at:

http://connexons.aecom.yu.edu/Home.htm

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feliksas.bukauskas@einstein.yu.edu
Sergei Kirov

Seminar Title:
Evolution of Astroglial and Neuronal Disruption in Stroke and TBI Revealed by in vivo Two-photon Imaging

Biography:
Sergei Kirov is an Associate Professor at the Department of Neurosurgery, Brain & Behavior Discovery Institute, Georgia Health Sciences University, Augusta, Georgia. In 1987 he obtained his M.S. and in 1991 Ph.D. in biophysics at St.Petersburg State University being trained at Pavlov Institute of Physiology of the Academy of Sciences of Russia. After obtaining postdoctoral training in Laboratory of Behavioral Sciences, National Institute on Aging, NIH, and in Gerontology Research Center, Baltimore, Maryland (1992-1995), he occupied a position of Senior Postdoctoral Fellow, Department of Physiology, University of Maryland (1995-1997). As a Research Associate at Division of Neuroscience, Harvard Medical School, Children's Hospital, Department of Neurology, Boston (1997-1999) and then as Research Assistant Professor, Department of Biology, Boston University (1999-2003). He is supported by an R01 grant on synaptic formation and glial cell morphology/function in brain. His major interests are: (i) Properties and changes in human synapses during epilepsy, stroke, brain tumors and traumatic head injury; (ii) Mechanisms of synapse formation and maintenance in the immature and especially mature brain and (iii) Cellular mechanisms of memory.

Elucidating mechanisms of structural plasticity of neurons and glia in the mature brain is specifically important under pathological conditions. Neuronal activity sculptures synaptic networks throughout the lifespan, however, more profound restructuring of the brain circuitry is caused by an excessive activation during epilepsy, stroke and traumatic brain injury (TBI). While important for maintaining ionic balance, preventing neurotoxicity, and providing trophic support for neurons, the dynamic contributions of astrocytes to the early stages of stroke and TBI are not well understood. Reorganization of surviving synaptic networks and astrocytes could provide support for synaptogenesis and recovery of function. Furthermore, rapid activation of microglia, the resident immune cells in the brain during the early stages of ischemic/traumatic brain injury might have a crucial benefit as well as detrimental effects on synaptic circuitry which remain to be elucidated. This complex sequence of spatiotemporal cellular events during pathological conditions such as stroke and TBI is an area of current intense studies of his laboratory.

Dr. Kirov's lab is using ex vivo (murine and human neocortical slices) and in vivo (transgenic mice) model systems to perform advanced functional brain imaging (2-photon laser scanning microscopy (2PLSM), intrinsic optical signal (IOS) imaging, laser speckle imaging) coupled with electrophysiology (whole-cell recordings, extracellular field potentials, cortical surface potentials) and serial section transmission electron microscopy (ssTEM). This unique array of sophisticated methods represents a powerful suite of techniques that are not widely used in combination, as each of them requires significance expertise.

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Web: http://www.georgiahealth.edu/medicine/neurosurgery/faculty/kirov.html
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| **1.** Spermine and IEM1460 reveal two pharmacologically distinct populations of interneurons in CA1 hippocampus.  
| **2.** Sodium accumulated in glia during glutamate transport increases polyamine dependent block of Kir4.1 channels.  
| **3.** TREK-2 potassium channels in astrocytes are functionally up-regulated during ischemia through a mechanism requiring de novo protein synthesis.  
| **4.** Polyamine fluxes through Cx43-hemichannels in freshly isolated astrocytes and Mueller glia.  
| **5.** Microglia promote glioma cell migration and dispersal by activating the PLCγ1 and Pyk2 signaling cascade.  
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Spermine and IEM1460 reveal two pharmacologically distinct populations of interneurons in CA1 hippocampus

Authors: *J. BENEDIKT*¹, S. L. BULDAKOVA², M. Y. INYUSHIN¹, Y. V. KUCHERYAVYKH¹, R. W. VEH³, C. G. NICHOLS⁴, M. J. EATON¹, S. N. SKATCHKOV¹.


