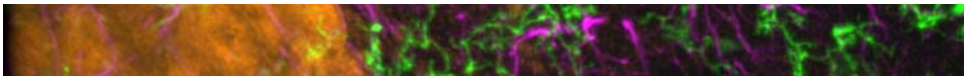
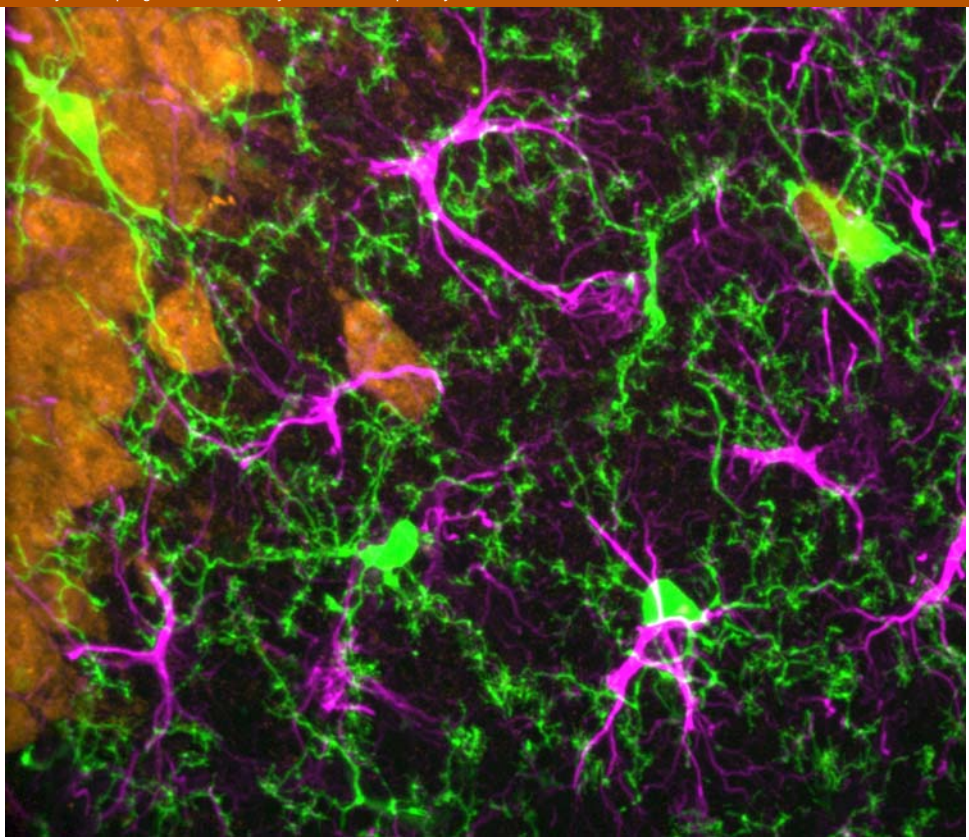
Abstracts of papers presented
at the 2010 meeting on

GLIA IN HEALTH & DISEASE

July 22–July 26, 2010





Cold Spring Harbor Laboratory
Cold Spring Harbor, New York

Abstracts of papers presented
at the 2010 meeting on

GLIA IN HEALTH & DISEASE

July 22–July 26, 2010

Arranged by

Beth Stevens, *Children's Hospital/Harvard Medical School*
William Talbot, *Stanford University*

Cold Spring Harbor Laboratory
Cold Spring Harbor, New York

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Front cover: Interactions between neurons (orange), microglia (green) and astrocyte (pink) in the mouse hippocampus. - Alain Bessis (INSERM, France)

Back cover: A CNS node showing sodium channels (blue), Neurofascin155 (green) and potassium channels (red). - Peter Brophy (University of Edinburgh, UK)

GLIA IN HEALTH & DISEASE

Thursday, July 22 – Monday, July 26, 2010

Thursday	7:30 pm	**1** Genetic Analysis of Glial Function
Friday	9:00 am	**2** Myelinating Cells
Friday	2:00 pm	**3** Poster Session I
Friday	4:30 pm	Wine and Cheese Party *
Friday	7:30 pm	**4** Axon-Glial Interactions
Saturday	9:00 am	**5** Glial Function at Synapses
Saturday	1:30 pm	**6** Microglia Function in Health and Disease
Saturday	4:30 pm	**7** Poster Session II
Saturday	8:00 pm	Roundtable Discussion: Glia in Health and Disease—Open Questions and Future Directions
Sunday	9:00 am	**8** Gliovascular Interactions and Astrocyte Function
Sunday	1:30 pm	**9** CNS Injury and Disease
Sunday	6:00 pm	Banquet
Monday	9:00 am	**10** Glial Development and Epigenetic Mechanisms

Poster sessions are located in *Bush Lecture Hall*

* *Airslie Lawn*, weather permitting

Mealtimes at Blackford Hall are as follows:

Breakfast 7:30 am-9:00 am
Lunch 11:30 am-1:30 pm
Dinner 5:30 pm-7:00 pm

Bar is open from 5:00 pm until late

Printed on 100% recycled paper.

PROGRAM

THURSDAY, July 22—7:30 PM

SESSION 1 GENETIC ANALYSIS OF GLIAL FUNCTION

Chairpersons: **B. Popko,** University of Chicago, Illinois
M. Freeman, University of Massachusetts Medical School, Worcester

7:30 pm **ZFP191 is required for CNS myelination**
Brian Popko.
Presenter affiliation: The University of Chicago, Chicago, Illinois. 1

8:00 pm **Gpr126 regulates Schwann cell development and myelination in mammals**
Kelly R. Monk, William S. Talbot.
Presenter affiliation: Stanford University School of Medicine, Stanford, California. 2

8:20 pm **Synaptic activity controlling locomotory pattern, pausing frequency and speed is regulated by *C. elegans* CEPsh glia**
Menachem Katz, Shai Shaham.
Presenter affiliation: The Rockefeller University, New York, New York. 3

8:40 pm **Astrocyte heterogeneity revealed by comprehensive genetic analysis of in vivo astrocyte in astroglial reporter mice**
Yongjie Yang, Svetlana Vidensky, Lin Jin, Jeffrey Rothstein.
Presenter affiliation: Johns Hopkins University, Baltimore, Maryland. 4

9:00 pm BREAK

9:20 pm **Cellular and molecular biology of astrocyte morphogenesis**
Tobias Stork, Marc R. Freeman.
Presenter affiliation: UMASS Medical School, Worcester, Massachusetts. 5

FRIDAY, July 23—9:00 AM

SESSION 2 MYELINATING CELLS

Chairpersons: **K. Jessen,** University College London, United Kingdom
P. Brophy, University of Edinburgh, United Kingdom

FRIDAY, July 23—2:00 PM

SESSION 3 POSTER SESSION I

***Drosophila* glial glutamate transporter Eaat1 is regulated by Fringe-mediated Notch signaling and is essential for larval locomotion**
Stephanie M. Stacey, Nara I. Muraro, Emilie Peco, Alain Labbé, Richard A. Baines, Donald J. van Meyel.
Presenter affiliation: McGill University Health Centre Research Institute, Montreal, Canada. 55

Acutely grafted embryonic progenitors result in more extensive oligodendroglial production and myelination in *shiverer* forebrain compared to purified and expanded embryonic oligodendrocyte precursor cells
Magda Petryniak, Gregory Potter, John Rubenstein, David Rowitch.
Presenter affiliation: UCSF, San Francisco, California. 56

FRIDAY, July 23—4:30 PM

Wine and Cheese Party

FRIDAY, July 23—7:30 PM

SESSION 4 AXON-GLIAL INTERACTIONS

Chairpersons: **J. Chan,** University of California, San Francisco
E. Peles, Weizmann Institute of Science, Rehovot, Israel

7:30 pm **Nogo-A establishes spatial segregation and extent of myelination during development**
SY Christin Chong, Sheila S. Rosenberg, Yun-An A. Shen, Angela T. Hahn, Binhai Zheng, Li I. Zhang, Aaron McGee, Q Richard Lu, Jonah R. Chan.
Presenter affiliation: USC, Los Angeles, California. 57

8:00 pm **Schwann cell mitochondrial dysfunction disrupts axo-glial interactions without interfering with Schwann cell survival**
Andreu Viader, Judith P. Golden, Robert H. Baloh, Jeffrey Milbrandt.
Presenter affiliation: Washington University in St. Louis, St Louis, Missouri. 58

SATURDAY, July 24—9:00 AM

SESSION 5 GLIAL FUNCTION AT SYNAPSES

Chairpersons: **D. Bergles,** Johns Hopkins University, Baltimore, Maryland
G. Corfas, Children's Hospital, Boston, Massachusetts

SESSION 7 POSTER SESSION II

Sculpting circuits and cleaning up messes—Glial Crk-II/Dock-180/Elmo regulate axon pathfinding during development, and clearance of degenerating axons from the adult brain
Jennifer S. Ziegenfuss, Johnna Doherty, Marc R. Freeman.
Presenter affiliation: UMass Medical School/HHMI, Worcester, Massachusetts. 116

SATURDAY, July 24—8:00 PM

ROUNDTABLE DISCUSSION

Glia in Health & Disease—Open Questions and Future Directions

Discussion Leaders:
B. Barres, Stanford University School of Medicine
M. Raff, University College London

SUNDAY, July 25—9:00 AM

SESSION 8 GLIOVASCULAR INTERACTIONS AND ASTROCYTE FUNCTION

Chairpersons: **R. Daneman,** University of California, San Francisco
M. Nedegaard, University of Rochester, New York

9:00 am **Cellular and molecular regulation of the blood-brain barrier**
Richard Daneman.
Presenter affiliation: UCSF, San Francisco, California. 117

9:30 am **The cAMP effector PKA mediates GPCR signaling in glial blood-brain barrier development**
Xiaoling Li, Ulrike Gaul.
Presenter affiliation: University of Munich, Munich, Germany. 118

9:50 am **VEGFR3 signaling in subventricular zone astrocytes is required for postnatal neurogenesis**
Jean-Léon Thomas, Charles-Félix Calvo, Romain Fontaine, Jihane Soueid, Tuomas Tamella, Kari Alitalo, Anne Eichmann.
Presenter affiliation: INSERM, Paris, France. 119

10:10 am BREAK

10:30 am **Twenty years of gliotransmission—Experimental artifact or reality?**
Maiken Nedergaard.
Presenter affiliation: University of Rochester, New York. 120

11:00 am **Cx32 and Cx47 have distinct roles in oligodendrocyte—Astrocyte gap junction coupling**
Sam Wasseff, Steven S. Scherer.
Presenter affiliation: University of Pennsylvania, Philadelphia, Pennsylvania. 121

