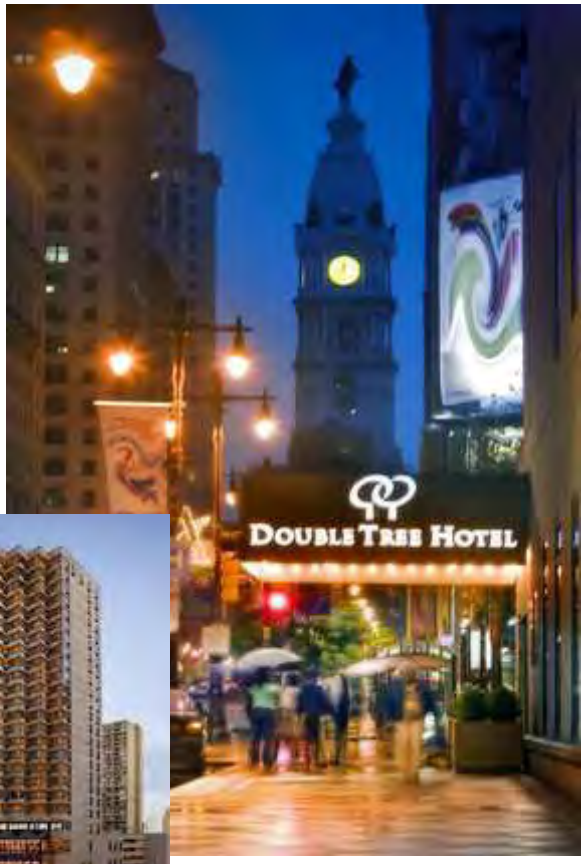
USA-JAPAN joint meeting for Glial Research

March 17th-20th 2008



The organizers gratefully acknowledge the financial support of our sponsors

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US-JAPAN BRAIN RESEARCH COLLABORATIVE PROGRAM (BRCP)

The governments of the USA and Japan provide funds through a bi-national agreement to support small meetings between scientists of each nation. In addition funds are also available to support binational collaborations. The announcement can be found at http://grants.nih.gov/grants/guide/notice-files/NOT-NS-03-024.html

The US-JAPAN BRAIN RESEARCH COLLABORATIVE PROGRAM (BRCP) supports:

1) Research collaboration (funding of this activity is not a research award, but is intended to cover travel expenses and living costs of the US scientists during their research visit to the Japanese collaborators' laboratories (up to three months);

2) Scientific training for junior and senior US scientists in Japanese laboratories (up to three months); and

3) Meetings or workshops held in the US, which are organized and attended by collaborators and trainees under the BRCP.

Our goals in holding this meeting are manifold: First and foremost is the opportunity to exchange information between the groups of scientists in each country. Second, to identify critical issues in the field requiring our joint attention and finally, to foster new collaborations.

We hope that you enjoy the meeting and find both cultural and scientific rewards from getting together in Philadelphia.

Best regards from the organizers

Philip Haydon,
Yoshihisa Kudo
Harold Sontheimer
Kazuhide Inoue

Wednesday March 19th: Dinner will be on the decks of the Moshulu.

Since the launching of the Moshulu (pronounced Mo-shoe'-loo) in 1904, she has had a long and exciting career on the seas working the ports of Europe, South America, Australia, America and Africa. She was confiscated by the Americans in one war and by the Germans in the next. She has traveled around Cape Horn 54 times. She has hauled coal and coke, copper ore and nitrate, lumber and grain. In lesser days, she has served as a floating warehouse. In grander days, she won the last great grain race in 1939. Today, the Moshulu is the largest four-masted sailing ship in the world still afloat.

The Moshulu was purchased in 1968 in Naantale, Finland for restoration and conversion into a restaurant. In the fall of 1974, she was towed to Philadelphia and opened as a restaurant on Philadelphia's Penn's Landing in 1975 until she was damaged by fire in 1989. In 1994 the Moshulu was purchased by HMS Ventures, Inc and restored in the style of a turn-of-the-century luxury liner. The Moshulu was re-christened by Philadelphia Mayor Ed Rendell on July 24, 1996. In 2002, Martin Grims, owner of Passarelle and many other restaurants, took over the operation of the Moshulu.

2003: The new Moshulu Restaurant opens under the direction of operator Marty Grims. Great food and service are the trademarks of Grims who presents a South Seas flair in this dining and entertainment adventure.

2003: The Moshulu gains prestigious "Three Bells" from Inquirer Food Critic Craig LaBan. He said that the Moshulu offers “a rare harmony of first-class food and service with stunning views and ambiance.”

US Japan Meeting Program

All meeting and breaks will be held in the Orchestra Room, located on the 2nd floor

Monday March 17, 2008

12:00-1:15	**Lunch – Academy Café, 2nd floor**
1:20-1:30	*opening comments by Phil Haydon and Yoshihisa Kudo*
Session I	**Astrocytes and Synapses I**
1:30-2:00	Hiroshi Kato – Yamagata University, Japan Mutual interactions between perineuronal astrocytes and interneurons in stratum radiatum of rat hippocampus
2:00-2:30	Ken McCarthy – University of NC, Chapel Hill, USA GPCR-mediated increases in astrocytic calcium are not sufficient to stimulate glutamate release in situ
2:30-3:00	Yoshihisa Kudo – Tokyo University of Pharmacy & Life Sciences, Japan Roles of astrocytes on synaptic plasticity
3:00-3:20	coffee break – Orchestra Room
Session II	**Astrocytes and Synapses II**
3:20-3:45	Takayuki Suzuki – Tokyo University of Pharmacy & Life Sciences, Japan A plateau potential generated by the activation of extrasynaptic NMDA receptors in rat hippocampal CA1 pyramidal neurons
3:45-4:10	Taiko Imura – Jikei University, Japan Presynaptic P2X receptors as an interface between glia and neurons
4:10-4:35	Miho Terunuma – University of Pennsylvania, USA Characterization of astrocytic $GABA_B$ receptors and their functional modulation by purino receptors
4:35-5:05	Stephen Traynelis – Emory University, USA Control of synaptic NMDA receptor function by astrocytic serine protease receptors
	GENERAL DISCUSSION

Evening Activities 6:30pm – 9:30pm
Welcome to Philly

Lucky Strike
Located at 1336 Chestnut Street
Food, drinks, bowling and karaoke

Tuesday March 18, 2008

7:45 – 9:00	Continental Breakfast – Orchard Room
Session III	**Transmitter release and uptake**
	Masami Takahashi – Kitasato University, Japan
9:00-9:30	Roles of Protein Phosphorylation in Neuronal and Glial Functions
	Vladimir Parpura – University of Alabama-Birmingham, USA
9:30-10	Spatio-Temporal characteristics of exocytosis in astrocytes
	Kaoru Sato – National Institute of Health Science, Japan
10-10:30	Estrogens inhibit L-glutamate uptake by astrocytes by membrane estrogen receptor alpha
	Hiroko Baba – Tokyo University of Pharmacy & Life Science, Japan
10:30-10:50	Modulation of axonal functions by glial cells
10:50-11:05	Coffee break – Orchard Room
Session IV	**Astrocytes Circadian Rhythms Sleep**
	F. Rob Jackson – Tufts University, USA
11:05-11:35	Glia are critical elements of the circuitry regulation circadian activity rhythms
	Phil Haydon – University of Pennsylvania, USA
11:35-12:05	Endogenous non neuronal modulators of synaptic transmission control cortical slow oscillations
	Joowon Suh – Tufts University, USA
12:05-12:25	Genetic interactions suggest a role for dopamine metabolism in the ebony circadian phenotype
	Michael Halassa – University of Pennsylvania, USA
12:25-12:45	Astrocytes are essential for mammalian sleep
12:45-2:00	**LUNCH – Academy Café**
Session V	**Calcium Signaling**
	Mitsuhiro Morita – Tokyo University of Pharmacy & Life Science, Japan
2:00-2:30	Intrinsic calcium oscillation and metabotropic glutamate receptor-induced calcium
	Tycho Hoogland – Princeton University, USA
2:30-3:00	Network activity of Bergmann glial cells in anesthetized and mobile rodents.
	Michael Szulczewski – Prairie Technologies, USA
3:00-3:25	New Developments for Laser Microscopy Imaging for Neuroscience
3:25-3:45	Coffee break – Orchard Room
Session VI	**Glia and disorders of the nervous system I**
	Hajime Hirase – RIKEN-BSI, Japan
3:45-4:15	Impact of S100B on neuronal activities in anesthetized and kainic acid induced seizure conditions *in vivo*
	Yasushi Enokido – Tokyo Medical and Dental University, Japan
4:15-4:45	Disruption of amino acid metabolism in astrocyte and neuropsychiatric disorders.
	Douglas Coulter – Children's Hospital of Philadelphia, USA
4:45-5:15	Epilepsy-Induced Reduction in Glutamate-Glutamine Cycle Efficacy Depletes Vesicular Release of GABA from Hippocampal Inhibitory Synapses
	GENERAL DISCUSSION