Abstract: Polyamines, such as spermine (SP) and spermidine, are predominantly stored in glial cells and can be released to the neuronal environment by specific stimuli (ischemia, depolarization, increased extracellular K+). Polyamine release might play an important role in modulation of excitatory and inhibitory neurotransmission, since SP is found in astrocytes surrounding interneurons in hippocampus. We suggest that astrocytic SP can modulate glutamate receptors expressed in CA1 hippocampal interneurons and ultimately, block feedback inhibition of pyramidal cells. The purpose of this study is to compare the effects of application of external SP and IEM1460 (impermeable open AMPA channel blocker) on non-enzymatically isolated interneurons of 15-24 days old rats and on interneurons in 18-30 days old rat patched in hippocampal slices.

We found that application of 200 µM SP hyperpolarized and depressed the firing of interneurons patched from CA1 s. radiatum, whereas pyramidal cells were either insensitive or slightly activated. On the other hand, interneurons located in s.oriens were depolarized and their firing rate was increased in 67% of cells. In isolated cells, SP blocked kainate-induced currents in s. radiatum interneurons (Vh = -70 mV) by 51±10%, while in s.oriens, currents were increased by 16±12% at the same holding potential. IEM1460, a specific blocker of GluR2-lacking, Ca2+-permeable AMPA receptors, also revealed the presence of two distinct populations of neurons: in isolated cells, IEM1460 reduced currents in s. radiatum by 48±8%, whereas in s. orien IEM1460 increased kainate induced current by 14±5%. In rat brain slices, IEM1460 increased firing in 100% cells in s. orien, while in s. radiatum 27% of cells showed increase of firing and 73% decrease in firing, suggesting the prevalence of GluR2- lacking interneurons in s. radiatum. Intriguingly, when s. orien interneurons were held at -5 mV, currents were increased from 26±9% to 123±8%, if internal Cs+ was substituted to K+. Internal SP (200 µM) did not play a role suggesting that IEM1460 and SP have extracellular K+-dependent potentiation sites.

Based on our findings, we propose a CA1 cell circuit model which explains paired-pulse facilitation in pyramidal cells that is always observed when astrocytes release SP. Furthermore, our model explains the neuromodulatory and potentially neuroprotective effect of endogenous SP as well as a potential role of IEM1460 as a pharmacological tool to regulate not only GluR2-subunit lacking interneurons but other types of interneurons whose receptors IEM1460 potentiates.
Sodium accumulated in glia during glutamate transport increases polyamine dependent block of Kir4.1 channels

Authors: *Y. V. KUCHERYAVYKH*, S. M. ANTONOV, Y. M. SHUBA, Y. RIVERA, M. Y. INYUSHIN, R. W. VEH, A. VERKHRATSKY, C. G. NICHOLS, M. J. EATON, S. N. SKATCHKOV

1Univ. Central Del Caribe, Bayamon, PR; 2Sechenov IEPb, St. Petersburg, Russian Federation; 3Int. Ctr. for Mol. Physiology, NASU, Kiev, Ukraine; 4Inst. of Integrative Anatomy, Charite, Berlin, Germany; 5The Univ. of Manchester, Manchester, United Kingdom; 6Washington Univ. Sch. of Med., St. Louis, MO

Abstract: In the tripartite synapse, astrocytes surround the peri-synaptic area to provide homeostatic control over synaptic transmission. Using high-affinity uptake transporters and Kir4.1 channels, glial cells influence neuronal activity. Mutations in Kir4.1 potassium channels cause severe disorders: EAST/SeSAME syndrome, epilepsy, deafness, seizure and early death (Scholl et al., 2009; Bockenhauer et al., 2009). Downregulation of Kir4.1 channels compromises glutamate transport (Kucheryavykh et al., 2007; Djukic et al., 2007) that may contribute to seizure susceptibility (Inyushin et al., 2010). The reciprocal effect of the glutamate transporter on Kir4.1 channels has not been studied. In fact, Kir4.1 channels are weakly rectifying by the polyamine spermine (SPM) (Oliver et al., 1998) and SPM may leak through the Kir4.1 pore in symmetrical K+ (Kucheryavykh et al., 2006). Under physiological conditions, however, Kir4.1 becomes strongly rectifying showing an unusual second blocking site induced by interaction between SPM and Na+ (Kucheryavykh et al., 2009). This feature is different in nature from the “biphasic” block observed by SPM alone in Kir2.1 and Kir2.2 channels (Ishihara & Yan, 2007).