11:20 am **Anti-depressive effects of sleep deprivation require the astrocyte-dependent sleep-homeostat**
Dustin J. Hines, Luke I. Schmitt, Rochelle M. Hines, Philip G. Haydon.
Presenter affiliation: Tufts University School of Medicine, Boston, Massachusetts. 122

11:40 am **Astrocyte stimulation can trigger cortical UP states**
Kira E. Poskanzer, Rafael M. Yuste.
Presenter affiliation: Columbia University/HHMI, New York, New York. 123

12:00 pm **Development of a novel method to purify and culture mature rat brain astrocytes**
Lynette C. Foo, Nicola J. Allen, John D. Cahoy, Ben A. Barres.
Presenter affiliation: Stanford University School of Medicine, Stanford, California. 124

12:20 pm LUNCH

SESSION 9 CNS INJURY AND DISEASE

Chairpersons: **M. Sofroniew,** UCLA School of Medicine, Los Angeles, California
H. Zong, University of Oregon, Eugene

1:30 pm **Molecular dissection of reactive astrogliosis and glial scar formation**
Michael V. Sofroniew.
Presenter affiliation: UCLA, Los Angeles, California. 125

2:00 pm **Deciphering the damaging and protective roles of glia in glaucoma**
Gareth R. Howell, Ileana Soto, Xianjun Zhu, Simon W. John.
Presenter affiliation: The Jackson Laboratory, Bar Harbor, Maine. 126

2:20 pm **Using embryonic stem cells to study motor neuron/glia interactions in ALS**
Hemali P. Phatnani, Brad A. Friedman, Monica A. Carrasco, Flo Pauli, Tim Reddy, Michael Muratet, Richard M. Myers, Tom Maniatis.
Presenter affiliation: Columbia University Medical Center, New York, New York. 127

2:40 pm BREAK

3:00 pm **The application of MADM, a mouse genetic mosaic system, for glioma modeling**
Hui Zong.
Presenter affiliation: University of Oregon, Eugene, Oregon. 128

3:30 pm **The lineage-restricted transcription factor Olig2 opposes p53 responses to genotoxic damage in neural progenitors**
Shwetal Mehta, Emmanuelle Huillard, Santosh Kesari, Cecile L. Maire, Diane Duby, Emily Harington, John A. Alberta, Michael Kane, Matthew Theisen, Keith L. Ligon, David H. Rowitch, Charles D. Stiles.
Presenter affiliation: Dana-Farber Cancer Institute, Boston, Massachusetts. 129

3:50 pm **Glial progenitor cells as the origin and essence of glioblastoma**
Lei Liang, Adam Sonabend, Thomas Ludwig, Steven Rosenfeld Steven, Jeffrey Bruce, Peter Canoll.
Presenter affiliation: Columbia University, New York, New York. 130

4:10 pm **The NG2 protein expressed by oligodendrocyte progenitor cells binds to the mitochondrial serine protease Omi/HtrA2—A role for NG2 in stress-protection?**
Frank Maus, Khalad Karram, Judith Stegmüller, Hauke Werner, Klaus-Armin Nave, Rejko Krüger, Jacqueline Trotter.
Presenter affiliation: University of Mainz, Mainz, Germany. 131

4:30 pm **The glial regenerative response to central nervous system injury is enabled by Pros-Notch and Pros-NFκB feedback**
Kentaro Kato, Manuel G. Forero, Stephanie Fannell, Janine C. Fenton, Alicia Hidalgo.
Presenter affiliation: University of Birmingham School of Biosciences, Birmingham, United Kingdom. 132

4:50 pm BREAK

SUNDAY, July 25

BANQUET

Cocktails 6:00 PM Dinner 6:45 PM

SESSION 10 GLIAL DEVELOPMENT AND EPIGENETIC MECHANISMS

Chairpersons: **D. Rowitch,** University of California, San Francisco
P. Casaccia, Mount Sinai School of Medicine, New York, New York

11:20 am **Schwann cell survival and myelination are critically dependent on HDAC1 and HDAC2 function**
Claire Jacob, Carlos N. Christen, Jorge Pereira, Christian Somandin, Arianna Baggiolini, Pirmin Loetscher, Murat Oezçelik, Nicolas Tricaud, Dies Meijer, Teppei Yamaguchi, Patrick Matthias, Ueli Suter.
Presenter affiliation: ETH Zurich, Zurich, Switzerland. 138

11:40 am **CHD4 is required for efficient myelination of the peripheral nervous system**
Holly A. Hung, John P. Svaren.
Presenter affiliation: University of Wisconsin, Madison, Wisconsin. 139

12:00 pm **Glial cell differentiation and neural stem reprogramming in *Drosophila melanogaster***
Hakima Flici, Berra Erkosar, Orban Komonyi, Omer F. Karatas, Angela Giangrande.
Presenter affiliation: IGBMC, Illkirch, Strasbourg, France. 140

12:20 pm MEETING END

ZFP191 IS REQUIRED FOR CNS MYELINATION

Brian Popko

The University of Chicago, Neurology, Chicago, IL, 60637

We have used a forward genetics approach in mice to uncover factors critical to the myelination process. This effort has identified a mouse strain that is severely hypomyelinated in the CNS due to a mutation in the gene that encodes the zinc finger protein191 (*Zfp191*). ZFP191 is a widely expressed, nuclear localized protein that belongs to a family whose members contain protein-protein interacting SCAN domains in addition to the DNA-binding zinc finger domains. Despite the absence of CNS myelin *Zfp191* mutants contain normal numbers of oligodendrocyte progenitor cells, as well as mature, process-extending oligodendrocytes, suggesting that the mutation blocks late stage oligodendrocyte maturation. Our data also indicates that ZFP191 acts in a cell autonomous fashion to promote the myelinating function of oligodendrocytes. Interestingly, global gene expression studies reveal that a limited number of genes are expressed at significantly reduced levels in the *Zfp191* mutant CNS, and that many of the differentially expressed genes have been previously identified as participants in the myelination process. Thus, the Zfp191 mutants provide a novel model system that should provide insight into the molecular control of the final stages of oligodendrocyte maturation and function.

GPR126 REGULATES SCHWANN CELL DEVELOPMENT AND MYELINATION IN MAMMALS

Kelly R Monk, William S Talbot

Stanford University School of Medicine, Developmental Biology, Stanford, CA, 94305

During the development of myelinated axons, promyelinating Schwann cells associate with one segment of an axon, and later become mature Schwann cells that repeatedly wrap their membranes around axons to form the myelin sheath. Incompletely understood signals between axons and Schwann cells initiate and control myelination. Using mutational analysis in zebrafish, we previously reported that *gpr126*, which encodes a member of the G protein-coupled receptor superfamily, is required for Schwann cell myelination in zebrafish. We now show that loss of *Gpr126* in mouse causes a severe congenital hypomyelinating peripheral neuropathy. *Gpr126*-/- mice possess abnormal limb posture and die before weaning. Schwann cells in *Gpr126*-/- mice fail to express several developmental markers, axonal sorting is delayed, and Schwann cells are ultimately arrested at the promyelinating stage of development. Additionally, invasive perineurial fibroblasts form aberrant fasicles throughout the endoneurium of the sciatic nerve. This work shows that Gpr126 is required for Schwann cell myelination in mammals.

SYNAPTIC ACTIVITY CONTROLLING LOCOMOTORY PATTERN, PAUSING FREQUENCY AND SPEED IS REGULATED BY *C. ELEGANS* CEPSH GLIA

Menachem Katz, Shai Shaham

The Rockefeller University, Developmental Genetics, New York, NY, 10065

Information processing by the nervous system, leading to precise animal behavior, is complex. Recent studies suggest the possibility that glial cells participate in controlling synaptic activities, and may thus contribute to behavior. However, the precise role of synaptic glia in control of animal behavior is poorly understood. *C. elegans* glia share morphological, functional and genetic features with their vertebrate counterparts. However, in contrast to vertebrate glia, *C. elegans* glia are not essential for neuronal survival, offering a unique arena for exploring the involvement of glia in neuronal functions in a live animal. The CEP sheath glia (CEPsh) of *C. elegans* envelope the nerve ring (the animal's brain), and, reminiscent of mammalian astrocytes, extend processes that abut synapses within the nerve ring. Here we demonstrate that animals in which the CEPsh glia are genetically ablated during the first larval stage display behavioral defects including reduced locomotion speed and extended locomotory pausing. Ablated animals also display exaggerated small-angle turns and frequent reversals that limit their dispersal. To understand how these locomotory behaviors are regulated by CEPsh glia, we focused on a synapse between the ALA and AVE neurons, which is ensheathed by these glia (White et al., 1986). In line with the previously described role of ALA in behavioral quiescence and locomotory pausing (Van Buskirk and Sternberg, 2008), we found that inactivation of the ALA neuron reduces the pausing frequencies of glia-ablated animals. Inactivation of the AVE neurons in wild-type animals, induced extended pausing periods similar to those seen in glia ablated animals. Our results suggest that AVE is a key regulator of speed and pausing frequency in the *C. elegans* nervous system, and that ALA functions to inhibit the activity of AVE. Moreover, our results indicate that the CEPsh glia provide important negative regulation on the activity of this tripartite synapse.

To understand the molecular basis of CEPsh glia function we have begun to identify genes expressed in these cells using an mRNA-tagging method, with the aim of examining the roles of enriched genes in *C. elegans* locomotory behavior.