Evening Activities – Dinner at Doubletree (Academy Café) starts 6:30pm

Wednesday March 19, 2008

7:45 – 9:00	Continental Breakfast – Orchard Room
Session VII	**Functional roles of Astrocytes I**
	Shigeo Okabe – University of Tokyo, Japan
9:00-9:30	Formation of excitatory synapses and astrocytic contacts
	Maiken Nedergaard – University of Rochester, USA
9:30-10	A Central Role of Connexins in Astrocytes
	Yoshihiko Yamazaki – Yamagata University, Japan
10-10:30	Properties of oligodendrocytes and its modulatory effects on conduction of action potentials in alveus of rat hippocampus
10:30-10:50	coffee break – Orchard Room
Session VIII	**Functional roles of Astrocytes II**
	Eric Newman – University of Minnesota, USA
10:50-11:20	Conversations Between Glia, Neurons and Blood Vessels in the Retina
	Erlend Nagelhus - Center for Molecular Biology(Norway)/University of Rochester, USA
11:20-11:50	Mislocalization of aquaporin-4 water channels does not have a major impact on synaptic transmission in mouse hippocampal slices
	Anusha Mishra – University of Minnesota, USA
11:50-12:15	Nitric Oxide and Oxygen Modulate Neurovascular Coupling
12:15-2:00	**LUNCH – Academy Café**
Session IX	**Glia and disorders of the nervous system II**
	Ajay Verma – Merck & Co, Inc., USA
2:00-2:30	
	Takahiro Takano – University of Rochester, USA
2:30-3:00	Redox state of neurons and glia during cortical spreading depression
	Mark Forman – Merck & Co, Inc., USA
3:00-3:30	Astrocytes and the pathogenesis of tau-based neurodegenerative disease
3:30-3:50	coffee break – Orchard Room
Session X	**Microglia I**
	Izumi Hide – Hiroshima University, Japan
3:50-4:20	α7 nicotinic acetylcholinergic receptor signaling and modulation of cytokine production in microglia.
	Schuichi Koizumi – University of Yamanashi, Japan
4:20-4:50	Microglial phagocytosis mediated by the extracellular nucleotide UDP
	Keiko Ohsawa – National Institute of Neuroscience, Japan
4:50-5:20	Molecular mechanisms of ATP-induced microglial chemotaxis
	GENERAL DISCUSSION

Evening Activities: Coach pickup at 6:30pm; Meet in Front Lobby
Dinner at Moshulu Penn's Landing
401 S. Columbus Blvd
Transportation Provided

Thursday March 20, 2008

7:45 – 9:00	Continental Breakfast – Orchard Room
Session XI	**Glia and disorders of the nervous system III**
9:00-9:30	Kohichi Tanaka – Tokyo Medical and Dental University, Japan The role of glial glutamate transporters in the pathophysiology of major mental illnesses
9:30-9:55	Mika Nishimoto – National Institute of Neuroscience, Japan The functional regulatory mechanism in astrocytes via G-protein coupled receptor systems
9:55-10:20	Michelle Olson – University of Alabama-Birmingham, USA Kir4.1 in spinal cord astrocytes
10:20-10:40	coffee break – Orchard Room
Session XII	**Glia and disorders of the nervous system IV**
10:40-11:10	Keiji Wada – National Institute of Neuroscience, Japan Essential players in neuro-gliology: GPCRs and deubiquitinating enzymes
11:10-11:40	Makoto Tsuda – Kyushu University, Japan Role of microglial ATP receptors in neuropathic pain
11:40-12:20	Harald Sontheimer – University of Alabama-Birmingham, USA Ion channels and amino acid transporters aid the biology of glial-derived brain tumors
12:20-12:30	*wrap up*
12:45-	**Lunch – Academy Café**
	End of Program – Thank you for your participation – Safe Travels

Hiroshi Kato
Department of Neurophysiology, Yamagata University School of Medicine, Yamagata, 990-9585, Japan

Mutual interactions between perineuronal astrocytes and interneurons in the stratum radiatum of rat hippocampal CA1 region

Recently, it has been demonstrated that glial cells express a variety of ion channels and neurotransmitter receptors and can modulate the neuronal activities. We focused on interneuron (IN) / perineuronal glial cell pairs in the rat hippocampus, because of their close apposition of these cells, to have direct evidence for mutual interactions between them. Based on the size of cell body under the differential interference microscope and electrophysiological properties, INs and perineuronal glial cells could be easily identified. Furthermore, perineuronal glial cells were roughly classified into two types, astrocyte and oligodendrocyte, according to the resting membrane potential and input resistance. In this presentation, I focus on perineuronal astrocytes (PNACs), all of which were confirmed by morphological and immuno-histochemical studies. The mutual interactions of IN and PNAC were investigated by dual whole cell recordings from IN and PNAC in sliced hippocampal preparations.

Direct depolarization of PNAC suppressed the excitatory postsynaptic currents in IN and decreased the paired-pulse ratio, indicating that PNAC has a suppressive effect on presynaptic elements. Moreover, depolarization of PNAC modulated the directory activated firing pattern of IN, with initial facilitation and subsequent suppression. Direct firing of IN, in tern, depolarized the membrane potential and reduced the input resistance of PNAC. These results indicate the existence of bidirectional interactions between IN and PNAC, playing some roles of modulation of hippocampal neuronal information processing.

Ken McCarthy
Department of Pharmacology
University of North Carolina at Chapel Hill
Chapel Hill, NC

GPCR-mediated increases in astrocytic calcium are not sufficient to stimulate glutamate release in situ

There is absolutely no doubt that pharmacological stimuli that lead to increases in astrocytic calcium lead to glutamate release *in vitro* and *in situ.* Pharmacological stimuli that have been used to increase astrocytic calcium and glutamate release *in situ* include mechanical stimulation with glass electrodes, uncaging of either IP3 or calcium, and the stimulation of Gq-coupled GPCRs assumed to be restricted to astrocytes. Neither mechanical stimulation nor uncaging experiments remotely reproduce the type of calcium finger print associated with GPCR mediated increases in astrocytic *in situ*. While certain Gq-coupled GPCRs appear to exert their effects primarily through astrocytic receptors, it is very difficult to rule out the possibility that the effects of activating these receptors are not due to direct effects on neurons. We have taken a different approach to selectively activating astrocytic signaling cascades *in situ*. This approach is based on the availability of genetically engineered mice that either express unique Gq-coupled GPCRs in astrocytes or contain a knockout of the IP3 receptor responsible for calcium mobilization in hippocampal astrocytes. Surprisingly, data obtained using these genetically modified mouse lines suggest that increases in astrocytic calcium do not lead to glutamate release *in situ* and that mice lacking GPCR-mediated changes in astrocytic calcium are phenotypically normal. Overall, our findings suggest that while it is possible to make astrocytes release glutamate *in situ*, this may not occur when activating Gq-coupled GPCRs restricted to astrocytes.

Yoshihisa Kudo
School of Life Sciences, Tokyo University of Pharmacy and Life Sciences,
1432-1 Horinouchi, Hachioji, Tokyo 192-0392, Japan

Roles of astrocytes on neuronal plasticity

Long term potentiation (LTP) found in hippocampal synapses has been paid attention as an important model for synaptic plasticity and thus memory consolidation. Since the Ca2+ dependent mechanisms on its establishment have been demonstrated, the hypothesis has been examined by electrophysiological methods and Ca2+ imaging. Now the Ca2+ entry into the neuronal cells through the activation of NMDA receptor has been recognized as the most plausible mechanisms as the first step for synaptic plasticity. We established our first Ca2+ imaging system for neuronal cells as early as 1985 and applied the methods on hippocampal slice preparation to examine the Ca2+-dependent mechanisms for LTP. We found the long lasting increase in the [Ca2+] i at the synaptic region of the slice by the tetanic stimulation to cause LTP. Although we believed that the increase might come from the neuronal cells, one of our collaborators showed that the neuronal cells never showed such long lasting increase in [Ca2+] i by the stimulation. Subsequently we encountered the dynamic Ca2+ responses in astrocytes to t-ACPD, an agonist for metabotropic glutamate receptor, but not to NMDA. Then we examined the effects of t-ACPD on hippocampal slice preparations and found the specific responses on the site close to the pyramidal cell layer. We confirmed the increase in [Ca2+] i by the tetanic stimulation in a single astrocyte in hippocampal slice loaded with a Ca2+ indicator (fluo-4/free form). The results indicated that the tetanic stimulation to cause LTP would induce the increase in [Ca2+] i of astrocytes locating in vicinity of the neural pathway through the activation of metabotropic receptors. Using slice culture preparations stained by fluo-4/AM we found the co-activation of neuronal cells during activation of astrocytes by metabotropic receptor agonists which had no direct effects on neuronal cells. Those results demonstrated possible involvement of neuron-glia mutual informational interaction in the establishment of LTP.

Takayuki Suzuki
Lab. Cellular Neurobiology
Tokyo Univ. Pharmacy & Life Sciences
1432-1 Horinouchi, Hachioji, Tokyo 192-0392

Hippocampal pyramidal neurons express various extrasynaptic glutamate receptors.