We hypothesize that the interaction between SPM and Na+ is linked to either glutamate transporters or ionotropic glutamate receptors in glia which provide Na+ entry into the glial cytoplasm. Using simultaneous (i) whole cell patch clamp recording from glial cells with intracellular SPM=300 µM, (ii) external glutamate application, (iii) intracellular Na+ recordings with fluorescent dye and (iv) blockers of glutamate transporters (TBOA) or receptors (CNQX), we tested our hypothesis. In freshly isolated retinal Mueller glial cells and cultured cortical astrocytes we found (1) that the degree of Kir4.1 rectification was dependent upon cytoplasmic Na+ and SPM concentrations; (2) increased intracellular Na+ (up to 30 mM) was observed immediately after application of external glutamate (100 µM); (3) TBOA (0.1µM), but not CNQX (20 µM), inhibited the glutamate-induced increase in intracellular Na+.

This suggests that glutamate transporter mediated Na+ accumulated in glia provides sufficient increase in cytosolic Na+ entry to cause nearly total block of Kir 4.1 channel-mediated outward currents. Physiologically, glutamate clearance and the co-transport of Na+ convert “leaky” Kir4.1 channels to "regulated" Kir4.1 channels ideally suited to maintain extracellular potassium homeostasis during synaptic activity.
TREK-2 potassium channels in astrocytes are functionally up-regulated during ischemia through a mechanism requiring de novo protein synthesis

Authors: *A. F. RIVERA-PAGAN*¹, D. E. RIVERA-APONTE¹, L. Y. KUCHERYAVYKH¹, Y. V. KUCHERYAVYKH¹, L. A. CUBANO¹, S. N. SKATCHKOV¹,², M. J. EATON¹.

¹Biochem. and ²Physiol., Univ. Central Del Caribe, Bayamon, PR

Abstract: Astrocytes, the most numerous cells in brainstem and cortex, are essential for neuronal viability, in part, by maintaining extracellular homeostasis. The ability of astrocytes to regulate the extracellular concentrations of neuroactive substances such as K+ and glutamate depends upon the presence of K+ channels in their membranes and the hyperpolarized membrane potential of these cells. We have previously shown that TREK-2 potassium channels in astrocytes support glutamate clearance during ischemia and are functionally upregulated by ischemia.

The purpose of the present study was to determine the mechanism leading to upregulation of functional TREK-2 channels in astrocytes during an ischemic insult.

Using real time RT-PCR, the levels of TREK-2 mRNA were not increased in response to ischemic conditions. In contrast, ischemia increased TREK-2 protein levels and this effect was reversed by the protein synthesis inhibitors emetine (60nM) or cycloheximide (1µg/ml). Similar up-regulation of TREK-2 protein is observed in both the cytoplasm and astrocytic membrane after ischemic conditions using a cell surface biotinylation assay. In addition, we found that TREK-2 channels in astrocytes are open during ischemic conditions, perhaps due to the ability of astrocytes to maintain ATP levels using anaerobic glycolysis. This differs from what had been previously reported by Kréneisz et al., 2009, where TREK-2 channels heterologously expressed in HEK cells were closed during metabolic inhibition through phosphorylation by AMP-activated protein kinase. Our data are supported by the following findings: quinine (a blocker of TREK-2 currents) was able to significantly block currents in ischemic treated astrocytes and the quinine sensitive currents were much greater in astrocytes treated with ischemia as compared with control astrocytes. Furthermore, wogonin and baicalein (activators of TREK-2 channels) were able to activate currents in 30% of control astrocytes, but were unable to activate currents in any astrocytes exposed to ischemia, suggesting that these channels were already fully open. Finally, the AMPK agonist AICAR was able to inhibit currents in astrocytes exposed to ischemia indicating that the TREK-2 channels were not already phosphorylated by AMPK.