ASTROCYTE HETEROGENEITY REVEALED BY COMPREHENSIVE GENETIC ANALYSIS OF IN VIVO ASTROCYTE IN ASTROGLIAL REPORTER MICE

Yongjie Yang, Svetlana Vidensky, Lin Jin, Jeffrey Rothstein

Johns Hopkins University, Neurology, Baltimore, MD, 21205

Astrocytes are the most abundant cells in the CNS, not only provide essential metabolic support to neurons, but also participate in the information processing by active modulation of neuronal/synaptic activity in the CNS. In pathophysiological conditions, astrocytes undergo dramatic morphological and molecular changes that can cause potentially both beneficial and detrimental effects. Although astrocytes are essential in normal CNS function and are involved in many neuropathological conditions, astrocyte heterogeneity remains largely unknown in the CNS, partially due to the lack of the specific astroglial makers. In this study, molecular identity of in vivo astrocytes was compared in BAC ALDH1L1 eGFP and BAC GLT1 eGFP transgenic mice by astroglial RNA transcriptome analysis. ALDH1L1 promoter is selectively activated in adult cortical and spinal cord astrocytes, indicated by the overlap of eGFP expression with ALDH1L1 and GFAP, but not with NeuN, APC, Oligo2, IbaI, PDGFRα immunoreactivity in BAC ALDH1L1 eGFP reporter mice. Interestingly, ALDH1L1 expression levels (protein, mRNA, and promoter activity) in spinal cord were selectively decreased during postnatal maturation. In contrast, its expression was up-regulated in reactive astrocytes in both acute neural injury and chronic neurodegenerative (G93A mutant SOD1) conditions, similar to GFAP, but opposite of GLT1. ALDH1L1+ and GLT1+ cells isolated through fluorescence activated cell sorting (FACS) from BAC ALDH1L1 and BAC GLT1 eGFP mice share a highly similar gene expression profile, suggesting ALDH1L1 and GLT1 are co-expressed in the same population of astrocytes. This observation was further supported by overlap of the eGFP driven by the ALDH1L1 genomic promoter and the tdTomato driven by a 8.3kb EAAT2 promoter fragment in astrocytes of BAC ALDH1L1 eGFP X EAAT2-tdTomato mice. In contrast, astrocytes isolated from brain or spinal cord show highly differential expression pattern, providing potential clues for their differential functions and astrogliogenesis in the CNS. These studies compared the molecular identity of in vivo astrocytes, as the first attempt to investigate the molecular differences of heterogeneous astrocytes in the CNS.

CELLULAR AND MOLECULAR BIOLOGY OF ASTROCYTE MORPHOGENESIS

Tobias Stork, Marc R Freeman

UMASS Medical School, Neurobiology/ HHMI, Worcester, MA, 01605

Astrocytes fulfill diverse roles in the development, function, and maintenance of the nervous system. However, the molecular mechanisms which govern the establishment and maintenance of their intricate morphology and intimate association with neurons remain largely unknown. We have identified a novel subtype of glial cells in *Drosophila* which shows a striking morphological, molecular, and functional similarity to vertebrate astrocytes. For example, fly astrocytes have a highly tufted morphology, associate closely with synapses, and express neurotransmitter clearing proteins (e.g. glutamate and GABA transporters). We are using this cell type as model system to elucidate basic principles of astrocyte morphogenesis, dynamics, and function.
Drosophila astrocytes infiltrate the synaptic neuropil at the end of embryogenesis. By the time the larva hatches from the egg shell, a dense meshwork of glial ramifications, showing close apposition to synapses, has been established throughout the neuropil. Similar to mammalian astrocytes, individual *Drosophila* astrocytes occupy discrete spatial domains with little overlap of their processes with neighboring cells. Although astrocyte-like glial cell bodies tend to occupy fairly stereotyped positions in the CNS, the tufted morphology of their cellular processes can vary substantially from cell to cell, suggesting dynamic cell-cell communication and cell competition pathways. Strikingly, we find that ablation of adjacent astrocytes lead to tremendous overgrowth of individual astrocytes, suggesting that astrocyte-astrocyte communication limits cell growth.
How do astrocytes invade the neuropil and elaborate membrane processes? We utilized forward genetics to identify molecular signals governing the coordinated infiltration of astrocyte-like glial cell projections into synaptic regions. In mutant animals for the FGF receptor *heartless*, or its ligands *pyramus* and *thisbe*, astrocytes are specified normally but fail to infiltrate the neuropil. Heartless is expressed on the astrocytes while Pyramus and Thisbe appear to be secreted from neurons. We are currently testing whether FGFs act as trophic signals which promote general glial outgrowth or if they directionally attract glial processes to dendrites and synapses.
Together our initial work indicates that *Drosophila* astrocytes show remarkable morphological, molecular, and functional conservation with their vertebrate counterparts and will serve as an excellent model to study fundamental aspects of astroglial development and function.

GLIA DELIMIT SHAPE CHANGES OF SENSORY NEURON RECEPTIVE ENDINGS IN *C. ELEGANS*

Carl Procko, Yun Lu, Shai Shaham

The Rockefeller University, Laboratory of Developmental Genetics, New York, NY, 10065

Neuronal receptive endings such as dendritic spines and sensory protrusions are remodeled by experience, and these morphological changes can alter downstream neuronal responses. The mechanism of receptive-ending remodeling is not well understood. In response to environmental stressors, the nematode *Caenorhabditis elegans* enters a diapause state, termed dauer, which is accompanied by sensory organ remodeling. We have found that proper remodeling of the sensory receptive endings of the AWC neurons in dauers is dependent on concomitant remodeling of the AMsh glial cell that envelops these endings. Remodeling of AMsh glia requires the Otd/Otx transcription factor TTX-1, the fusogen AFF-1, and the VEGFR-related protein VER-1, all acting within the glial cell. *ver-1* expression requires direct binding of TTX-1 to *ver-1* regulatory sequences, and is induced in dauers and at high temperatures. Our results raise the possibility that glia may function as general regulators of neuronal receptive ending shape.

MANIPULATING DROSOPHILA GLIA TO MODEL HUMAN GLIOMA

Renee D Read[1], Webster K Cavenee[2], Frank B Furnari[2], John B Thomas[1]

[1]The Salk Institute for Biological Studies, Molecular Neurobiology Laboratory, La Jolla, CA, 92037, [2]University of California at San Diego, Ludwig Institute for Cancer Research, La Jolla, CA, 92093

Glioma formation is a complex process involving accumulation of mutations in many genes, some of which normally regulate proliferation and differentiation of neuro-glial progenitor cells, such as EGFR. Constitutive activation of the EGFR-Ras and PI-3 kinase (PI3K) signaling pathways is common in gliomas and is sufficient to cause glioma in mouse models, although how these pathways specifically regulate glial pathogenesis is unclear. To investigate the genetic basis of this disease, we developed a glioma model in *Drosophila*. This builds on observations that the *Drosophila* brain contains multiple glial cell types that are strikingly similar to their mammalian counterparts in terms of function, development, and gene expression. We found that constitutive co-activation of EGFR-Ras and PI3K signaling in *Drosophila* glia gives rise to highly proliferative, neoplastic, and invasive glial cells that create transplantable tumor-like growths. Genetic analyses demonstrate that EGFR-Ras and PI3K initiates neoplastic transformation via a combinatorial genetic network composed of pathways activated in human glioma, which act synergistically to coordinately stimulate cell cycle entry and progression, protein translation, and inappropriate cellular growth and migration. We have used our *Drosophila* model in forward genetic screens to identify new genes that drive gliomagenesis and to characterize the cell types that give rise to glial neoplasia. These functional screens have identified over 40 genes, including novel kinases and cell-type specific transcription factors, that govern normal and/or neoplastic glial proliferation. Using lineage analysis and cell-type specific markers, we have found that neoplastic glial cells originate from lineage-restricted glial progenitor cells, rather than from differentiated glia or multipotent neuroblasts. Genes that are required for normal development and function of these glia are required for their transformation by EGFR and PI3K signaling. Mammalian orthologs of genes uncovered by these screens are now being examined in mammalian glioma model systems. These studies may bring new insights into the genetic and cellular origins of human glioma.

SCHWANN CELL C-JUN CONTROLS THE GENERATION OF THE DENERVATED SCHWANN CELL AND NERVE REPAIR.

Kristjan R Jessen, Rhona Mirsky

University College London, CDB, London, WC1E6BT, United Kingdom

Following nervous system injury, the glial response is a key determinant of repair. In the CNS, this response prevents recovery, but in the PNS Schwann cells foster repair. This is because injury prompts myelinating and non-myelinating Schwann cells to generate the denervated Schwann cell. This cell, which has a distinct phenotype related to that of immature Schwann cells in developing nerves, provides essential support for axon growth and later myelinates the regenerated fibres. Therefore injury generates the repair cell needed for healing and functional recovery. Given their importance, remarkably little is known about the transcriptional programmes that control the generation of the denervated cell. In recent studies, we have implicated c-Jun and Notch in this process and have defined the concept of negative transcriptional regulation of myelination (Jessen and Mirsky, Glia 56: 1552-1565, 2008; Woodhoo et al. Nat Neurosci 12: 839-847, 2009). We find that c-Jun, in particular, is an essential regulator of the distinct molecular and morphological phenotype of the denervated cell. It is also necessary for the key function of these cells, since after injury, neuronal survival, axon regrowth and functional recovery are severely and permanently compromised in mice with genetic inactivation of c-Jun specifically in Schwann cells. (In contrast, development and myelination are unaffected.) Because Schwann cell c-Jun also directly and indirectly controls the rate of injury-related myelin breakdown, c-Jun appears to act as a global regulator of the regenerative response in injured nerves.

EPHB SIGNALLING DIRECTS PERIPHERAL NERVE REGENERATION THROUGH SOX2-DEPENDENT SCHWANN CELL SORTING

Simona Parrinello[1], Ilaria Napoli[1], Patrick Wingfield Digby[1], Sara Ribeiro[1], David Parkinson[2], Alison C Lloyd[1]

[1]University College London, MRC Laboratory for Molecular Cell Biology, LONDON, WC1E 6BT, United Kingdom, [2]University of Plymouth, Peninsula College of Medicine and Dentistry, Plymouth, PL6 8BU, United Kingdom

The peripheral nervous system has astonishing regenerative capabilities in that cut nerves are able to reconnect and re-establish their function. Schwann cells are important players in this process, during which they dedifferentiate to a progenitor/stem cell and promote axonal regrowth. Here we report that fibroblasts also play a key role. Upon nerve cut, ephrinB/EphB2 signalling between fibroblasts and Schwann cells results in cell-sorting, followed by directional collective-cell migration of Schwann cells out of the nerve stumps to guide regrowing axons across the wound. Mechanistically, we show that cell-sorting downstream of EphB2 is mediated by the stemness factor Sox2 through N-cadherin relocalisation to Schwann cell-cell contacts. In vivo, inhibition of EphB2 impaired organised migration of Schwann cells, resulting in misdirected axonal regrowth. Our results identify a new link between Ephs and Sox proteins, providing a mechanism by which progenitor cells can translate environmental cues to orchestrate the formation of new tissue.