It has been reported that these receptors can be activated by glutamate spillover from synaptic cleft and the glutamate released from astrocytes. To investigate the effect of activation of extrasynaptic receptors, we recorded changes in membrane potentials of CA1 pyramidal neurons induced by strong synaptic stimulation or iontophoretic application of glutamate. When glutamate spillover was facilitated by blocking glutamate uptake, repetitive stimulation of Schaffer collaterals evoked a persistent membrane depolarization that consisted of an early Ca2+-independent component and a late Ca2+-dependent component. The early component, which we refer to as a plateau potential, was accompanied by an increase in membrane conductance. The I-V relationship of the membrane conductance showed the same property as that of the NMDA currents reported previously. The plateau potential was suppressed by NMDA receptor antagonists. After blocking synaptically located NMDA receptors using MK801, an open channel blocker for NMDA receptors, and the plateau potential was still generated synaptically when spillover was facilitated. A plateau potential was also evoked by iontophoretic application of glutamate in the presence or absence of a glutamate uptake blocker. This potential was not affected by Na+ or Ca2+ channel blockers, but was suppressed by an NMDA receptor antagonist. The I-V relationship of the current during this potential was similar to that obtained during the synaptically induced plateau potential. These results show that CA1 pyramidal neurons generate plateau potentials mediated by activation of extrasynaptic NMDA receptors. It is conceivable that this depolarization potential connects the action of astrocytes to that of neurons.

Taiko Imura, Fusao Kato
Lab Neurophysiology, Department of Neuroscience,
Jikei Univ. School of Medicine, Tokyo, Japan

Presynaptic P2X receptors as an interface between glia and neurons

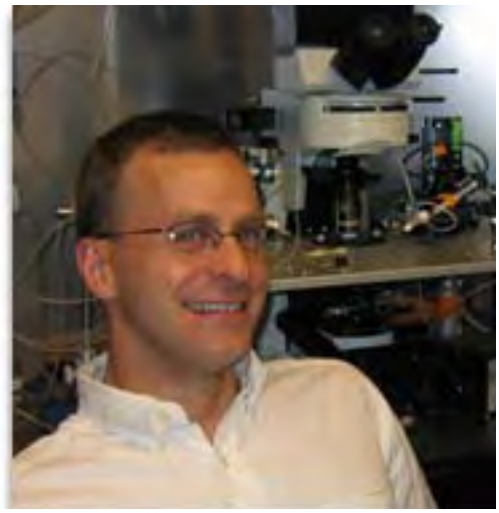
The physiological significance of the ATP-gated receptor channels (P2X receptors) remains undetermined despite their abundant expression in the central nervous system. The high Ca2+ permeability of P2X receptors makes it likely to be a presynaptic source of Ca^{2+} entry that trigger exocytosis. For example, in the nucleus of the solitary tract (NTS) of the brainstem, bath application of P2X receptor agonists (Shigetomi and Kato, 2004) or space- and time-delimited ATP application with laser photoactivation of caged ATP (Imura et al., 2007) facilitates action potential-independent glutamate release, leading to a postsynaptic excitation. Accumulating evidence demonstrates that ATP of glial origin modulates synaptic transmission, rendering it one of the “gliotransmitters”. Here we examined a possibility that ATP released from astrocyte processes surrounding excitatory synapses could activate these presynaptic P2X receptors in the NTS. Electron-microscopic observation revealed that 99% of presynaptic terminals of excitatory synapses had partial or full contact with astrocyte processes, most of which expressed GLT1 or GLAST. Pharmacological activation of P2Y1 receptors, which are abundantly expressed in astrocytes, in the acute brain slices of the young rats increased the frequency of miniature excitatory postsynaptic currents (mEPSCs) in a manner sensitive to P2Y1 receptor antagonist. Interestingly, this increase was almost completely suppressed also by P2X receptor antagonist, indicating that this P2Y1 receptor-mediated release facilitation involves P2X receptor activation. Moreover, the increase in mEPSC frequency was attenuated in the presence of fluoroacetate, an agent inhibiting the glial TCA cycle, suggesting an essential role of astrocytes in this facilitation. Taken together, these data indicate that presynaptic P2X receptors in the NTS could function as an interface between astrocytes and neurons through intermediary of ATP release, thus enabling glial control of neuronal excitability in response to various humoral influences that might activate gap junction-linked astrocyte network.

Miho Terunuma, Philip G. Haydon and Stephen J. Moss.
Department of Neuroscience
University of PA

Characterization of astrocytic $GABA_B$ receptors and their functional modulation by purino receptors

While a role for glutamate in gliotransmission has emerged, the significance of γ-aminobutyric acid (GABA), the major inhibitory neurotransmitter in the brain, is less well defined. To address this issue, we have begun to assess the roles that $GABA_B$ receptors play in communication between neurons and glia. $GABA_B$ receptors are heterodimeric G-protein coupled receptors that mediate slow and prolonged inhibitory signals in the brain. We know that modified $GABA_B$ receptor signaling plays a critical role in the etiology of schizophrenia, depression, anxiety, cognitive deficits, epilepsy, nociception, and mental retardation. However, the relative roles that astrocytic and neuronal populations play in these phenotypes remains to be established.

To begin to address this issue we have characterized the structure and functional properties of astrocytic $GABA_B$ receptors. Using biochemical and immunological methods we reveal that cell surface astrocytic $GABA_B$ receptors are heterodimers composed of R1 and R2 subunits similar to their neuronal counterparts. Using imaging with Fluo-4 derivatives we show that astrocytic $GABA_B$ receptors are able to enhance intracellular accumulation of Ca^{+2}, but only after the pre-activation of purino-receptors. In addition purino-receptors enhance the phosphorylation of astrocytic $GABA_B$ receptors on Serine 783 in the R2 subunit dependent upon the activity of AMP-dependent protein kinase (AMPK). Critically we have previously illustrated that S783 is important in regulating the effector coupling of neuronal $GABA_B$ receptors. Therefore our studies suggest that $GABA_B$ effector coupling in astrocytes is dependent upon prior activation of purino receptors in a mechanism dependent upon the activation of AMPK, and the subsequent phosphorylation of the R2 subunit. We are currently analyzing the significance of this functional cross talk in regulating gliotransmission and animal behavior.



Stephen F Traynelis
Department of Pharmacology
Emory University
Atlanta, GA USA

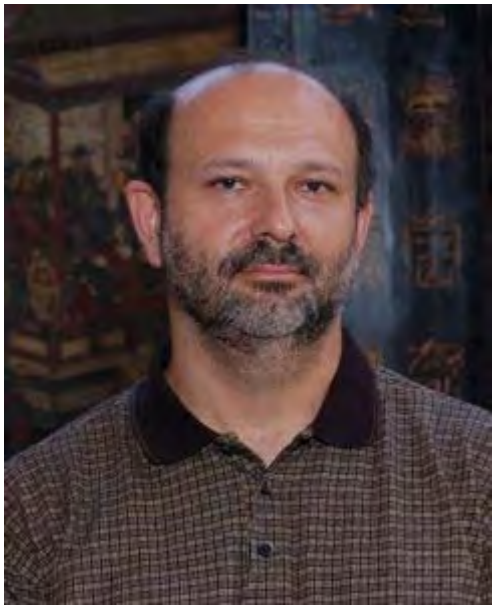
Control of synaptic NMDA receptor function by astrocytic serine protease receptors

Astrocytes have long been known to provide numerous supportive functions for neurons. Over the last decade it has become clear that astrocytes additionally communicate with neurons by a variety of different mechanisms, including their response to neuroactive substances that activate G-protein coupled receptors. More recently, it has been proposed that astrocytes can reciprocally control neuronal signaling by mechanisms that are currently under investigation. One important manner by which astrocytes can impact neuronal function is through the control of excitatory synaptic signaling. Astrocyte uptake systems terminate the signal mediated by synaptic release of glutamate. Furthermore, astrocytes themselves can release glutamate in response to receptor stimulation, raising the possibility that they can engage metabotropic and ionotropic glutamate receptor signaling on neurons to modify excitability and function. We have studied the role of protease activated receptor 1 (PAR1) in astrocyte signaling. PAR1 is a G-protein coupled receptor that responds to protease (thrombin, plasmin, Factor Xa) cleavage of its N-terminal at Arg41 with activation of at least three different classes of G-proteins: Gαi/o, Gαq/11, Gα12/13. PAR1 is primarily expressed on astrocytes, and activation of astrocytic PAR1 leads to the release of glutamate onto neurons. Our data suggest that this release of glutamate is capable of activating NMDA receptors on neurons, which depolarizes the postsynaptic membrane and potentiates synaptic NMDA receptor responses secondary to relief of voltage-dependent Mg^{2+} blockade. This enhancement of the NMDA component of excitatory synaptic transmission appears to shift the stimulus-response curve for activity-dependent synaptic plasticity, which may suggest a role in learning and memory. Consistent with this idea, PAR1 removal in vivo can impair two forms of emotional learning. We are currently studying the mechanisms of glutamate release and selective removal of PAR1 from astrocytes to further explore the physiological implications of this signaling pathway.