Taken together, these data suggest that TREK-2 channel activity in astrocytes is not inhibited by ischemia and TREK-2 channels are upregulated during ischemia through a mechanism requiring de novo protein synthesis.
Polyamine fluxes through Cx43-hemichannels in freshly isolated astrocytes and Mueller glia

Authors: *Y. RIVERA¹, Y. V. KUCHERYAVYKH¹, J. BENEDIKT¹, R. W. VEH², C. G. NICHOLS³, A. RIVERA¹, M. J. EATON¹, S. N. SKATCHKOV¹.

¹Univ. Central Caribe, BAYAMON, PR; ²Charite, Berlin, Germany; ³Washington Univ. Sch. of Med., St. Louis, MO

Abstract: Glio-transmitters such as ATP, glutamate and D-serine may regulate neuronal behavior when released from glia. Here we provide evidence that the polyamines, spermidine and spermine (SD/SP), are new candidate glio-transmitters for several reasons. We found that SD and SP (i) are accumulated predominantly in glia, (ii) are taken up as well as released from glia and (iii) act on polyamine-sensitive neuronal receptors and channels (AMPAR, NMDAR, TRP, kainate, Kir, Ca²⁺, and Na⁺-channels and receptors) to modulate neuronal activity.

We hypothesize that SD/SP can become unbound from ATP, RNA, DNA, proteins and other acid compounds in the glial cytoplasm and released upon depolarization via large pores in the glial membrane.

The purpose of the present study was to investigate the permeability of SD/SP through the glial membrane.

We (i) monitored biotinylated SP uptake into glia, (ii) measured SD and SP electrical currents through the glial membrane and (iii) used a novel SP-biosensor to measure SP release from glia. The SP-biosensor is based on excised patches from Cosm-6 cells transfected with cDNA encoding mutated Kir6.2 [N160D, C166S]/SUR1 channels. We found that (i) biotinylated SP permeates the membrane and accumulates in cultured astrocytes; (ii) SD and SP electrical currents can be recorded in freshly isolated cortical and hippocampal astrocytes and retinal Mueller glial cells; (iii) SD and SP fluxes can be blocked by siRNA against Cx43 in glial cells. Comparing freshly isolated astrocytes and Mueller cells with cultured astrocytes, we found that SP currents were 60% less pronounced in freshly isolated cells than in culture. This suggests that enzymatic treatments needed to isolate cells (papain and DNase) can affect the external Cx43 pore and/or disrupt fine glial processes where Cx43 is predominantly localized. Using our SP-biosensor, we measured SP release from astrocytes and Mueller cells in culture and in retina and brain slices. Depending on the stimulation protocols and the amount of previously accumulated SP in glia and tissues, SP release from 4 to 100 µM was observed.

We conclude that SD and SP are novel glio-transmitters which not only modulate synaptic transmission but also can be neuroprotective when blocking Ca²⁺-permeable channels in neurons.
Microglia promote glioma cell migration and dispersal by activating the PLCγ1 and Pyk2 signaling cascade.


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Abstract: Glioblastoma multiforme is one of the most aggressive and fatal brain cancers mostly because of the highly invasive nature of these glioma cells. The ability of glioma cells to disperse through the healthy brain stroma makes surgical and chemotherapeutic treatments mostly ineffective. Microglia infiltrate most gliomas and release factors, which favor tumor growth and dispersal. We hypothesize that microglia residing within the tumor release soluble factors that directly stimulate glioma cell migration and dispersal.

Specifically, we hypothesize that microglia stimulate migration of glioma cells through the phospholipase C (PLC)γ1 and proline rich tyrosine kinase 2 (Pyk2) signaling cascade. In the present study, we investigated two different human glioma cell lines with varying levels of invasiveness: U-87MG and HS683.

Using Western blot, we demonstrated that treatment of glioma cells with microglia conditioned medium upregulated phosphorylation of Pyk2 protein at Tyr579/580 in both glioma cell lines. Also, using a standard Boyden chamber invasion assay, we demonstrated that microglia significantly activated invasion of both glioma cell types, and this effect was reversed after knock-down of PLCγ1 and Pyk2 proteins using siRNA in glioma cells. Pharmacological blockers of PLCγ1 (U73872 1nM) and Pyk2/focal adhesion kinase (FAK) (PF562271 20nM) also completely eliminated the ability of microglia to stimulate glioma cell migration.