CLAW PAW MEETS THE ADAMS FAMILY: THE ROLE OF LGI4 AND ADAM22 IN PERIPHERAL NERVE DEVELOPMENT

Ekim Ozkaynak[1], Gina Abello[1], Siska Driegen[1], Linde Kegel[1], Martine Jaegle[1], Koji Sagane[2], John R Bermingham[3], Dies Meijer[1]

[1]ErasmusMC, Cell Biology and Genetics, Rotterdam, 3015GE, Netherlands, [2]Eisai Co. Ltd, Tsukuba Research Laboratories, Tsukuba, 300-2635, Japan, [3]McLaughlin Research Institute, Genetics, Great Falls, MT, 59405

The development, homeostasis and function of the nervous system depend on intimate cellular interactions between neurons and glia cells. Disturbances of these interactions result in neurological disease. We are interested in defining the molecular interactions between Schwann cells and neurons in the peripheral nervous system that govern cellular differentiation and myelin formation. Analysis of the natural mouse mutant Claw Paw (Clp) has revealed a novel signalling pathway operating in nerve development. Claw paw mice are characterized by congenital hypomyelination and limb posture abnormalities. We have demonstrated that a mutation in the Leucine rich Glioma Inactivated (Lgi) gene 4, underlies the claw paw phenotype (1). Lgi4 is a member of the Lgi family of secreted proteins and is expressed by Schwann cells and neurons. Recently, it has been shown that Lgi1 and Lgi4 can bind to ADAM22, a member of the type I transmembrane A Disintegrin And Metalloproteinase family of cell surface receptors (2). It is likely that this interaction is functionally significant as ADAM22 mutant mice exhibit a peripheral nerve phenotype similar to that observed in clp mice(3). Adam22 is expressed in Schwann cells and neurons. To determine whether Lgi4-Adam22 function involves a paracrine and/or an autocrine mechanism of action we performed heterotypic Schwann cell sensory neuron cultures and cell-type-specific ablation of Lgi4 and Adam22 in mice. We have shown that Schwann cells are the principal cellular source of Lgi4 in the developing nerve and that Adam22 is required on axons (4). Our results revealed a novel paracrine signalling axis in peripheral nerve myelination in which Schwann cell secreted Lgi4 functions through binding of axonal Adam22 to drive the differentiation of Schwann cells.

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4.Özkaynak, E., et al. Adam22 is a major neuronal receptor for lgi4-mediated schwann cell signaling. J Neurosci 2010 March; 30:3857-3864.

FORMATION OF AXONAL DOMAINS IN MYELINATED NERVES OF THE CNS

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The paranodal axoglial junction, an adhesion zone whose structural integrity is vital for the propagation of the action potential, is the major site of axon-glia contact in myelinated nerves. The adhesion complex is composed of the axonal proteins Caspr/Paranodin and Contactin and the glial receptor Nfasc155, an isoform of the cell adhesion molecule Neurofascin. This was the first glial cell adhesion molecule shown to mediate axon-glia interaction in the myelinated vertebrate nerve. We have generated mice lacking a functional Neurofascin gene which revealed that Nfasc155 is indeed a glial component of the junctional complex. Furthermore, these mutants also lack Nfasc186, the neuronal Neurofascin isoform, which is a major component of the node of Ranvier. In this mutant nodes of Ranvier are disrupted and rescue experiments have shown that Nfasc186 is required for their assembly in the CNS. More recently, we have shown in the CNS that clustering of sodium channels at the node of Ranvier can be promoted either by an intact axoglial junction or by the expression of Nfasc186.

DICER1 AND MIR-219 ARE REQUIRED FOR NORMAL OLIGODENDROCYTE DIFFERENTIATION AND MYELINATION

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To investigate the role of microRNAs in regulating oligodendrocyte (OL) differentiation and myelination, we utilized transgenic mice in which microRNA processing was disrupted in OL precursor cells (OPCs) and OLs by targeted deletion of Dicer1. We found that inhibition of OPC-OL miRNA processing disrupts normal CNS myelination, and that OPCs lacking mature miRNAs fail to differentiate normally in vitro. We identified three miRNAs, miR-219, miR-138, and miR-338, that are induced 10-100x during OL differentiation; the most strongly induced of these, miR-219, is necessary and sufficient to promote OL differentiation, and partially rescues OL differentiation defects caused by total miRNA loss. miR-219 directly represses the expression of PDGFRα, Sox6, FoxJ3, and ZFP238 proteins, all of which normally help to promote OPC proliferation. Together, these findings show that miR-219 plays a critical role in coupling differentiation to proliferation arrest in the OL lineage, enabling the rapid transition from proliferating OPCs to myelinating OLs.

THE TETRASPANIN KAI1/CD82 REGULATES OLIGODENDROCYTE PROGENITOR MIGRATION, DIFFERENTIATION AND MYELINATION

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Mechanisms that regulate the migration and differentiation of oligodendrocyte precursors are of great importance in understanding normal development and demyelinating/remyelinating conditions. In a microarray analysis comparing adult and neonatal O4+ cells, we found that Kai1/CD82 is far more highly expressed in the adult than in the neonatal cells. CD82 is a transmembrane tetraspanin that suppresses tumor metastasis and its expression is often down-regulated or lost in the advanced stages of metastatic cancer. We hypothesized that CD82 could be a factor that restricts migration and promotes differentiation of maturing oligodendrocytes.
+, olig2+ or NG2+, or became CC1+ non-myelinating oligodendrocytes or GFAP+ astrocytes.αWe have previously shown that in the adult rat white matter CD82 is co-expressed with CC1 and olig2 but not with NG2 or GFAP. CD82 is also expressed in isolated adult O4+ cells and continues to be expressed as these become O1+ in vitro. When we forced constitutive expression of CD82 in immature cells of the neonatal SVZ in vivo, the cells did not remain immature but differentiated either into CC1+ and MBP+ myelinating oligodendrocytes in the white matter or zebrinII+ astrocytes in the cortex. Their migration from the SVZ was severely restricted. In contrast, downregulation of CD82 in SVZ cells in vivo using retroviral-expressed shRNAs, prevents their differentiation into myelinating oligodendrocytes. shRNA-expressing cells remained PDGFR
Our new data confirm that preventing CD82 expression in either adult or neonatal O4+ cells in vitro prevented the cells from accumulating MBP, an observation that correlates with the inability of CD82 shRNA-expressing cells to myelinate in vivo. CD82 has been physically linked to c-Met, the hepatocyte growth factor receptor, in tumor cell membranes and this interaction decreases signalling through c-Met. Accordingly, we performed immunoprecipitation and found that c-Met and CD82 bind each other directly in acutely isolated adult O4+ cells. Since c-Met is a positive regulator of oligodendrocyte precursor migration and is expressed in both neonatal and adult O4+ cells, the expression of CD82 may help to restrict migration. CD82 interaction with integrins, as occurs in other cell types, may also play a role in restricting precursor migration.

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NEUREGULIN SWITCHES OLIGODENDROCYTES BETWEEN TWO MODES OF MYELINATION

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Neuregulin expressed by axons can regulate myelination by signalling to ErbB receptors on myelinating cells. Furthermore, in CNS grey matter, neuregulin increases the expression of NMDA receptors. Oligodendrocytes at all developmental stages in the white matter exhibit NMDA evoked currents, mediated by receptors which show very weak magnesium block and are expressed in the myelin (Káradóttir et al., 2005, 2008), indicating that they might play a role in myelination.

We now use an assay in which cortical oligodendrocytes ensheath dorsal root ganglion cells (Wang et. al., 2006) to show that neuregulin and NMDA receptors interact to regulate myelination. Without neuregulin, blocking NMDA receptors had no effect on myelination. Adding neuregulin (in the form of the extracellular domain of NRG1-β1, 10ng/ml) increased myelination by 60%, and led to the majority of myelination becoming dependent on activation of NMDA receptors: NMDA receptor block decreased myelination by 80%. Blocking action potentials with TTX also had no effect in the absence of neuregulin, but greatly reduced myelination in the presence of neuregulin. Neuregulin's effect was associated with a 4-fold increase in NMDA receptor current and an increase in phosphorylation of Creb in oligodendrocyte lineage cells. Thus, neuregulin apparently switches myelination from a default programme, that is independent of neuronal activity, to a mechanism that is regulated by glutamate released from active axons.

These data reveal a function for oligodendrocyte NMDA receptors, and could provide a novel white matter explanation for how the linkage of neuregulin to schizophrenia can be reconciled with schizophrenia involving a malfunction of NMDA receptors. The absence of neuregulin in multiple sclerosis lesions, and enhanced remyelination by added neuregulin, suggest a role for neuregulin/NMDA receptor dependent remyelination after pathology.

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BASIC CHARACTERIZATION OF A NOVEL CLN1/CLN5 DEFICIENT MOUSE MODEL

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The neuronal ceroid lipofuscinoses (NCLs) represent the most common group of severe neurodegenerative diseases in children. NCLs are inherited, autosomal recessive lysosomal storage diseases (LSD), which are characterized clinically by epileptic seizures, progressive psychomotoric decline, visual failure, variable age of onset, and premature death.
We have previously generated the Cln1 and Cln5 deficient mouse models [1, 2]. These mice suffer from several neuronal defects, such as retinal atrophy leading to blindness, motor dysfunction, weight loss, and difficulties in movement. The brain analyses show localized astrocytosis and neuronal loss in the thalamocortical system.
In this study we have developed a novel Cln1 and Cln5 deficient double knock-out (KO) mouse model, The Cln1/Cln5 double KO offspring is viable, and the first phenotypical defects (myoclonic jerks, balance problems, spontaneous seizures) are observed at 3 months, which is earlier than in Cln1 or Cln5 deficient mouse, which show the first symptoms around the age of 5 months. The double KO mice revealed a massive accumulation of autofluorescence storage material in the brain, especially in cortex and thalamus. The histopathological analysis showed the loss of Purkinje cells in the cerebellum, and the presence of glial cells (astrocytes and microglia) in the cortex indicates astrocytosis in the Cln1/Cln5 double KO mouse brain. However, the loss of myelin, which is commonly seen in Cln1 and Cln5 deficient mouse brain, was not observed in double KO mouse brains. These results will be confirmed by immunohistochemical straining and stereological analysis of the brain sections.
Together the results suggest that loss of both Cln1 and Cln5 genes leads to a more severe phenotype that the loss of only one of these genes. The basic disease phenotype of the novel mouse model will be characterized in more detail, and the gene expression profiles will be analyzed and compared to the existing data of Cln1 and Cln5 deficient mouse models. Since Cln1 KO mouse has disturbances in cholesterol synthesis, the lipid metabolism in the double KO mouse will be also analyzed. In addition, the possible defects in oligodendrocyte biology and mitochondrial function will be investigated.
The data obtained from this study will provide a comprehensive view of NCL diseases and should lead to further understanding of neurodegenerative mechanisms involved in the development of brain disorders.