Masami Takahashi
Department of Biochemistry, Kitasato University School of Medicine, Sagamihara, Kanagawa 228-8555, Japan

Regulation of glial functions by protein phosphorylation

Neurons communicate each other through synaptic connections, and exocytotic release of neurotransmitters plays essential role for the communication. A series of recent studies revealed that glial cells also release various bioactive substances including “gliotransmitters” and cytokines by an exocytotic mechanism, and these bioactive substances play important roles in neuronal survival, brain inflammatory response, and regulation of neuronal networks. Exocytosis involves docking and fusion of secretory vesicle membrane with plasma membrane, and so-called SNARE proteins play crucial roles in these process. Many isoforms of SNARE proteins are expressed in mammalian brain, and VAMP-2 in synaptic vesicle membrane, and syntaxin 1 and SNAP-25 in presynaptic plasma membrane function in neuronal synapses as v-SNARE and t-SNARE, respectively. A different set of SNARE proteins are involved in the exocytosis of gliotransmitters, and VAMP-2, syntaxin 4, and SNAP-23 are likely to be involved in astrocyte. In neurons, neurotransmitter release is positively regulated by protein kinase C (PKC) and the phosphorylation of SNAP-25 at $Ser^{187}$is likely to be involved in this regulation. We studied the PKC-dependent regulatory mechanisms of exocytosis in cultured rat brain astrocyte and in clonal rat glioma C6 cells. Phorbol 12-myristate 13-acetate (PMA), an activator of protein kinase C (PKC), suppressed the Ca^{2+}-evoked peptide release from astrocyte and C6 cells, and this suppression was reversed by a PKC inhibitor, bisindolylmaleimide I (BIS). PMA-treatment caused a mobility shift of SNAP-23 in SDS-PAGE, and this change was also abolished by BIS. All of these results suggested that PKC regulate exocytotic release of transmitters in opposite directions in neurons and glial cells.



Vladimir Parpura
Department of Neurobiology, Center for Glial Biology in Medicine, Atomic Force Microscopy & Nanotechnology Laboratories, Civitan International Research Center, Evelyn F. McKnight Brain Institute, University of Alabama, Birmingham, AL USA

SPATIO-TEMPORAL CHARACTERISTICS OF EXOCYTOSIS IN ASTROCYTES

The mechanism underlying Ca^{2+}-dependent release of various transmitters from astrocytes is exocytosis. Astrocytes express the protein components of the SNARE complex, including synaptobrevin 2, syntaxin and SNAP-23, but not SNAP-25. Using astrocytes expressing synapto-pHluorin, exocytotic sites can be fluorescently imaged. Solitary astrocytes predominantly exhibit exocytotic fusion sites at the plasma membrane in the perinuclear region of the astrocytes, while astrocytes in contact with other cells show their fusion sites evenly distributed between the central and peripheral (location of cell-cell contact) regions; this contact-directed distribution of fusion sites is regulated by the gap junction protein, connexin 43. Fusions of synapto-pHluorin labeled vesicles with the plasma membrane can be observed using total internal reflection fluorescence microscopy; the time course of fusion events (burst vs. sustained), their type ("kiss-and-run" vs. full fusion) and spatial relationship between different fusion sites is discussed. The spatio-temporal characteristics of exocytosis might be in part due to intrinsic properties of the ternary SNARE complex in astrocytes.

Kaoru Sato1*, Norio Matsuki2, Ken Nakazawa1
1. Div. Pharmacol., Natl. Inst. Hlth. Sci.,
2. Lab. Chemical Pharmacol., Grad. Sch. Pharmaceut. Sci., Univ. Tokyo

Estrogens inhibit L-glutamate uptake by astrocytes by membrane estrogen receptor alpha.

Recently we discovered that estrogens inhibited L-glu uptake by cultured astrocytes via membrane ERα (mERα). Here we show further evidences indicating that the effect is mediated by mERα and the signal transduction pathway mediating the effect. Western blotting of biotinylated membrane proteins showed the distribution of ERα on the plasma membrane. ERα-specific antibody completely inhibited the effects of β-estradiol (E2) and E2 conjugated with BSA (E2BSA). E2BSA increased Km of GLAST without affecting Vmax and did not cause the internalization of GLAST, suggesting the modification of GLAST activity. The effect of E2BSA was significantly inhibited by LY294002, wortmannin, Akt inhibitor, LNA and LNAME. Western blotting of phosphorylated Akt and quantification of NO indicated the existence of the following signal transduction pathway: mERα→PI3K activation→Akt activation→NO generation→down-regulation of GLAST.

Hiroko Baba
Tokyo University of Pharmacy and Life Sciences, Japan

Axon-glia interaction on myelinated fibers.

In myelinated fibers, axons interact with surrounding glial cells, including oligodendrocytes and astrocytes in the central nervous system (CNS), and Schwann cells in the peripheral nervous system (PNS). Sulfatide is one of the major glycolipids in myelin. To know the roles of sulfatide, we investigated the cerebroside sulfotransferase (CST) knockout mice, in which this glycolipid is specifically eliminated. During development, the numbers of oligodendrocyte-lineage cells were significantly increased in the CST knockout optic nerves compared with wild type. These differences were continuously observed in the adult animals, suggesting that this glycolipid has some role on regulation of oligodendrocyte-lineage cell numbers. In myelin, compact myelin was normally formed but paranodal axo-glial junctions were disappeared. Specific localizations of voltage-gated ion channels on axons were progressively disorganized especially in the CNS, and at the same time, the changes of white matter astrocytes were observed. In the PNS, Schmidt-Lanterman incisures (SLI), which are thought to act as highways for transportation of small molecules between periaxonal myelin membranes to Schwann cell bodies, increased their numbers, and Kv channel clusters were formed on the apposed axonal surface. In addition, the axonal shapes at the PNS nodes were deformed by accumulation of enlarged mitochondria. Thus, loss of sulfatide causes structural and functional changes of axons and surrounding other cells in addition to myelin, probably by influencing the interactions between myelin or myelin forming cells and other cell types.

F. Rob Jackson, Joowon Suh and Fanny Ng
Department of Neuroscience and Center for Neuroscience Research
Tufts University School of Medicine, Boston, MA USA

GLIA ARE CRITICAL ELEMENTS OF THE CIRCUITRY REGULATING CIRCADIAN ACTIVITY RHYTHMS

Our recent work has highlighted the role of ebony, a glia-specific factor, in the regulation of Drosophila circadian behavior (Suh and Jackson, Neuron 55, 2007). To extend that work, we are currently employing sophisticated genetic techniques to perturb specific physiological activities in defined classes of glia with the goal of defining the glial populations and processes that are essential for circadian behavior. This work is making use of methods that permit conditional perturbations of physiology during adulthood in a glia-specific manner. A variety of tools are available in this model genetic system which permit a temperature-dependent and/or developmental stage-specific activation or inhibition of exocytosis, glia cell membrane potential or calcium signaling. In initial experiments, for example, we have disrupted exocytosis in all lateral glia (some of which contain ebony) while monitoring adult behavior. Those studies reveal an obvious role for glia in the ongoing modulation of circadian behavior – the conditional, temperature-dependent perturbation of glial exocytosis for 6 h, for example, causes significant arrhythmicity in locomotor activity. We will present these and other results from recent studies that have examined glial cell activities and circadian behavior. Such studies are beginning to define physiological roles of glia in circadian and other types of behavior.

Tommaso Fellin, Michael Halassa, Miho Terunuma, Francesca Succol, Hajime Takano, Stephen J. Moss, **Philip G. Haydon**

Silvio Conte Center for Integration at the Tripartite Synapse
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Endogenous non neuronal modulators of synaptic transmission control cortical slow oscillations

Gliotransmission, the release of molecules from astrocytes, regulates neuronal excitability and synaptic transmission *in situ*. Whether this process affects neuronal network activity *in vivo* is not known. Using a combination of astrocyte-specific molecular genetics, with *in vivo* electrophysiology and pharmacology we determined that gliotransmission modulates slow oscillations, the main cortical rhythm under anesthesia. Inhibition of gliotransmission by the expression of a dominant negative SNARE domain (dnSNARE) in glia significantly decreased the power of slow oscillations, reduced the duration of neuronal depolarizations and caused prolonged hyperpolarizations. These network effects resulted from the astrocytic modulation of intracortical synaptic transmission at two sites: a hypofunction of postsynaptic NMDA receptors, and by reducing extracellular adenosine, through a loss of tonic A1 receptor-mediated inhibition. These results represent the first demonstration that neuronal rhythms are generated by the coordinated activity of neuronal and glial networks in which synaptic transmission generates the rhythm that is regulated by gliotransmission-dependent modulation.

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Genetic interactions suggest a role for dopamine metabolism in the ebony circadian phenotype

Drosophila ebony mutants exhibit arrhythmic circadian locomotor activity but normal population eclosion rhythms, suggesting a selective lesion of a clock output pathway controlling activity. Our published studies demonstrate that Ebony protein is exclusively localized in glial cells of the larval and adult nervous systems, and that glia-specific expression of Ebony protein can rescue behavior of *ebony* null mutants. The same studies showed that *ebony* mRNA and protein exhibit circadian changes in abundance, within glia, that are regulated by the *per/tim*-based molecular clock. Surprisingly, however, the Ebony molecular rhythm is not dependent on the neuronal release of PDF, the best-characterized circadian neurotransmitter, and our current studies are focused on identifying which neural cell types are relevant for glial cycling of Ebony. Immunostaining results indicate that certain Ebony-containing glia contain PER and TIM whereas others are adjacent to clock-containing neurons or glia. Thus, Ebony cycling in certain glia may be dependent on output from clock cells whereas cycling of the protein in other glia may be driven by autonomous circadian mechanisms.