Taken together, these data indicate that microglial cells activate glioma cell migration/dispersal through the pro-migratory Pyk2 and PLCγ1 signaling pathway in glioma cells.
Morpho-physiological characterization of retinal Müller cells from the Spectacled caiman

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Abstract: The Spectacled caiman (Caiman crocodilus fuscus) is a medium-sized crocodilian widely distributed in America. This unique animal develops a complex retina and has perfect night vision as well as day vision with a large scale of light adaptation. The caiman’s retina is composed of five different regions: (1) ventral, for upper vision; (2) dorsal, for lower and underwater vision; (3) nasal, for side and backward vision; (4) ventral-temporal, for frontal vision; and (5) central. In addition, its retina possesses a tapetum lucidum in the superior part of the fundus that extends from the nasal region to the central and ventral region.

Our objective is to describe the precise morphology of Müller cells in each of the five regions of the caiman's retina. For this purpose, eyes of a light adapted caiman (length = 50cm) were fixed by vascular perfusion of 4% paraformaldehyde in potassium buffered solution (pH 7.4). After eye enucleation, the retinas were isolated, sectioned, and prepared for immunohistochemical evaluation.

Retinal tissues were evaluated for glial markers: vimentin, S100B, GFAP, glutamine synthetase, TASK-1, -2, -3, Kir2.1, Kir6.1, and Kir4.1. Among all the markers, glutamine synthetase and TASK-1 best delineated the overall structure of Müller cells. We observed pronounced morphological cell diversity among the different regions of the retina. In the central region, the Müller cells are longer, thinner and divide into multiple (up to five) stalks, while the cells in the dorsal region are thick and short and have a single stalk. In contrast to mammalian retinas, antibodies against the inwardly rectifying potassium channel Kir4.1 did not label Müller cells; instead, photoreceptor cells were labeled. Markers for the 2P-domain pore channels TASK-1, -2, and -3, however, provided a strong staining of Müller cells. The I/V-curves recorded from vitreal endfeet of Müller cells in retinal whole mounts as well as from enzymatically isolated Müller cells demonstrated mostly outwardly rectifying K+ currents. This is different from mammalian Müller cells in which robust inward currents can be recorded. Also in contrast to data from mammalian Müller cells, the endfoot currents were poorly sensitive to barium (100 µM).

This suggests that in the caiman’s retina, 2P-domain pore channels may play a major role in glial K+ homeostasis. Future studies will evaluate the hypothesis that caiman Müller cells may act as light-guiding fibers towards the photoreceptor cells, similar as has been shown in the guinea-pig's retina.
Astrocyte participation in thyroid hormone conversion

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Abstract: Thyroid hormone (TH) has diverse effects on mammals, including neonatal and adult brain. In the developing animal, TH regulates various events such as neuronal processing, glial cell proliferation, myelination, and neurotransmitter synthesis. THs are produced by thyroid gland and released to the blood. The metabolically active form of thyroid hormone, 3,5,3'-triiodothyronine (T3), acts by binding to nuclear receptors. Although small amounts of 3,5,3'-triiodothyronine (T3) are produced directly by the thyroid gland, more than 80% of this form of the hormone in the brain derives from the local conversion of thyroxine (T4), catalyzed by a cAMP-inducible membrane-bound enzyme, the type II iodothyronine 5'-deiodinase (D2). Neurons do not possess D2 and express only type III deiodinase (D3) that degrades T3 to its inactive metabolite diiodothyronine (T2). Because, in the brain D2 is predominantly expressed in astrocytes and tanyocytes we hypothesized that T4 is captured by non-neuronal cells from the blood-brain or blood-cerebrospinal fluid (CSF) barriers and converted into T3 for neuronal use. We used cultured astrocytes and mass-spectrometry to test our hypothesis of “Glial TH conversion”.