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GFAP POSITIVE PROCESSES ENTER A SCHWANN CELL BRIDGE AND CORRELATE WITH REGENERATED BRAINSTEM AXONS AND HIND LIMB LOCOMOTION FOLLOWING SPINAL CORD TRANSECTION INJURY

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Complete transection of the thoracic spinal cord and implantation of a Schwann cell (SC) bridge is a rigorous model to assess the regeneration of CNS axons. Previous work with this model found that implantation of a pre-gelled bridge of SCs and Matrigel results in the regeneration of intra-spinal but not supra-spinal axons into the bridge. We now report that, in contrast to pre-gelled bridges, an initially fluid bridge of SCs and Matrigel is sufficient for up to ten percent of AAV-GFP labeled brainstem axons to regenerate into the bridge. The host spinal cord/SC bridge interfaces varied from sharp boundaries to irregular ones where long GFAP positive astrocyte processes were found to enter and cross the bridge for at least 2.5 mm. The processes were finger-like, with more robust ones found close to the interface and very fine ones found farther into the bridge. At the rostral interface, the astrocyte processes were also in close association with brainstem axons as well as SCs, and together formed a triumvirate. The brainstem axons were often found to regenerate beyond the astrocyte processes. The percentage of brainstem axon regeneration into the bridge correlated positively with the number of GFAP positive processes 0.25 mm past the rostral interface ($p<0.005$; $r2=0.65$). In addition, the number of GFAP positive processes found at all measured distances across the SC bridge was greater in the initially fluid bridges compared to those that were pre-gelled ($p<0.0001$; two-way ANOVA). At 0.25 mm past the rostral interface, there was an average of 182 GFAP positive processes/mm2 in the initially fluid bridges compared to only 16 GFAP positive processes/mm2 in the pre-gelled ($p<0.01$; Bonferroni posttest). After six weeks, scores for an open-field hind limb locomotion test, the BBB, also correlated with the total number of GFAP positive processes found at both the caudal and rostral interfaces ($p<0.05$; $r2=0.31$). Thus, the presence of GFAP processes crossing both interfaces defines an environment that may lead to improvements in hind limb locomotion after complete transection and implantation of a SC bridge. In this way, a host spinal cord/SC bridge interface may or may not be permissive for brainstem axon regeneration and functional recovery, and this appears to be dependent upon the nature and morphology of the GFAP positive astrocytes at the interface.

CHARCOT-MARIE-TOOTH 1C NEUROPATHY AND EXOSOMAL TRAFFICKING

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Charcot-Marie-Tooth (CMT) peripheral neuropathy is the most common inherited disorder of the peripheral nervous system. Approximately, 1 in 2500 people exhibit CMT defects. The high prevalence of the CMT disease brings enormous burdens onto our health-care system and severely affects the economy of the United States. Formulating new therapeutic approaches are critical for improving the quality of life of CMT patients.

CMT1C is a subclass of the CMT family. Mutations in protein SIMPLE (small integral membrane protein of the lysosome/late endosome) account for the etiology found in CMT1C patients. Molecular basis of SIMPLE mutations in causing demyelination in CMT1C, however, remain elusive. The lack of knowledge on the role of SIMPLE further complicates our understanding of SIMPLE in CMT1C demyelination.

We have recently discovered that SIMPLE participates in vesicular trafficking to extracellular milieu. Indeed, secreted SIMPLE is found in exosomes, extracellular fractions that contain 50-90 nm nano-vesicles. Exosomes are arisen from membrane invagination of late endosomes. Exosomal trafficking, therefore, is an extracellular continuum of the complex endosomal pathway.

To provide the molecular foundation for subsequent examination of SIMPLE in CMT1C neuropathy, we investigated the role of SIMPLE in exosomes *per se*. We found that a composite signal is required for exosomal targeting of SIMPLE. Exosomal SIMPLE contributes to the making of these *nano*-vesicles. SIMPLE is also evolutionarily conserved and SIMPLE ortholog in *C. elegans* is expressed along the worm intestinal tract, located at the apical/basal membranes and exhibited vesicular punctate localization. Notably, we found that all mutations identified in CMT1C patients impair targeting of SIMPLE to exosomes. Together, our data demonstrate that defective exosomal localization is a new disease-causing mechanism and could account for CMT1C neuropathy. To further unravel the pathomechanisms of CMT1C neuropathy, we will discuss molecular studies of CMT1C mice, *Simple*$^{-/-}$ null mice and genetic mutagenesis in *C. elegans*.

THE FUNCTION OF ASTROCYTES IN NEURAL CIRCUIT MATURATION AND SYNAPSE ELIMINATION

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To achieve precise neural connectivity and form mature neural circuits, pre-formed axons and synapses need to be remodeled extensively. These remodeling processes include elimination of excess axons, dendrites, synapses and their debris. Growing evidence suggests that elimination processes are essential in shaping neural circuits during development as well as in regulating synaptic plasticity in response to experience and memory in adulthood. Moreover, certain neurodegenerative diseases, such as Alzheimer's disease (AD), are associated with a profound loss of synapses early in the disease process, underscoring the importance of understanding the mechanism for this synapse loss. Studies in mammals, flies and worms have previously demonstrated that glial cells play central roles in clearing apoptotic neurons and degenerating axons through an engulfment process called phagocytosis. However, the cellular and molecular mechanisms that drive these phenomena are largely unknown, particularly in the mammalian CNS. Recently by gene profiling, we have found that several phagocytic pathways--the MEGF10/draper/ced1 pathway that mediates axon pruning in flies and the MERTK pathway that mediates outer segment clearance by retinal pigment epithelial cells--are specifically and highly expressed by mouse astrocytes in both the developing and adult CNS. Although it has been assumed that microglia in the mammalian CNS are largely responsible for clearing neural debris, our findings suggest that mammalian astrocytes may actively participate in eliminating excess axons, dendrites, synapses and their debris. In our studies, we have found that astrocytes in fact have high phagocytic activity in clearing axonal debris in vitro. Currently, we are investigating the hypothesis that astrocytes phagocytose neural debris and synapses through the MEGF10 and/or MERTK phagocytic receptor pathways expressed by astrocytes.

PRENATAL COCAINE EXPOSURE ALTERS HUMAN AND RODENT INFANT VOCALIZATIONS: IMPLICATIONS FOR UNDERLYING NEURAL-GLIAL INTEGRITY.

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Maternal cocaine abuse is correlated with decreased mother-infant attachment, increased neglect and altered infant behavioral development. Increased neglect may derive from effects prenatal cocaine has on infant cues that promote maternal response. Perhaps most salient among these cues is the cry of the newborn and young infant. In both human and rodent infants auditory stimuli (cries and ultrasonic vocalizations) elicit maternal care. This study examined human and rodent infant vocalizations to determine if prenatal cocaine alters vocalizations which might influence maternal care and if vocalizations might be a sensitive tool in predicting infant glial and neuronal integrity. Limbic regions of the neonatal brain are plastic and mature during the postnatal period correlating with altered social behavior in human infants. Prenatal cocaine has been found to delay astrocyte maturation but the impact this has on neuronal development or behavior has not been explored. Healthy control mothers, mothers who abused cocaine, and mothers who abused drugs but not cocaine during pregnancy brought in their infants at 4 weeks + 4 days. Infants were placed on a chilled scale and 35 seconds of crying was obtained and subsequently analyzed. In our animal model Sprague-Dawley rats, upon conception, were divided into three groups: untreated, chronic-cocaine treated (30mg/kg/day from gestation days (GDs) 1-20), or chronic-saline treated (2mg/kg/day from GDs 1-20). On GDs 13-15 pregnant rats were injected with Bromodeoxyuridine (10mg/kg) to label proliferating cells in the periaqueductal gray, amygdala, and nucleus accumbens. On postnatal day 14 pups were tested for vocalizing behavior by placement on a metal scale mirroring our human cry elicitation protocol. Vocalizations were altered in both species following cocaine-exposure, including alterations in pitch, duration, and amplitude. Rodent brains are now being assessed for astrocyte and neuronal maturation rates with fluorescent immunohistochemistry to explore if prenatal cocaine exposure delays neural-glial development and if this developmental delay correlates with altered vocalizing behavior. Vocalizations may serve as a behavioral and biological marker for infants with developmental vulnerability to maternal neglect.

REGULATION OF OLIGODENDROCYTE PRECURSOR CELL MIGRATION BY HOMEOPROTEIN PAX6

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Homeoprotein (HP) transcription factors play fundamental roles in development, ranging from embryonic polarity to cell differentiation and migration. Their cell autonomous activity has been extensively documented. Less importance has been granted to their other properties, notably their ability to undergo intercellular transfer. Recent years, have underscored functions of HP transfer in eye field development (Lesaffre 2007), axon guidance (Brunet 2005), retino-tectal patterning (Wizenmann 2009) and visual cortex plasticity (Sugiyama 2008).
More recently, we investigated a possible role of HP transfer in cell migration. We started by looking at the role of certain HPs, notably Pax6 and Nkx2.2, in migration in the embryonic spinal cord, using the chick neural tube as a model. In the developing spinal cord, Nkx2.2 and Pax6 are involved in the regulation of specification and/or differentiation of oligodendrocytes. The oligodendrocyte precursors (OPCs) arise in the Olig2-positive ventral domain of the ventricular zone and disperse in successive ventral and latero-dorsal waves. In analogy with the work on axon guidance (Brunet et al., 2005), the presence of extracellular Pax6 and Nkx2.2 in the neural tube at E6 suggested a possible non-cell autonomous role of these factors in OPC migration.
This hypothesis was tested by loss and gain of function experiments to neutralize or enhance extracellular Pax6 activity, respectively. Loss of function was achieved through the expression by electroporation of secreted antibodies designed to antagonize Pax6 HP transfer. Secreted Pax6 antibody expression at E3 resulted in a reduction in the number of migrating Olig2+OPCs at E6. In contrast, electroporation of the protein Pax6 with a signal peptide increased the number of migrating OPCs. Identical conclusions were reached by culturing open book neural tubes in the presence of Pax6 neutralizing antibodies or purified Pax6 protein.
All in all, these findings suggest a paracrine effect of Pax6 on OPCs, specifically promoting their migration, which provides a new example of non-cell autonomous HP activity.