The *ebony* gene encodes N-β-alanyl-biogenic amine synthetase (BAS), which conjugates β-alanine to biogenic amines including dopamine and serotonin to produce N-β-alanyl-biogenic amines (e.g., NBAD for dopamine). Consistent with a close proximity of Ebony glia to dopaminergic neurons, a genetic interaction is observed between *ebony* and *Dopamine Transporter (dDAT)* mutant alleles. Whereas *ebony* mutant have slightly reduced activity compared to wild type, *dDAT* mutants are hyperactive, similar to the phenotype of comparable mouse mutants. Remarkably, the hyperactivity of *dDAT* mutants is genetically suppressed by an *ebony* mutation, suggesting a role for NBAD in the regulation of locomotor activity. We will test this idea by directly feeding NBAD to flies and monitoring locomotor activity profiles. The *dDAT; ebony* double mutant also displays a striking circadian phenotype: the bimodality of daily activity is altered and the evening bout of activity is significantly diminished or eliminated. Using cell biological and behavioral methods, we are currently investigating if this is caused by abnormal synchrony among the communicating clock neuronal populations that govern the “morning” and “evening” bouts of activity.

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Astrocytes are essential for mammalian sleep

Adenosine is thought to play a critical role in mediating sleep homeostasis. However, the cellular source and mechanism of action of adenosine are still debated. Because our previous work has shown that extracellular adenosine is derived from astrocytes, a sub-type of glial cell, (Pascual et al, 2005) we asked whether the inhibition of accumulation of extracellular adenosine from an astrocytic source impairs sleep homeostasis. We used a tet-off line of transgenic mice in which the release of chemical transmitters from astrocytes (gliotransmission) is attenuated by overexpression of a dominant negative SNARE domain (dnSNARE), selectively and conditionally (doxycycline control) in astrocytes. dnSNARE mice (N=8) and wildtype littermate controls (N=9) (8-10 weeks of age) were implanted with EEG and EMG electrodes and acclimated to a 12:12 light-dark cycle; lights on at 6 A.M. The average vigilance states across 24hrs were similar between the two groups. Baseline recordings were acquired for 48 hrs before mice were sleep-deprived for 6hrs (starting at 6 A.M.) and allowed to recover. Following sleep deprivation, wildtype mice showed a significant sleep rebound: a decrease in wakefulness, and an increase in both nonREM sleep and REM sleep. In contrast, in comparison to baseline measurements, dnSNARE mice showed no change in wakefulness or nonREM sleep following sleep deprivation, but only a small increase in REM sleep. This impaired sleep homeostasis phenotype was fully reversed by feeding doxycycline to dnSNARE mice to inhibit transgene expression. Cortical adenosine rises following sleep deprivation implicating the cortex as a region involved in mediating the homeostatic action of adenosine. To determine whether the expression of dnSNARE in astrocytes impairs the accumulation of cortical adenosine we monitored intracortical synaptic transmission and found that astrocytic dnSNARE expression significantly reduced a tonic A1 receptor-mediated presynaptic inhibition of synaptic transmission. In conclusion, these studies indicate an important role for astrocyte-dependent gliotransmission (possibly purinergic) in sleep homeostasis.

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Metabotropic glutamate receptor-induced and intrinsic calcium oscillation in astrocyte

Intracellular calcium increase is believed as the main trigger for astrocyte functions modulating neuronal activities and vasculature. As in many other cell types, it is considered that astrocyte calcium is a multifunctional signal, and an appropriate spatial and temporal pattern of calcium increase is required to elicit a specific function. In addition to diverse calcium release patterns (transient, sustained and oscillatory) following G-protein coupled receptor activation by neurotransmitters, astrocyte is known to show intrinsic calcium oscillation. In our studies using cultured astrocytes, metabotropic glutamate receptor-induced calcium oscillation was shown to require appropriate growth factor / cytokine treatment and neurotransmitter concentration, supporting the notion that astrocyte senses environmental parameters and determine its calcium behavior and following function. This calcium oscillation was accompanied by increase of sarco-endoplasmic reticulum calcium ATPase expression and enlargement of calcium store, which were mediated by MAP kinase cascade. In our preliminary studies using acute and cultured slice preparations, a group I mGluR agonist, DHPG induced calcium increase in a limited astrocyte population lacking intrinsic calcium oscillation, whereas whole pyramidal neurons responded to DHPG even at a lower concentration which was insufficient for astrocyte. Nevertheless the same growth factor / cytokine treatment was required for DHPG-induced astrocyte calcium oscillation in slice culture, suggesting astrocyte calcium behavior and subsequent function is under the control of brain environment.

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Network activity of Bergmann glial cells in anesthetized and mobile rodents

In vivo calcium imaging of neural circuits using two-photon microscopy has heralded a new era in our understanding of the population activity of neurons and how they encode sensory information. The network behavior of the most numerous cells in the brain, glial cells, has remained elusive. I will present calcium imaging data that shows how individual and small networks of Bergmann glial cells (BGs) are active in the intact cerebellum, a brain structure involved in sensory-motor encoding. Using multi-cell bolus loading of fluorescent calcium indicators (Fluo4 and Fluo-5F/AM) in the molecular layer of the cerebellum and in vivo two-photon microscopy, signals in individual BG processes and wave-like signals that spanned multiple BGs were observed. We confirmed that such waves occurred in glial cells by injecting mice with an adenovirus carrying the sequence for the calcium-sensor protein G-CaMP2. G-CaMP2 was only expressed in BGs in these animals.

We also uncovered a mechanism underlying the initiation and propagation of the waves. Local pressure ejection of ATP triggered synchronized activity in multiple Bergmann glial cell processes comparable in shape and time course to waves that occurred spontaneously. ATP-triggered waves could be blocked with PPADS suggesting that P2Y receptors are critical for wave propagation.

Spontaneous waves initiated at the pial surface, the molecular layer and at BG somata in the Purkinje cell layer. Likewise, ATP could trigger waves at all these locations, suggesting that the molecular layer is a continuously excitable medium.

We are currently imaging cerebellar circuit activity including glial waves in awake mobile mice. Our goal is to determine whether glial waves are correlated with the behavioral state of an animal and more specifically how the onset of these waves relates to network activity in interneurons and Purkinje cells.

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New Developments for Laser Microscopy Imaging for Neuroscience

Recent advancements in microscope design to increase the sensitivity of the collection of the fluorescence emission from two photon laser excitation and the addition of multiple (uncaging and imaging) excitation paths has led to an increase in applications and experiments currently being performed using two photon laser microscopy for Neuroscience. Now the next engineering challenge is to speed up the point source point excitation laser microscope to allow the researcher to collect neuronal responses in a volume of interest at the speeds of the biology. The use of multiple AODs holds promise for allowing the use of these optical techniques for increasing the understanding neural and glial networks.

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Impact of S100B on neuronal activities in anesthetized and kainic acid induced seizure conditions *in vivo*

S100B is a calcium binding protein predominantly synthesized by astrocytes and secreted to the extracellular space. Previous studies using gene manipulated animals have suggested that the protein has a role in synaptic plasticity and learning. In order to assess the physiological roles of the protein in active neural circuitry, we have recorded spontaneous neural activities from the neocortex and hippocampus of urethane anesthetized S100B knockout (KO) and wildtype control (WT) mice. Typically occurring local field oscillation patterns including the slow (0.5-2 Hz) oscillations in the neocortex, theta (3-8 Hz) and sharp wave associated fast ripple (120-180 Hz) oscillations in the hippocampus were observed in both genotypes and appeared virtually indistinguishable. When seizure was induced by intraperitoneal injection of kainic acid, gamma (30-80 Hz) oscillation in hippocampal CA1 *str. radiatum* was significantly smaller in S100B KO mice.

To assess the contribution of extracellular S100B to the gamma oscillation, we combined local field potential recording with local infusion of dimeric S100B at CA1 *str. radiatum* in S100B KO mice *in vivo*. Infusion of S100B in S100B KO resulted in an increase of the gamma oscillation, reversing S100B knockout effect. Similarly, infusion of anti-S100B antibody in WT resulted in a decrease of the gamma oscillation. Both results indicate that the presence of extracellular S100B contributes to the increase in the amplitude of kainate-induced gamma oscillation. Next, we asked whether the effect of extracellular S100B was due to activation of S100B receptors by functionally blocking receptor for advanced glycation end products (RAGE) by a specific antibody. The amplitude of kainate-induced gamma oscillation decreased significantly by blocking RAGE in WT mice. The result suggests that activation of RAGE significantly contributes to the gamma oscillation amplitude.