Primary cortical astrocyte cultures were prepared from 1-4 day old rats and treated with 50 mM leucine methyl ester for 60 minutes (pH=7.4) to kill the microglial cells. Then astrocyte cultures were allowed to recover for at least one day before being plated for experiments. After that all cultural media were removed from dishes and replaced by TRIS buffered (pH=7.4) physiological solution. The astrocytes were incubated with 200 nM of T4 and at 30 min and 60 min samples of the media were analyzed by direct infusion into a Mass Spectrometer-Quadruple Time of Flight TOF (MS-QTOF) with an electrospray ion source in the negative ion mode (ESI-). The spectrum were acquired by 2 minutes and then correct by a reference (D(+)-Raffinose, 250 pg/uL). T3 spectrum was detected at 0 (control), 30 and 60 minutes samples, showing the conversion from T4. There were no T3 accumulation recorded at the beginning of the experiments, then the difference in T3 spectrum amplitudes increased between 30 and 60 minutes samples showing substantial (more than 50%) accumulation of converted T3.

We can conclude that astrocytes (1) can convert T4 to T3 and (2) may represent an important pool of cells participating in TH conversion in brain. The work for additional quantification of experiments using MS-QTOF method would allow to determine if astrocytes are a main source of T3 in brain.
High Glucose Decreases Kir4.1 and TREK-2 Potassium Channels Expression and Function in Astrocytes

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Abstract: Both type I and type II diabetes affect the central nervous system (CNS) and are coupled with increased incidence of seizure and stroke. Astrocytes are critical for normal CNS function and dysfunction or loss of astrocytic potassium channels increases seizure susceptibility and is associated with ischemic brain damage.

The purpose of the present experiment was to test the hypothesis that high glucose alters the expression of potassium channels in astrocytes. To test this hypothesis, we prepared primary astrocyte cultures from Sprague-Dawley rats in low (5.5 mM) and high glucose (25 mM)-containing DMEM. After two weeks in culture, we performed RT-PCR and Western blot to determine gene and protein expression of Kir4.1 and TREK-2 potassium channels and whole-cell electrophysiological recording to measure potassium channel currents. For comparison, we determined the mRNA and protein levels of glial fibrillary acidic protein (GFAP). GFAP is a marker of reactive astrocytes that has been shown to be downregulated in astrocytes grown in high glucose. Our results revealed that astrocytes grown in high glucose had lower levels of Kir4.1, TREK-2 and GFAP mRNA and protein when compared with astrocytes grown in low glucose. Furthermore, electrophysiological studies demonstrated that Kir and TREK channel function is impaired in astrocytes grown in high glucose.

Taken together, our results suggest that downregulation of astrocytic potassium channels by elevated glucose may contribute to the underlying pathophysiology of diabetes-induced CNS disorders.
Polyamines and brain signaling: Use of a novel spermine biosensor

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Abstract: The concept of neuronal-glial communication is a novel frontier in neuroscience. Glio-transmitters such as ATP, glutamate, D-serine and a few others may regulate neuronal activity when released from astrocytes; however, the commonly accepted glial signaling has been questioned recently (Science 327(2010)1250; Nat. Rev. Neurosci. 11(2010)227). The purpose of this study was to test an alternative/supplemental “polyamine hypothesis”. Accordingly, (i) the polyamines, spermidine and spermine, are accumulated in glia, (ii) bound with ATP, RNA, proteins and other acid compounds in the glial cytoplasm and then (iii) released upon glial depolarization via large pores. After release, polyamines (iv) act on key neuronal receptors and channels. This results in (v) a switch of synaptic excitation and change of spiking rate while (vi) at higher doses of release, neuroprotection is expected due to block of calcium permeable neuronal channels preventing apoptosis.

We used (i) immunocytochemistry to determine the localization, uptake and release of polyamines such as spermine and spermidine; (ii) immuno-labeling of spermidine-synthase (J. Neurochem. 103(2007)679) and spermine-synthase, the enzymes producing these polyamines; (iii) a spermine bio-sensor to measure spermine release in brain slices and in retina; (iv) patch-clamp to study neuronal activity when glial cells release spermine/spermidine.