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AKT REGULATES MYELIN SHEATH THICKNESS AND AXON WRAPPING IN THE PNS

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We are investigating the signaling pathways that regulate myelination. Previous studies have shown that the amount of Neuregulin1 (NRG1) type III on the axon surface triggers myelination by Schwann cells and regulates myelin sheath thickness (Michailov et al, 2004; Tavveggia et al, 2005). Type III NRG1 binds to the erbB2/3 heterodimer on Schwann cells, activating downstream signaling pathways including PI 3-kinase, which is critical for myelination (Maurel and Salzer, 2000). In this study, we have examined the role of the serine/threonine kinase, Akt, a key downstream effector of PI 3-kinase. We have generated and are characterizing transgenic mice in which constitutively active (CA) Akt constructs (DD-Akt and myr-Akt) are expressed in Schwann cells and oligodendrocytes under the control of the 2', 3'-cyclic nucleotide phosphodiesterase (CNPase) promotor. These mice exhibit hypermyelination, i.e. increased myelin sheath thickness in both the CNS and PNS. Some myelinated fibers in the PNS show outfoldings similar to that observed in CMT4 patients. In unmyelinated fiber tracts, CA Akt expression does not convert Remak Schwann cells to a myelinating phenotype but does drive dramatic membrane expansion and axon wrapping by these cells. Conversely, inhibiting Akt activity with perifosine blocks Schwann cell myelination in cocultures in a Krox20 independent manner. Taken together, these results suggest that Akt is a key signal downstream of NRG1 that drives wrapping of axons by Remak Schwann cells and the sheath thickness of myelinating Schwann cells, independent of effects on transcription.

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ONGOING EXPRESSION OF THE OLIGODENDROCYTE TRANSCRIPTIONAL REGULATOR MYELIN GENE REGULATORY FACTOR (MRF) IS REQUIRED FOR THE MAINTENANCE OF MYELIN INTEGRITY IN THE ADULT CNS

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In the past decade much progress has been made in elucidating the transcriptional control of oligodendrocyte differentiation and myelination, with factors such as Sox10, the Oligs and Nkx family members shown to have pivotal roles in aspects of oligodendrocyte development. In addition, we have recently identified a novel transcriptional regulator, Myelin Gene Regulatory Factor (MRF) that is essential for the transition of a pre-myelinating oligodendrocyte into a myelinating oligodendrocyte expressing the full complement of myelin genes. Less is known, however, about whether maintenance of myelin in the adult CNS requires ongoing expression and activity by the same set of transcription factors that are required during oligodendrocyte development. Here, we make use of PLP-CreERT2 transgenic mice to conditionally inactive the MRF gene within mature oligodendrocytes in adult mice. This results in a severe CNS-specific demyelination that occurs over an eight-week period, accompanied by significant microglial activation and functional impairment. These results indicate that in addition to being required for the final stages of oligodendrocyte differentiation, MRF expression in oligodendrocytes is required on an ongoing basis to maintain the integrity of CNS myelin. These observations will form the basis for ongoing work identifying the immediate target genes of MRF, and may also provide a reliable non-immune mediated model of CNS demyelination/remyelination.

GAMMA-SECRETASE IS REQUIRED FOR MICROGLIA ALZHEIMER'S BETA AMYLOID CLEARANCE

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The cleavage of amyloid precursor protein (APP) by gamma-secretase, plays an important role in the pathogenesis of Alzheimer's disease (AD). Thus, gamma-secretase is a target for the development of AD therapeutics and specific inhibitors are currently in clinical trials. Nevertheless, gamma-secretase also cleaves other receptors such as notch receptors, which control cell development and homeostasis. Our aim was to investigate whether gamma-secretase can also mediate microglial phagocytosis of Alzheimer's disease beta amyloid. We have demonstrated that gamma-secretase inhibitors impair microglial activity, as seen in gene expression, protein expression, migration ability and which resulted in a reduction of soluble beta amyloid phagocytosis. We further showed that dysfunction gamma secretase catalytic site lead to microglia impairment in clearing insoluble beta amyloid from brain sections taken from an Alzheimer's disease mouse model, as compared to microglia from wild-type mice. In this work, we suggest for the first time, a dual role of gamma-secretase in Alzheimer's disease. One role is the cleavage of amyloid precursor protein towards beta amyloid production and the other is to regulate microglia activity that is important for clearing amyloid deposits.

BERGMANN GLIA MODULATE PATHOGENESIS IN A TRANSGENIC MURINE MODEL OF SPINOCEREBELLAR ATAXIA TYPE 7.

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Spinocerebellar ataxia type 7 (SCA7) is a dominantly inherited disease resulting in the progressive degeneration of specific neuronal populations including cerebellar Purkinje cells (PCs). SCA7 is caused by the expansion of a CAG/polyglutamine(polyQ) repeat in the ataxin-7 gene. We have previously reported that directing expression of polyQ-expanded ataxin-7 to Bergmann glia (BG) in transgenic mice leads to ataxia and non cell-autonomous PC degeneration. We set out to further examine the BG pathology and its contribution to the behavioral and histological phenotype of SCA7 mice. We generated a novel SCA7 mouse model in which polyQ-expanded ataxin-7 flanked by loxP sites is ubiquitously expressed by the mouse prion protein transgene promoter in a bacterial artificial chromosome. This floxed-SCA7-92Q line demonstrated progressive ataxia and PC degeneration on a time course similar to previously published SCA7 transgenic mouse models with wide spread expression of the mutant protein. Additionally, our model exhibited a measurable reduction in the number of BG processes within the cerebellar molecular layer. To conditionally inactivate polyQ-expanded ataxin-7 expression in BG, we generated mice expressing our floxed mutant ataxin-7 transgene and a transgene in which Cre recombinase is controlled by the human glial fibrillary acidic protein (GFA2) cassette, a promoter known to direct expression to murine BG. In floxed-SCA7-92Q;GFA2-Cre bigenic mice, the mutant transgene was excised in approximately 70% of BG. Using a composite phenotype score and accelerating rotarod analysis, we determined the removal of polyQ-expanded ataxin-7 from BG partially ameliorates the SCA7 behavioral phenotype. Concurrently, we generated mice bigenic for the floxed-SCA7-92Q transgene and a Cre transgene with PC-specific expression. Excision of the mutant SCA7 transgene from the majority of PCs also partially reversed the SCA7 behavioral phenotype. Analysis of BG morphology in both bigenic lines is ongoing. In summary, polyQ-expanded ataxin-7 expression is required in both PCs and BG to recapitulate SCA7 pathogenesis in mice, suggesting that neurodegeneration in SCA7 results from dysfunction that develops in both neurons and glia.

MICE DEFICIENT FOR THE EXPRESSION OF SCHIP-1 PRESENT MILD AGE-DEPENDENT ABNORMALITIES OF PERIPHERAL MYELINATED FIBERS AND DISPLAY SOME DEGREE OF ATAXIA

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Myelination provides an insulating myelin sheath, regularly interrupted at nodes of Ranvier (NR) where voltage-gated Na+ channels are concentrated. The Na+ channels are localized in the axonal membrane of the nodes where they are part of multimolecular complexes which present striking similarities with those identified in the axonal initial segment of neurons (AIS) where action potentials are generated.
SCHIP-1 is a protein highly enriched at NRs and AISs. To shed light on the possible role of SCHIP-1 in vivo we generated mice deficient for its expression. Adult mutant mice appeared normal, but suffered from a mild growth delay. Analysis of PNS myelinated fibers revealed morphological anomalies of NR and a progressive decrease in their number within the nerve. Additionally, nodal accumulation of some of the NR proteins was progressivly perturbed with age. Electron microscopy of semi-thin sections of phrenic nerve of old mice revealed a slight decrease in axonal diameter, g-ratio, and fibers number, as compared to wild type mice, suggesting also axonal alterations in mutant mice. Sciatic nerve conduction was not significantly different between mutant and wild type littermates. However, CAPs duration was increased in mutant mice, suggesting a mild conduction defect in small caliber fibers. Therefore our results show that SCHIP-1 has a minimal importance in the formation and function of the NRs in young mice, but support the hypothesis that it plays a critical role in their maintenance. Analysis of the clinical phenotype of the mutant mice revealed subtle walking problems, including abnormal hind limb spreading. Tests of motor coordination also revealed impairments. However, the muscular strength of mice did not show any deficit. We also showed that mutant mice displayed slightly reduced pain sensitivity. Thus, SCHIP-1 mutant mice presented some degree of ataxia which could correspond to sensory defects of the PNS nerves. However, since histological and physiological alterations of peripheral nerves appeared to be minor, these symptoms may also involve defects of the CNS.

ANTI-INFLAMMATORY AND NEUROPROTECTIVE EFFECTS OF C/EBPS INHIBITION IN MICROGLIAL CELLS

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Neuroinflammation, a process in which reactive glial cells are involved, is suggested to play a role in the development of neurodegenerative diseases. The CCAAT/enhancer binding protein (C/EBP) family of transcription factors is involved in glial activation by regulating the expression of pro-inflammatory genes. Our objective in this study was to investigate the role of C/EBPs in neuroinflammation and neurotoxicity induced by reactive glial cells.

As a model of neuroinflammation, we induced neuronal death in neuronal-microglial co-cultures using LPS/IFNγ to activate microglia. Specific pharmacological inhibitors of C/EBPs are not available, making it difficult to study their role in neuroinflammation. As an alternative, we studied the neuroprotective role of an anti-inflammatory agent, the flavonoid chrysin, and the involvement of C/EBPs in its mechanism of action. Chrysin pre-treatment inhibited the pro-inflammatory phenotype in LPS/IFNγ-treated microglia (NO and TNF-α release, and iNOS expression) and the resulting neurotoxicity. These effects occurred in the presence of a decrease in C/EBPδ mRNA and protein expression induced by LPS/IFNγ, while no effect on C/EBPβ and p65 nuclear expression or DNA binding was observed.

We also studied glial activation in C/EBPβ KO mice using mixed glial cultures treated with LPS or LPS/IFNγ. We observed an inhibition of the inflammatory response in C/EBPβ KO cultures (COX-2, IL1β, and iNOS mRNA expression, iNOS and COX-2 protein expression and NO release). Our results show that the inhibition of C/EBPs expression results in a decreased glial activation. In addition, we show for the first time that C/EBPδ inhibition protects from the neurotoxicity induced by reactive microglial cells. We suggest C/EBPs as possible therapeutic targets against neurodegenerative processes occurring in the presence of neuroinflammation.

CONTROL OF MICROGLIA ACTIVATION THROUGH THE INNATE IMMUNE RECEPTORS CD200R1 AND TREM-2

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Neuroinflammation, in which reactive glial cells (mainly microglia) are involved, is suggested to play a role in the development of neurodegenerative diseases. Microglia are the principal immune cells of the CNS. In the control brain, these cells are continually surveying their microenvironment, ready to detect changes that could compromise neuronal function. In response to brain tissue injury, they develop morphological and functional changes, among them the production of pro-inflammatory mediators with potentially neurotoxic consequences. This reactive/pro-inflammatory phenotype is inhibited in the normal brain through a strict control through a series of "off-on" signals. CD200 (neuronal) and CD200R1 (microglial) constitute an "off-signal". It is present in control conditions and its absence determines the onset of pro-inflammatory microglial activation. TREM-2 (microglial) activation is an "on-signal". It is inducible and its activation determines the onset of an anti-inflammatory microglial response. The mechanism of action of these signals is still unknown.