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Disruption of amino acid metabolism in astrocyte and neuropsychiatric disorders

Astrocytes are the most abundant glial cells in mammalian CNS and play a crucial role for maintaining brain metabolism. Here we show the regional and cellular distribution of cystathionine β-synthase (CBS; EC 4.2.1.22), a key enzyme for homocysteine (Hcy) metabolism, in the adult and developing mouse brain. A deficiency of CBS leads to homocystinuria (MIM 236200), an inherited human disease characterized by mental retardation, seizures, psychiatric disturbances, skeletal abnormalities and vascular disorders. In the adult mouse brain, CBS was expressed ubiquitously, but most intensely in the cerebellar molecular layer and hippocampal dentate gyrus. Immunohistochemical analysis revealed that CBS is preferentially expressed in cerebellar Bergmann glia and in astrocytes throughout the brain. At early developmental stages, CBS was expressed in neuroepithelial cells in the ventricular zone, but its expression changed to radial glial cells and then to astrocytes during the late embryonic and neonatal periods. CBS was most highly expressed in juvenile brain, and a striking induction was observed in cultured astrocytes in response to some growth factor stimuli. Moreover, CBS was significantly accumulated in reactive astrocytes after kainic acid-induced seizures, and brain mal-formation was also observed in CBS-deficient mice. These results suggest that CBS plays an important role for the development and maintenance of the CNS, and that radial glia/astrocyte may be a key target for understanding the complex neuropathogenesis associated with abnormal brain metabolism.

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Epilepsy-Induced Reduction in Glutamate-Glutamine Cycle Efficacy Depletes Vesicular Release of GABA from Hippocampal Inhibitory Synapses

Recent studies have demonstrated that glutamate-glutamine cycle function is severely compromised in seizure foci of patients with temporal lobe epilepsy. In addition to limiting glutamate excitotoxicity, the astrocytic glutamate-glutamine cycle provides the majority of synaptic GABA released during sustained activity. Inhibitory synaptic efficacy is critical in maintaining the excitatory/inhibitory balance in brain, which, when disrupted, can cause seizures and epilepsy. Here we show that inhibition is compromised in the hippocampus of epileptic animals, due to reduced quantal release of GABA. This compromised release is mediated by attenuation in glutamate-glutamine cycle contribution to GABA synthesis. In epileptic animals, inhibitory synapses upregulate use of alternate amino acids for GABA synthesis. However, these alternate substrates are inadequate to maintain inhibitory tone. Provision of exogenous glutamine restores normal synaptic GABA release in epileptic brain. These findings provide insight into pathogenic mechanisms of epilepsy, and suggest novel therapeutic strategies for amelioration of this disorder. Supported by grants from NINDS and NIMH to DAC.

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Regulation of synapse development by astrocytic contacts

Several lines of evidences indicate roles of astroglia in synaptogenesis, possibly mediated by either cell adhesion or diffusible factors. However, structural evidences supporting this claim are virtually lacking, mainly due to technical limitations in simultaneous imaging of neuronal and astroglial structures. We visualized astroglia and pyramidal neurons in hippocampal slice cultures by combining adenovirus-mediated, Cre-dependent expression of GFP with electroporation of rhodamine-dextran. Two-photon time-lapse imaging of immature dendritic protrusions and astroglial processes revealed longer lifetime of dendritic protrusions having experienced astroglial contacts than those without contacts. Dendritic protrusions with astroglial contacts also showed higher tendency to form spines. Inhibition of astroglial motility and interference of ephrin-Eph signaling affected normal stabilization and maturation of spines. These findings suggest an involvement of direct astroglia-filopodia contacts in subsequent maturation of dendritic spines.

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A Central Role of Connexins in Astrocytes

Over the past few years, a virtual revolution has occurred in our understanding of the cell biology and physiology of astrocytes, and in our understanding of their interactions with neurons and the vasculature. Astrocytes are electrically non-excitable cells, which communicate predominantly by P2Y receptor mediated calcium signaling. Astrocytes release ATP through connexin-hemichannels, and possible other pathways, highlighting the key role of connexins in non-synaptic signaling in brain.

Connexin-expression also plays important roles in the adhesive properties of astrocytes, as well as their responses to ischemia and traumatic brain injury. The complex roles of gap junctions and connexin hemichannels in protoplasmic astrocytes will be discussed focusing on in vivo imaging
approaches using 2-photon laser scanning microscopy.

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Properties of oligodendrocytes and its modulatory effects on conduction of action potentials in alveus of rat hippocampus

Oligodendrocytes, a subtype of glial cells in the central nervous system, are myelin forming cells, and enable rapid salutatory conduction of action potentials. Like neurons and astrocytes, oligodendrocytes have a variety of neurotransmitter receptors and ion channels. However, except for facilitating the rapid conduction of action potentials by myelin formation and extracellular K^+ buffering along axons, little is known about the direct involvement of these cells in neuronal activities. To investigate their physiological roles, we focused on oligodendrocytes in the alveus of the rat hippocampal CA1 region. As a first step, we attempted to identify and characterize the oligodendrocytes in this region. For this purpose, we performed whole-cell recordings from oligodendrocytes, and examined electrophysiological and morphological properties of the recorded cells. These cells were found to respond by depolarization to exogenously applied glutamate through *N*-methyl-D-aspartate (NMDA) receptors and non-NMDA receptors. Electrical stimulation of the border between the alveus and stratum oriens evoked inward currents through various routes involving glutamate receptors and inward rectifier potassium channels. Moreover, electrical stimulation resembling in vivo activity evoked long-lasting depolarization. To examine the modulatory effects of oligodendrocytes on neuronal activities, we performed dual whole-cell recording on CA1 pyramidal neurons and oligodendrocytes. Direct depolarization of oligodendrocytes shortened the latencies of action potentials evoked by antidromic stimulation. These results suggest that oligodendrocytes play more active roles in conduction of action potentials than previously considered.

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Conversations Between Glia, Neurons and Blood Vessels in the Retina

Neuronal activity in the central nervous system elicits localized changes in blood flow, a response termed functional hyperemia. The cellular mechanisms that underlie functional hyperemia, however, are not well understood. We have investigated the role of glial cells in mediating functional hyperemia using an ex vivo preparation of the isolated rodent retina.

We investigated whether neuronal activity stimulates glial cells in the retina. We found that flickering light, which activates retinal neurons, also evokes Ca2+ increases in Müller cells, the principal glial cells of the retina. Light-evoked glial Ca2+ increases are blocked by purinergic antagonists, demonstrating that neuron to glia signaling is mediated by neuronal release of ATP.
We have also investigated two proposed mechanisms of glial control of the vasculature: glial K+ siphoning and glial induction of vasoactive arachidonic acid metabolites. In K+ siphoning, current flowing through glial cells transfers K+ released from active neurons to blood vessels. Our results suggest that glial K+ siphoning does not contribute significantly to neurovascular coupling in the retina.

Instead, our experiments indicate that glial cells mediate neurovascular coupling by inducing the production of two types of arachidonic acid metabolites, EETs and 20-HETE, which dilate and constrict vessels, respectively. We show that both light flashes and direct glial stimulation produce vasodilation or vasoconstriction mediated by EETs and 20-HETE. The type of vasomotor response observed (dilation or constriction) depends on retinal levels of nitric oxide and oxygen. Our data also demonstrate that glial cells are necessary intermediaries for signaling from neurons to blood vessels, as functional hyperemia does not occur when neuron to glia communication is interrupted. These results indicate that glial cells play an important role in mediating functional hyperemia and suggest that the regulation of blood flow may involve both vasodilating and vasoconstricting components.

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Mislocalization of aquaporin-4 water channels does not have a major impact on synaptic transmission in mouse hippocampal slices

Aquaporin-4 (AQP4) is concentrated in perivascular astrocyte endfeet together with the inwardly rectifying potassium channel Kir4.1. α-syntrophin, a member of the dystrophin associated protein complex, plays a critical role for the subcellular distribution of AQP4 and for the clearance of extracellular potassium following neuronal activation. We show by high-resolution immunogold cytochemistry that genetic inactivation of α-syntrophin reduces by 90% the expression of AQP4 in astrocyte endfoot membranes adjacent to blood vessels in stratum radiatum of the hippocampal CA1 region. However, recordings in hippocampal slices from α-syntrophin knockout and control mice did not reveal significant differences neither in CA3-to-CA1 synaptic transmission nor in synaptic excitability. Furthermore, both paired pulse facilitation and long term potentiation were of similar magnitude in the two genotypes. Similar data were obtained in AQP4 knockout mice. Conclusion: Mislocalization or loss of AQP4 fails to have a major impact on excitatory synaptic transmission in the mouse hippocampal slice. This bodes well for the usefulness of future therapies based on pharmacological targeting of AQP4.

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Nitric Oxide and Oxygen Modulate Neurovascular Coupling

Cerebral blood flow is spatially and temporally controlled to provide increased nutrients and oxygen to sites of high neuronal activity. The fundamental cellular mechanisms underlying this functional hyperemia are not well understood. Recent evidence has shown that glial cells mediate neurovascular coupling in the brain and we have demonstrated in the whole mount preparation of the rat retina, that light and glial stimulation can evoke both vasodilations and vasoconstrictions. The generation of bi-directional vasomotor activity raises the question of how such responses are controlled. We have shown that nitric oxide (NO) plays an important modulatory role in this response. Low levels of NO in the retina result in a primarily vasodilatory response while high levels result in increased vasoconstriction. We have also investigated whether oxygen (O_2) modulates neurovascular coupling, as its concentration is an important factor in many biochemical pathways. Retinas perfused with 95% O_2 predominantly display light-evoked vasoconstrictions. Lowering the oxygen level to the atmospheric concentration of 20% resulted in an increase in the frequency and magnitude of vasodilations and a decrease in the magnitude of vasoconstrictions. Furthermore, we show that this O_2 effect remains in presence of the NOS inhibitor, L-NAME, when NO levels are minimal. These data suggest that NO and O_2 may act independently to modulate the polarity and magnitude of the vasomotor response.