We did not find biosynthetic enzymes for spermidine and spermine in glia (neither in retina or in brain), but we have found that polyamines permeate the glial membrane through Cx43 and Kir4.1, but not Panx-1 glial channels (using electromotive force) allowing polyamines to be taken up or released, depending on the membrane state. We, therefore, created a novel spermine biosensor based on two mutations: Kir6.2 [N160D, C166S]/SUR1 that render the channel (1) very insensitive to ATP inhibition, requiring millimolar levels for significant inhibition, (2) steeply voltage-dependent, with high-affinity binding for spermine, (3) insensitive to divalent cations and (4) considerably more stable against rundown. This biosensor is, therefore, ideal for our work, since ATP does not exceed low micromolar levels in the extracellular space of brain and retina after strong stimulation. Using inside-out patches, we measured spermine release from different populations of glial cells in retina (up to 10 µM during neuronal stimulation) and brain (up to 100 µM during ischemia). (5) Considering that spermine and its derivatives are key regulators of neuronal AMPAR, NMDAR, TRP, kainate, Kir, Ca2+, and Na+-channels and receptors, we tested spermine release from glial cells on neuronal firing and synaptic activity and found that interneurons from stratum radiatum and stratum oriens respond differently. These effects were blocked by AMPA/Kainate blockers. This suggests a complex action of glial polyamines and opens an avenue of further investigation.

Polyamines accumulated in and released from glia may act as novel glio-transmitters in both normal and diseased brain (i.e., during ischemia, stroke and epilepsy). Therefore, the amount of polyamines stored in glia would be a key condition to regulate the neuronal state in normal and traumatic brain. Glial cells could play a novel key role in CNS via their polyamine uptake and release mechanisms.
Spermine permeation is decreased and its block is increased by sodium in glial Kir4.1 channels

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Abstract: Dysfunction of Kir4.1 potassium channels causes severe disorders: lack of glutamate transport, hypokalemia, hypomagnesemia, metabolic alkalosis, ataxia, epilepsy, deafness, seizure and early death (Buono, et al., 2006; Djukic et al., 2007; Scholl et al., 2009). Spermine (SP) can block Kir4.1 channels (Oliver et al., 1998) but may also permeate the pore (Kucheryavykh et al., 2007). Recently, we identified a second blocking site in the Kir4.1 channel that blocks outward currents with extended depolarization. Similarly a “biphasic” block was found in Kir2.1 and Kir2.2 channels (Ishihara & Yan, 2007). Previously suggested mechanisms for such biphasic block include (i) an interaction of spermidine (SD⁴⁺) or Mg²⁺ with SP⁴⁺, (ii) the existence of two channel subpopulations (Ishihara & Ehara; 2004; Yan & Ishihara, 2005) or (iii) two separate sites with low and high affinity for SP in a single Kir2.x channel pore (Ishihara & Yan, 2007).

The purpose of this study was to investigate a nature of “biphasic block” in Kir4.1 glial channel. Because Kir4.1 in glia functions in a complex environment we asked what is the behavior of Kir4.1 channels under physiological conditions, and could Na⁺ (which accumulates to levels of ~10-40 mM in glia) be an effective modulator of these channels?

Using patch clamp and fluorescent methods to test sodium intracellular concentration with Na-dyes, we investigated the behavior of Kir4.1 rectification under different physiological conditions manipulating by sodium and spermine concentrations at different voltages. We also used fast application of glutamate to test physiological sodium entrance into astrocytes due to this glutamate-(Na)-transport.