The objective of the present work is to study the role of CD200-CD200R1 and TREM-2 in the modulation of the inflammatory response in reactive microglial cells and the resulting neurotoxicity. As a first approach we determined the response of CD200R1 and TREM-2 to pro-inflammatory and anti-inflammatory stimuli using microglial cell cultures. We also studied the modulation of CD200 (CD200R1 ligand expressed in neurons) in neuronal cultures in the presence of apoptotic or necrotic neuronal death, which are associated to anti-inflammatory or pro-inflammatory microglial activation respectively.

We believe that the modulation of the signal induced by these molecules may constitute a target to act against neurodegenerative processes occurring in the presence of neuroinflammation.

DEFICIENCY OF THE PROTEIN TYROSINE PHOSPHATASE SHP-1 IN NORMAL APPEARING WHITE MATTER OF MS SUBJECTS

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Multiple Sclerosis (MS) is a chronic immune mediated demyelinating disease of unknown etiology. Only recently has research begun to discover some of the molecular abnormalities that occur in the MS. We have previously shown that the protein tyrosine phosphatase SHP-1 is reduced in PBMC's and macrophages of multiple sclerosis patients. Studies in mice have demonstrated that SHP-1 is a crucial negative regulator of pro-inflammatory cytokine signaling, Toll-like receptor signaling, and inflammatory gene expression involved in demyelination. Furthermore, we have shown that mice lacking functional SHP-1 display myelin abnormalities and an extreme susceptibility to inflammatory demyelination relative to wild-type mice. We hypothesize that the increased vulnerability to demyelination may be accounted for in part by the loss of SHP-1 in oligodendrocytes. In order to determine if SHP-1 may also play a role in oligodendrocytes of MS subjects we examined oligodendrocytes in normal appearing white matter (NAWM) of MS and normal brains. We found that SHP-1 is particularly enriched in oligodendrocytes and that SHP-1 expression is reduced in NAWM relative to WM of controls. We and others have also found inflammatory cytokines to be increased in NAWM. SHP-1 has been demonstrated to be critical in terms of modulation the immune response and proper development of oligodendrocytes; therefore a reduction in SHP-1 may represent a crucial factor in the pathophysiology of MS.

IRON ACCUMULATION IN ALEXANDER DISEASE ASTROCYTES

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Alexander Disease (AxD) is a leukodystrophy caused by heterozygous mutations in *GFAP*. Infantile AD patients suffer from seizures, extensive de/dysmyelination, neuronal death, and hydroencephaly. Astrocytes in white matter and at the pial surface contain Rosenthal fibers (RFs), dense inclusions containing GFAP, small heat shock proteins, and other proteins. Mutant GFAP produces cell stress, including upregulation of MAPK pathways, autophagy, and proteasome inhibition. In this study we examined AxD CNS for iron, an important cofactor in oligodendrocytes, essential for myelination. Previously, mice with a knock-in mutation equivalent to that found in patients with a common *GFAP* mutation were found to have accumulated Fe 3+ iron in astrocytes (Hagemann et al., 2006). We stained AxD CNS tissues for Fe3+ by the enhanced Perl's method and by immunocytochemistry for ferritin and found both associated with RFs. Additionally we found ferroportin, putatively the sole protein allowing iron exit from astrocytes, accumulated in the cell bodies of astrocytes. In primary astrocyte cultures obtained from mice expressing both transgenic human *GFAP* and a knock-in mouse *GFAP* mutation, we found that ferroportin accumulated in large, perinuclear inclusions that also contained GFAP. In these cultures all astrocytes contained ferritin, while in cultures from wild type mice, ferritin was not detectable in all astrocytes. Sequestration of iron in AxD astrocytes may increase the levels and consequences of cellular oxidative stress and make astrocytes more toxic to oligodendrocytes and neurons.

ABERRANT ACTIVATION OF MTOR IN PERIPHERAL NEUROPATHY

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Demyelination is a significant source of morbidity in nerve injuries and neuropathies, however, the mechanisms involved have remained elusive. Demyelination is frequently associated with, and may result from dedifferentiation of Schwann cells and the consequent breakdown of their myelin sheaths. Our lab has developed an experimental model of demyelination that involves addition of the Neuregulin-1 (NRG1) growth factor to myelinating cocultures. This results in the activation of the erbB receptors and their downstream signaling pathways followed by Schwann cell dedifferentiation and demyelination. Among the pathways activated by NRG1 is the mammalian target of rapamycin (mTOR), a serine/threonine kinase that regulates the translational apparatus and integrates a number of upstream signals. We have found that inhibiting mTOR with rapamycin effectively prevents NRG1-induced demyelination. Recent results suggest that mTOR is also aberrantly activated in the PMP22 overexpressing transgenic rat, a murine model of the demyelinating human neuropathy Charcot-Marie-Tooth 1A (CMT1A). This activation leads to Schwann cell dedifferentiation as inhibition of mTOR with rapamycin rescues myelination in a tissue culture model of CMT1A. Current studies seek to elucidate further the role of mTOR in Schwann cell dedifferentiation, and the mechanism by which alterations in gene dosage of PMP22 lead to its activation.

RETINAL DEGENERATION ALTERS GLIAL GENE EXPRESSION IN THE VISUAL CORTEX

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Astrocytes in the CNS are putative mediators of synaptogenesis and neural plasticity. The outgrowth and retraction of astrocytic processes represent a mechanism by which these cells could influence the surrounding neural milieu. Specific subclasses of astrocytes have been identified on the basis of expression of cytoskeletal Type III intermediate filament (IF) genes such as glial fibrillary acidic protein (GFAP) and vimentin. These IFs are important in determining process length; the expression of IF genes is tightly regulated throughout the development of the CNS when neural connections are first established. We investigated whether astrocyte-specific IF genes were affected by loss of vision caused by retinal degeneration, a neurodegenerative disorder that causes aberrant connections within the visual pathway. We utilized two lines of mice: Pde6b- mice with progressive retinal degeneration due to a mutation in the gene that codes for phosphodiesterase 6 beta subunit, and Pde6b+ mice which served as congenic controls. In an earlier phase of this study, we found that Pde6b- mice began showing significant visual impairments between postnatal day (PND) 28 and PND 35. Using this timepoint as a guideline, we evaluated whether IF gene expression was altered in the visual cortex by quantifying GFAP and vimentin gene expression using real time PCR (N = 40). We found that expression of GFAP was reduced, and vimentin expression was altered transiently, between PND 28 and PND 49 in the visual cortex of Pde6b- mice. We are currently evaluating IF protein expression and process length in subclasses of astrocytes in the visual cortex with Western blotting and immunohistochemistry. Our findings tentatively support the conclusion that loss of sensory inputs to the visual cortex, caused by retinal degeneration, ultimately leads to transient alterations in the expression of GFAP and vimentin. Notably, this is associated with the onset of visual impairments. It remains to be determined whether transient alterations in IF gene expression enhance brain plasticity, and present a unique opportunity to optimize retinal implant interventions.

INFLAMMATORY RESPONSES ARE NOT SUFFICIENT TO CAUSE DELAYED NEURONAL DEATH IN ACUTELY INJURED BRAIN

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Generally, in injured brain, microglia have been known to produce inflammatory mediators and cause delayed neuronal death. However, the results in this study showed that in acutely injured brain, blood inflammatory cells as well as microglia participate in brain inflammation but that the inflammatory responses were not neurotoxic. When ATP was injected into the substantia nigra pars compacta (SNpc) and cortex, Iba-1+ microglia as well as neurons died in the injection core within 3 h, and morphologically activated thick process-bearing microglia were arranged around the injury sites. Round Iba-1+ cells highly expressing CD45 (a marker of monocytes) appeared in the penumbra at 1- 2 d, filled the core at 7 d, and then rapidly disappeared at 14 d. Interestingly, in ATP-injected brain, iNOS was barely detectable at all times. Although IL-1β was detectable from 3 h to 1 d, PBS also induced IL-1β as much as ATP did. CD68 that represents phagocytic activity was detectable in the area where CD45+ cells existed. Importantly, the total numbers of TH+ neurons at 3 h (about 80% of those in the contralateral sides) did not decrease further at 7 d. In the cortex, similar results were obtained: ATP induced acute death of neurons and microglia within 3 h but neuron-damage area did not increase for up to 7 d. These results suggest that microglia appear to isolate injury sites and blood monocytes appear to phagocyte damaged cells and debris, but their inflammatory responses are not sufficient to cause delayed neuronal death.

BEHAVIOR AND FUNCTIONS OF MICROENVIRONMENT-REGULATING CELLS IN SPINAL CORD INJURY: IMPORTANCE OF ASTROCYTES

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Traumatic spinal cord injury (SCI) causes acute neuronal death followed by delayed secondary neuronal damage. As a candidate to cause secondary damage, changes in microenvironment of the spinal cord could be condidered. Since microglia and astrocytes respond to the traumatic damage and blood cells infiltrate the injured tissue, we examined how these cells behave and affect secondary neuronal damage. We designated the sites of the injured spinal cord as epicenter (the damage site), C1 (0.9 mm caudal from the epicenter where delayed neuronal death occurred), and C2 (1.8 mm caudal from the epicenter where delay neuronal death did not occur). Major findings in this study is that first, neurons in the ventral region were much more vulnerable than neurons in the dorsal regions: at the epicenter and C1 region, neurons in the ventral region died within 3 h and at 14-18 h, respectively, but neurons in the dorsal region survived up to 3 d. Second, microglia and blood monocytes appeared not to be related to delayed neuronal death: microglia underwent death earlier than neurons in C1 ventral region, and activated in C2 region where neurons did not die. Monocyte infiltration occurred after neurons died, and monocytes showed alternatively activated phenotypes rather than classically activated ones. Third, astrocytes were different in the morphology and function in dorsal and ventral regions: dorsal astrocytes have highly branched and interacting processes while ventral astrocytes have long and thin processes. Furthermore, dorsal astrocytes had higher capacity to uptake potassium and glutamate in normal and injury-mimic state although mRNA levels of potassium and glutamate uptake system, GLT-1/GLAST and Kir4.1, respectively, were lower in the dorsal regions compared to the ventral regions. Accordingly, in the dorsal areas at the epicenter and C1 region, and dorsal and ventral areas of C2 region, neuron survival was correlated with astrocytes existence. Taken together, these results suggest that microenvironment maintained by astrocytes could be more important for neuronal survival than those regulated by microglia and monocytes.