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Redox state of neurons and glia during cortical spreading depression

Cortical spreading depression (CSD) is a self-propagating wave of large depolarization that has been implicated in migraine and progressive neuronal injury following stroke and head trauma. Using two-photon microscopy in live mouse cortex, the propagation of CSD was visualized as a progressive wave of transient calcium flux in astrocytes. Metabolic state can be monitored by NADH intrinsic fluorescence, which, unlike the imaging in acute slices, showed no clear differences between neurons and astrocytes in baseline fluorescence. During the passage of CSD, NADH imaging revealed that the tissue consists of two distinct areas of decrease and increase of the NADH. This distinctive NADH pattern followed neither the cellular organization nor cell distribution, but outlined the vasculature. Thus CSD caused a rapid increase of energy demand to the tissue, and only the perivascular tissue received adequate oxygen supply to increase oxidative metabolism, causing the rest of the tissue hypoxic. There were no clear differences between neurons and astrocytes in NADH fluorescence behaviors. Our results suggest that during CSD both neurons and glia experience high energy demand and hypoxic states.

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Astrocytes and the pathogenesis of tau-based neurodegenerative disease

Filamentous inclusions in both neurons and glia composed of the microtubule-associated protein tau are pathological hallmarks of a class of neurodegenerative disease termed “tauopathies” that manifest with diverse clinical phenotypes including dementia and movement disorders. The discovery of mutations in the tau gene (*MAPT*) in the disease frontotemporal dementia with parkinsonism linked to chromosome 17 provided confirmation of the central role of tau abnormalities in the pathogenesis of tauopathies. Filamentous aggregates in neurons composed of abnormally phosphorylated, insoluble tau protein are widely acknowledged to play a role in neurodegeneration; however, the contribution of tau pathology in astrocytes to disease pathogenesis remains largely unexplored. Recently, astrocyte dysfunction has been implicated in a variety of neurological disorders including neurodegenerative disease.

To study the unique role of astrocytes in the pathogenesis of tauopathies, we developed the first transgenic mouse model of tau pathology restricted to astrocytes by expressing human *MAPT* under the control of the glial fibrillary acidic protein (GFAP) promoter. These GFAP/tau transgenic mice manifest an age-dependent accumulation of tau pathology in astrocytes similar to the human disease. Functional consequences of this pathology include reduced expression and activity of the glial glutamate transporters, GLT-1 and GLAST, which correlated with compromised motor function. In mixed neuron/astrocyte cultures derived from the GFAP/tau transgenic mice, we observe reduced survival of neurons relative to control cultures and this toxicity is likely mediated by glutamate-mediated excitotoxicity. These studies provide early insights into the role of astrocytes in the pathogenesis of neurodegenerative disease, a field of study in its infancy.

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α7 nicotinic acetylcholine receptor signaling and modulation of cytokine production in microglia.

The α7 subtype of nicotinic acetylcholine receptors (α7 nAChRs) are homopentameric ion channels with high Ca^{2+} permeability. They exhibit low affinity for acetylcholine and nicotine, and rapid desensitization. Although the α7 nAChRs were thought to be expressed mainly in neuronal cells, recent evidence indicates that these receptors also function in some non-neuronal cell types. We have found that in rat primary cultured microglia, nicotine elicits a transient increase in intracellular Ca^{2+} levels, which is abolished by specific blockers of α7 nAChRs, methyllycaconitine and α-bungarotoxin. However, this response is not affected by the absence of extracellular Ca^{2+} and is blocked by U73122, an inhibitor of phospholipase C (PLC), and xestospongin C, a blocker of the IP_3 receptor. Extensive electrophysiological measurements failed to reveal any nicotine-induced currents in microglia. These results suggest that microglial α7 nAChRs drive a signaling process involving the activation of PLC and Ca^{2+} release from IP_3-sensitive stores, rather than operating as ion channels. Furthermore, the activation of α7 nAChRs enhanced BzATP-stimulated TNF release ($P2X_7$ receptor activation), but suppressed lipopolysaccahride (LPS)-induced TNF release (Toll-like receptor 4 activation), without affecting the expression of TNF mRNA. In LPS-stimulated microglia, the decreased TNF release induced by nicotine was associated with the suppression of the activation of JNK and p38 MAP kinases, which regulate the post-transcriptional steps of TNF synthesis. On the other hand, nicotine had no effect on the activation of either MAP kinase induced by BzATP, but enhanced BzATP-elicited Ca^{2+} influx. Given our previous findings that small amounts of TNF released from $P2X_7$ receptor-activated microglia can protect neurons, while by contrast the massive TNF release induced by LPS leads to inflammation and neurodegeneration, it is possible that α7 nAChRs have an important role in modulating microglial function, promoting neuroprotection and suppressing the inflammatory release of TNF.

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The “eat-me signal UDP” and microglial phagocytosis mediated by P2Y6 receptors.

Microglia, brain immune cells, engage in the clearance of dead cells or dangerous debris, which is a crucial event in maintaining brain functions. When neighboring cells are injured, microglia rapidly move toward or extend a process to engulf the injured cell. Because cells release or leak ATP when they are stimulated or injured, extracellular nucleotides are thought to be involved in these events. In fact, ATP triggers a dynamic change in the motility of microglia via $P2Y_{12}$ receptors *in vitro* and *in vivo*. These findings presented a novel mechanism underlying microglial chemotaxis, while microglial phagocytosis has received only limited attention. Here we show that microglia express the metabotropic $P2Y_6$ receptor, the activation of which by endogenous agonist uridine 5’-diphosphate (UDP) triggers microglial phagocytosis. UDP facilitated the uptake of microspheres in a $P2Y_6$ receptor-dependent fashion, which was mimicked by endogenous UDP that was leaked when hippocampal neurons were damaged by kainic acid *in vivo* and *in vitro*. In addition, systemic administration of kainic acid in rats resulted in neuronal cell death in the hippocampal CA1 and CA3 regions, where increases in mRNA for $P2Y_6$ receptors that were colocalized with activated microglia were observed. Thus, the $P2Y_6$ receptor is upregulated when neurons are damaged, and would function as a sensor for phagocytosis by sensing diffusible UDP signals, which is a novel pathophysiological function of P2 receptors in microglia.

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Molecular mechanisms of ATP-induced microglial chemotaxis

In response to pathological stimuli microglia rapidly cause morphological change and migrate toward the lesioned site. These microglial responses are crucial for tissue repair of a damaged CNS. Extracellular ATP is known to regulate physiological functions of microglia through various types of ATP receptors, which are divided into two families: ionotropic P2X receptors (P2Xs) and G-protein coupled P2Y receptors (P2Ys). We have previously shown that ATP induces membrane ruffling and chemotactic migration of microglia. The ATP-induced migration is mediated by P2Y12 and P2X4, and requires the activation of phosphatidylinositol 3-kinase (PI3K) and phospholipase C (PLC). Recently ATP has been reported to induce the extension of microglial processes through P2Y12 in response to neuronal death in a mouse brain. In order to investigate the signal transduction underlying the process extension, we established an in vitro experimental model system using rat primary cultured microglia and three-dimensional (3D) collagen gels. Microglia in 3D gels extended their processes toward ATP and experiments with agonists and antagonists against P2Y12 confirmed that P2Y12 mediates the process extension. The effects of PI3K and PLC inhibitors indicated that PI3K and PLC signaling pathways are involved in the process extension. We also found that microglia did not migrate into 3D gels within 2 h stimulation with ATP, however, they moved into 3D gels 4 h after ATP stimulation. These observations suggest that the cell migration of microglia in 3D matrices requires the activation of some other signals in addition to those underlying the process extension.

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The role of glial cell in the pathophysiology of major mental illnesses

Abnormalities in L-glutamate signal transmission have been postulated to play a role in major mental illnesses. Recent studies suggest that glial glutamate transporters play critical roles in normal glutamatergic signal transmission. The glial disruption results in decreased uptake of glutamate and an elevation of extracellular glutamate levels. Elevated extracellular glutamate may cause cytotoxic damage to neurons and glia. Significant down-regulation of glial glutamate transporters, GLT1 and GLAST, in major depressive disorder has been reported. In the present study we examined the role of glial glutamate transporters in the pathogenesis of autism. Autism is a neurodevelopmental disorder characterized by impairments in reciprocal social interaction, communication deficits and repetitive and restricted patterns of behavior and interests. Yet, the etiology of autism is largely unknown. Aberrant glutamate function is often cited as an important element of risk for autism, but little is known about the underlying molecular determinants and neural mechanisms. In the present study, we generated animal models in which glutamate receptors are overstimulated by genetic down-regulation of glial glutamate transporters. Resulting mutant mice showed abnormal social interaction and increased anxiety-like behavior. We observed enlarged amygdala and hippocampus. These mutant mice replicate many aspects of the behavioral and neuroanatomical abnormalities seen in autism. Scanning the genomes of the largest cohort of autism spectrum disorder families revealed that GLT1 falls close to one of linkage peaks. Thus, these mutants are new animal models of autism.