In inside-out patches expressing Kir4.1 channels, we found that (1) Na⁺ itself blocks outward current at the low affinity site for SP; (2) The K_d for Na⁺, estimated after depolarization from -100 to 0 mV is dramatically decreased from 562 ± 226 M at 80 msecs to 34.8 ± 8.5 mM at 1.5 secs; (3) Na⁺ also affects the high affinity site for SP: the K_d for SP is decreased from 3.5 ± 1.2 µM (in symmetrical K⁺) to 0.3 ± 1.1 µM (in physiological condition, with 147 mM Na⁺ and 3 mM K⁺ in the pipette and 150 mM K⁺ on the cytoplasmic side); (4) The biphasic nature of Kir4.1 rectification is apparent only with Na⁺ present. Because the K_d for Na is shifted by SP and the affinity for SP is shifted by Na⁺; (5) the interaction of Na⁺ and SP⁴⁺ enhances Kir4.1 channel rectification and (6) prevents SP permeation. When (7) glial cells were perfused with glutamate 0.1-1 mM, a block of Kir4.1 was dramatically increased with enhancement of biphasic type of rectification. This was due to Na-entrance into glial cells. The effect was completely blocked by TBOA, a blocker of Na-dependent glutamate transporter.

We concluded: The interaction of multiple blockers within the Kir4.1 channel pore reveals a hitherto unappreciated complexity to the rectification exhibited by this critical glial channel. Physiological relevance of this study is to highlight an importance and influence of glutamate transporter on Kir4.1 block.
L-DOPA accumulation in astrocytic endfeet surrounding blood vessels.

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Abstract: Astrocytes, glial cells that comprise the gliovascular interface, are an important part of the brain vascular system. Astrocytic endfeet surround blood vessels, forming a second barrier around endothelial cells. A basal lamina gel separates the astrocytic endfeet and endothelium cells layers; however, this does not prevent passage of macromolecules. Astrocytes possess a transport system that plays a role in the delivery of therapeutic drugs for Parkinson’s disease and hypothyroidism. For example, tyrosine and L-DOPA are taken up from the circulating blood by an amino acid transport system that consists of a complex of the L-type amino acid transporter 1 (LAT1) and 4F2 heavy chain (4F2hc). This transporter was found and characterized in cultured astrocytes (Tsai, Lee, 1996; Kim et al., 2004); however, there is no previous evidence of the presence of this transporter in brain astrocytes. On the other hand, it has been shown that L-DOPA and its products are accumulated in brain capillary endothelium and attached pericytes after systemic administration of L-DOPA (Bertlee et al., 1966; Wade, Katzman, 1975; Hardebo et al., 1980). The PURPOSE of the present study was to test astrocytic transport system for monoamines using a combination of patch-clamp and biochemical procedures, together with confocal microscopy.

We used (i) Falck-Hillarp method together with (ii) confocal to study uptake of the monoamine fluorescent analog ASP+ (4-[4-(dimethylamino)-styryl]-N-methylpyridinium]) that permeates glial membranes preferentially without considerable uptake in non-dopaminergic neurons. In addition, (iii) using voltage clamp, astrocytes in brain slices were tested for an electrogenic transporter current elicited by monoamines (norepinephrine, dopamine, ASP+ and other monoamines). Finally, we used histochemical methods to study monoamine oxidase (MAO) content in brain areas.

We found that L-DOPA is accumulated mainly in astrocytic endfeet surrounding blood vessels and in pericytes. Our data are supported by the following: 1) Accumulation of a fluorescent monoamine analog ASP+ is colocalized with L-DOPA fluorescence (as revealed by the Falck-Hillarp method in astrocytes in brain slices). 2) Polyamine uptake known to be specific for astrocytes and pericytes was also colocalized with ASP+ and L-DOPA uptake in astrocytes and pericytes in adult brain (Inyushin et al., 2009, SFN Abstract 138.7/C74). L-DOPA uptake was localized in glial somata and in endfeet attached to blood vessels. 3) Astrocytes were characterized electrophysiologically before the uptake of ASP+, and then fluorescence uptake was demonstrated in the same cells. 4) Using voltage clamp, astrocytes in brain slices were shown to have an electrogenic transporter current elicited by monoamines (norepinephrine, dopamine and ASP+), but not by L-DOPA, suggesting a different pathway for monoamines and L-DOPA. 5) The pattern of MAO (type B) allocation in pericytes and astrocytic endfeet was similar to that of L-DOPA accumulation.

We summarized that astrocytes (i) control L-DOPA uptake and metabolism via this gliovascular interface and, therefore, may play a key role in regulating brain dopamine. (ii) These data also suggest that different transporter mechanisms may exist for monoamines and L-DOPA.