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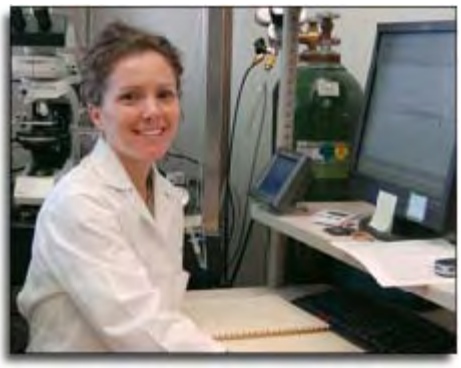
Functional role of gastrin-releasing peptide receptor in reactive astrocyte

Gastrin-releasing peptide receptor (GRP-R) is one of the G-protein coupled receptors (GPCRs) and known as a neuronal modulator in the CNS. GPCR signaling play key physiological roles and their dysfunction causes several diseases; however, functions of GPCRs in the brain diseases has not been elucidated. We found that the expression of GRP-R was increased among GPCRs in dibutyryl cAMP-stimulated cultured astrocytes by RT-PCR analysis with 343 primer sets for mouse GPCR genes. In vitro studies revealed that GRP-R activation induced the proliferation of astrocytes associated with EGF. GRP-R expression was also increased in reactive astrocytes of the mouse cerebral lesion after cold injury. In the cerebral lesion of GRP-R deficient mice, the Ki-65 immunoreactive astrocytes were decreased and wound healing after injury was retarded compared with that in the wild type. We concluded that GRP/GRP-R signaling induced the proliferation of astrocytes and consequently reactive astrocytosis in the injured brain. The production of reactive astrocytes may have an important role for tissue repair processes of the pathological conditions in the CNS.



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Kir4.1 in spinal cord astrocytes

Two signature features of differentiated astrocytes are a high resting K^+ conductance and a very negative resting potential. The former is believed to underlie the K^+ buffering by astrocytes, the latter provides the energetic force for glutamate transporters. Both features are due to the activity of inwardly rectifying potassium (Kir) channels, which are highly expressed in astrocytes. Using shRNA approaches in rat and knock-out mice, we have demonstrated that the principle Kir channel in spinal cord is Kir4.1, a weakly rectifying, Ba^{2+} sensitive channel. Kir4.1 demonstrates developmental up-regulation that correlates with an increasingly tight K^+ regulation in the spinal cord.

Kir channel activity in astrocytes has been shown to be downregulated following injury in several experimental models, mainly *in vitro*, and has been attributed to "reactive gliosis". We demonstrate by Western blot and immunocytochemistry that a crush injury to the spinal cord causes a marked downregulation of Kir4.1 expression near the site of the injury. Importantly, Kir4.1 channel expression can be increased when these animals are given time-release physiological doses of the neuroprotective hormone 17β-estradiol. We also demonstrate that the astrocytic glutamate transporter GLT-1 also increases following estrogen treatment. These findings suggest that some of the neuroprotective benefits of 17β-estradiol following injury may be due to increased Kir4.1 channel expression in spinal cord astrocytes which would lead to improved K^+ regulation and enhanced glutamate homeostasis. Furthermore, other CNS conditions in which a loss of K^+ and glutamate homeostasis are suspected to contribute to disease may benefit from 17β-estradiol treatment.

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Pathophysiological role of the protein degradation system in neurons and glia

The brain consists of the two major cell populations; neurons and glia. It is widely accepted that neurons are more vulnerable to cellular insults and glia are resistant to such stress. However, molecular basis for the idea is poorly understood, and recent literatures suggest that glia are more essentially involved in brain disorders than previously expected. Thus it is reasonable to ask how the difference of vulnerability and the glia-neuron communication contribute to the onset and subsequent progress of the disorders. To address the question, we decided to characterize the nature of each cell type and its response to cellular stimuli at molecular level (See also the talk by Nishimoto et al.). Since increased protein insolubility is believed to be one of the key events in cellular dysfunction, we first focused on the machinery for protein quality control in the two cell populations. We observed that differential expression of some molecular chaperon-associated proteins may contribute to the change of the vulnerability. Since the proteins show some functional relation to the ubiquitin system, we further studied the components of the ubiquitin system. Among various components, a deubiquitinating enzyme, UCH-L1, has a unique feature because its expression is observed in neurons but not in glia. We found that, compared with UCH-L3 that is a ubiquitously expressed isozyme of UCH-L1, UCH-L1 has multiple function and is involved in various biological phenomena including the regulation of a neurotransmitter receptor activity and neurogenesis. We also found that UCH-L1 is modified by oxidative stress and the modified form shows an increased association with other intracellular proteins. Unexpectedly, the mutant mouse that lacks the expression of UCH-L1 showed some alteration in astrocytes. These results suggest that 1) neurons may have a protein degradation system different from that of glia; 2) the presence of such unique system in neurons might result in the different vulnerability to insults such as oxidative stress; and 3) neuron-specific UCH-L1 might be considered as a regulator of glia-neuron interaction. Detailed data in this ongoing project are going to be presented and discussed at the meeting.

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Role of microglial ATP receptors in neuropathic pain

Neuropathic pain is a highly debilitating chronic pain that occurs after nerve injury and is generally resistant to currently available treatments. The mechanisms underlying the pathogenesis of neuropathic pain remain unknown, but a growing body of evidence indicates that extracellular nucleotides and their receptors (P2XR and P2YR) have important roles in neuropathic pain. Results of our laboratory have shown that activating P2X4R upregulated in spinal microglia after nerve injury contributes to neuropathic pain (Tsuda et al., Nature 424: 778-783, 2003). P2X4R-stimulated microglia release brain-derived neurotrophic factor (BDNF) as a crucial factor to signal to dorsal horn neurons, causing a collapse of their transmembrane anion gradient and the subsequent neuronal hyperexcitability (Coull et al., Nature 438: 1017-1021, 2005). We have demonstrated that the fibronectin/integrin system participates in the upregulation of P2X4R expression after nerve injury (Nasu-Tada et al., Glia 53: 769-775，2006; Tsuda et al., Glia in press) and that Lyn tyrosine kinase in microglia is a crucial kinase in the molecular machineries mediating the P2X4R upregulation (Tsuda et al., Glia 56: 50-58, 2008). Our recent study has also shown that P2Y12R is expressed in spinal microglia and that mice lacking the receptor display impaired neuropathic pain. Based on these results, we propose that understanding the roles of purinergic receptors in microglial functions in the spinal cord may provide us with exciting insights into pain mechanisms and clues to developing new therapeutic agents for treating neuropathic pain.

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Ion channels and amino acid transporters aid the biology of glial-derived brain tumors

Glial-derived tumors, commonly called gliomas, constitute a significant health challenge since effective treatments are currently entirely lacking. This is in parts due to the unique biology of the tumors. Unlike cancers in the body, the growth of gliomas is physically restricted by the skull and tumor expansion can only occur if normal brain is being destroyed. To do so, gliomas release glutamate at excitotoxic concentrations thereby vacating room in peritumoral brain. This occurs to a large part via system Xc, a transporter that imports cystine in exchange for glutamate. Imported cystine serves as an essential building block for the cellular antioxidant glutathione. Pharmacological inhibition of this transporter can be achieved with sulfasalazine, an FDA approved drug typically used to treat Crohn's disease. Sulfasalazine shows promising preclinical results as it inhibits tumor growth in animal models of glioma. A second unique aspect of glioma biology pertains to their spread within brain. Unlike other cancers, which spread hematogenously throughout the body, gliomas do not metastasize outside the central nervous system (CNS). Instead they invade the brain and spinal cord by active cell migration. To effectively navigate the tortuous and narrow extracellular spaces in the CNS, glioma cells have developed a remarkable ability to change their shape and reduce their overall cell volume. This cell shrinkage is initiated by the release of Cl^- and K^+ through ion channels together with obligated water diffusing through aquaporins. The channels involved in this process have been identified and in recent years and their contribution to cell invasion has been characterized. The principle K^+ channel expressed by gliomas is gBK, a splice variant of the hslo BK channel gene that contains a 34 amino acid insert near the Ca^{2+} sensor of the channel. gBK is highly sensitive to changes in cytosolic Ca^{2+} and the channels can be activated by growth factors and neurotransmitters. Ca^{2+} influx through Ca^{2+} permeable AMPA-Rs is believed to be a major physiological stimulus. To shrink, efflux of K^+ must be accompanied by anion flux, which in biological systems is mainly Cl^-. The main Cl^- channel involved in glioma cell shrinkage appears to be ClC-3, a channel more typically found on endocytotic vesicles of cells. Glioma Cl^- channels are sensitive to DIDS and NPPB. Moreover, whole cell glioma ClC currents are effectively inhibited by chlorotoxin (Cltx), a scorpion derived peptide. However, unlike other scorpion peptides that bind to K^+ or Na^+ channels, Cltx does not bind to the channel directly but to a surface associated matrix-metalloproteinase MMP-2. Upon binding, Cltx induces the endocytosis of ClC-3 channels and MMP-2 into caveoli. Ultimately Cltx causes depletion of Cl^- channels and from the cell membrane leaving the cell compromised in its ability to regulate its volume. Chlorotoxin has recently completed clinical evaluation in a phase II clinical study with promising results.

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