INDUCIBLE ABLATION OF NG2$^+$ GLIAL CELLS IN THE ADULT BRAIN

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NG2$^+$ cells constitute a fourth major glial population in CNS. Although they serve as progenitors for myelinating oligodendrocytes in early development, their roles in the mature CNS are less clear, as many do not undergo differentiation. To determine the functions of NG2$^+$ cells in the normal and diseased CNS, we generated *NG2-floxed EGFP-STOP-DTa* BAC transgenic mice, in which diphtheria toxin is expressed under the control of NG2 promoter only after tamoxifen-induced activation of Cre. After these mice were crossed to *Olig2-CreER* mice, we were able to ablate both cycling and non-cycling NG2$^+$ glial cells in the adult brain by administering 4-hydroxytamoxifen (4HT). In mice aged P70 or older, 80-95% of cortical NG2$^+$ cells disappeared within 3-4 days after exposure to 4HT (7mg in 4 days). The ablation of NG2$^+$ cells was not accompanied by changes in the number of astrocytes, oligodendrocytes, microglia, vascular cells (NG2-expressing pericytes) or neurons, indicating that these mice allow selective deletion of NG2$^+$ glial cells in the adult brain. Moreover, this ablation did not induce overt reactive changes in microglia or astrocytes, and did not result in obvious alterations in the behavior of the mice. In response to incomplete ablation, remaining NG2$^+$ cells rapidly began to proliferate, and eventually repopulated the entire cortex in 1-3 weeks, with the rate of repopulation varying with the extent of cell loss. Almost all NG2$^+$ cells incorporated BrdU during repopulation, suggesting that few, if any, NG2$^+$ cells are postmitotic in the adult brain. After crossing these mice with NG2-Lck-EGFP BAC transgenic mice, we monitored the behavior of NG2$^+$ cells *in vivo* using time-lapse, two-photon imaging for days to weeks after 4HT administration. These studies confirmed that administration of 4HT to these mice triggered the death of cortical NG2$^+$ cells, and revealed the behavior of newly generated NG2$^+$ cells during repopulation. These studies indicate that NG2$^+$ cells are capable of rapid cellular homeostasis in the absence of brain injury, demyelination or damage to oligodendrocytes, suggesting that each NG2$^+$ cell continually monitors the status of other neighboring NG2$^+$ cells. The ability to selectively remove NG2$^+$ cells *in vivo* may help define the mechanisms that regulate the growth and development of these progenitors, as well as reveal other roles of these ubiquitous glial cells. Supported by the NIH (NS051509, NS050274).

UDP INDUCES CHEMOKINE EXPRESSION IN MICROGLIA AND ASTROCYTES THROUGH ACTIVATION OF THE $P2Y_6$ RECEPTOR.

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Chemokines play critical roles in inflammation by recruiting inflammatory cells to injury sites. In this study, we found that UDP induced expression of chemokines, CCL2 and CCL3, in microglia, astrocytes, and slice cultures, by activation of $P2Y_6$. Interestingly, CCL2 was more highly expressed than CCL3. However, CCL2 synthesis kinetics in response to UDP differed in microglia and astrocytes; microglia rapidly produced small amounts of CCL2 whereas astrocytes slowly and continuously synthesized CCL2, resulting in a high ultimate level of the chemokine. UDP-induced chemokine expression was reduced in the presence of a specific antagonist of $P2Y_6$ (MRS2578), or siRNA directed against the $P2Y_6$ gene. Inhibition of phospholipase C (PLC) and calcium increase, downstream signaling pathways of Gq-coupled $P2Y_6$, reduced UDP-induced chemokine expression. UDP activated two calcium-activated transcription factors, NFATc1 and c2. Furthermore, inhibitors of calcineurin (a phosphatase activating NFAT) and NFAT reduced UDP-induced chemokine synthesis. We also found, using a transmigration assay, that UDP-treated astrocytes recruited monocytes. These results suggest that UDP induces chemokine expression in microglia and astrocytes of the injured brain by activation of $P2Y_6$ receptors.

EARLY ACTIVATION OF MICROGLIA HAS A CENTRAL ROLE IN THE DISEASE PATHOGENESIS OF UNVERRICHT-LUNDBORG DISEASE (EPM1)

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Unverricht-Lundborg disease type 1 (EPM1, OMIM 254800) is an autosomal recessively inherited neurodegenerative disorder with progressive course and the most common progressive myoclonic epilepsy. Onset lies between 6 and 16 years and the symptoms include tonic-clonic seizures, stimulus-sensitive progressive myoclonus, ataxia and a characteristic EEG. It is caused by loss-of-function mutations in the cystatin B gene (*CSTB*, OMIM 601145), which encodes an inhibitor of lysosomal cysteine proteases. However, the exact function of CSTB remains to be elucidated.
A mouse model for EPM1, Cystatin B-deficient (*Cstb -/-*) mouse, has been generated by targeted disruption of the mouse *Cstb* gene. *Cstb-/-* mice develop myoclonic seizures and progressive ataxia recapitulating the clinical features of the human disease. Mice also show severe loss of cerebellar granule cells by apoptosis and wide-spread gliosis.
Microglial activation is a common hallmark of neurodegenerative diseases. Activated microglia produce a variety of inflammatory mediators, such as cytokines, chemokines, reactive free radicals and proteases, which can be detrimental for neurons and brain homeostasis, if their release is not tightly regulated. Interestingly, in *Cstb-/-* mice, activated microglia showing atypical morphology is evident in selected brain areas already in presymptomatic mice.

In this study, we elucidate the microglial contribution to the neuronal dysfunction and selective neuronal death of cerebellar granule cells in EPM1. We aim to characterize the functional properties of *Cstb-/-* microglia and their effects on the survival of *Cstb-/-* neurons. Our results show that the Cstb mRNA level in cultured wild type mouse microglia shown by real time quantitative PCR is high in relation to astrocytes or neurons, suggesting that CSTB has a pivotal role in microglia. Moreover, LPS stimulation induces increased release of chemokines from *Cstb-/-* microglia into the media indicating altered inflammatory response due to the CSTB deficiency. Also, neurons cultured with *Cstb-/-* microglia display neuritic fragmentation and cell death.
Our data give insight into the role of microglia in EPM1 and provide a basis for further detailed studies on the pathophysiology of this devastating disease.

ASTROCYTE-SECRETED FACTORS MODULATE DENTATE GRANULE CELL MIGRATION.

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Proper migration and layering of the dentate granule cells are fundamental to the establishment of functional neural networks in the hippocampus. However, the mechanisms that regulate the incorporation of migrating granule cells to the cell layer remain unclear. Because the granule cells perform radial migration tightly attaching onto GFAP-positive glial scaffold, we hypothesized that astrocyte-derived factors modulate the cell migration. In this study, we show that astrocyte-secreted factors modulate granule cell migration by decreasing the expression of polysialic acid (PSA). PSA is a long linear homopolymer of alpha-2,8-linked sialic acid, which is added to the fifth Ig-like domain of the neural cell adhesion molecule (NCAM). The expression of PSA on the cell surface is known to reduce cell interactions and promote neuronal migration. We prepared hippocampal slices from postnatal day (P)14 rats and cultured them in the presence of NeuAc2en, an inhibitor of neuraminidases/sialidases. Although newborn granule cells terminated their migration in the inner granule cell layer in control cultures, a number of them were found in the outer granule cell layer in NeuAc2en-treated cultures. Next, we examined whether astrocytes regulate PSA expression of the granule cells. For this purpose, we prepared astrocyte-conditioned medium (ACM) from the postnatal rat dentate gyrus and applied it to explant cultures of the dentate hilus. We found that ACM increased the expression of neuraminidase and reduced PSA expression in the granule cells, inhibiting their emanation from the explants. Importantly, these phenomena were blocked by NeuAc2en, which suggests that astrocyte-secreted factors modulate the migration of granule cells via activating neuraminidases. These results indicate that astrocyte-secreted factors induce PSA degradation which is required for the proper incorporation of granule cells to the cell layer.

ALTERATIONS IN THE PROPERTIES OF OLIGODENDROCYTES IN THE NEURONAL CEROID LIPOFUSCINOSIS CLN8

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The neuronal ceroid lipofuscinoses (NCLs; CLN1-CLN10), a group of inherited lysosomal storage disorders, are the most common cause for pediatric neurodegeneration. Their clinical course includes progressive mental and psychomotor retardation, epilepsy, blindness and premature death. Mutations in the *CLN8* gene account for two types of NCL: Northern epilepsy in Finland and the late infantile form enriched in the Mediterranean. The motor neuron degeneration mouse, mnd, harbors a mutation in the *Cln8* gene and is used as a model to study CLN8 disease mechanisms.

Mechanisms of neuronal dysfunction in the *CLN8*-related NCLs are largely unknown. Based on sequence homology, CLN8 is linked to a TLC (TRAM, LAG1, CLN8) superfamily of proteins, which have been proposed to have a role in lipid biosynthesis, metabolism and sensing. Supporting this in silico finding, we have shown perturbed sphingolipid metabolism in Northern Epilepsy patient brains. In addition, we have shown a decrease in myelin-specific galactolipids in mnd mouse brains as well as delayed myelination. This was explained by the reduced activity and mRNA level of galactolipid-synthesizing enzyme UDP-galactose:ceramide galactosyltransferase (*Ugt8A*).

Oligodendrocytes are the main *Cln8* expressing cells in the CNS and responsible for synthesis of galactolipids as well as myelin formation. In this study, we have used neural progenitor cells from mnd to investigate whether the reduced levels of galactolipids have consequences on oligodendrocyte development and maturation. Our results show enhanced neural progenitor proliferation. Neural progenitors exit the cell cycle normally, and produce neurons, astrocytes and oligodendrocytes of different developmental stages. In vivo, by real-time PCR, we have observed decreased oligodendrocyte/myelin marker expression in mnd brains.

In conclusion, our study indicates oligodendrocytes as the key cell type in the CLN8 disease. These findings are critical for understaning the pathomechanisms of CLN8 disease as well as the physiological function of the CLN8 protein.

AN EVOLUTIONARY CONSERVED ENHANCER MEDIATES OLIG2-DEPENDENT SOX10 EXPRESSION IN OLIGODENDROCYTE PRECURSORS

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The HMG-domain transcription factor Sox10 is expressed throughout oligodendroglial development and is an important component of the transcriptional regulatory network in this glial cell type of the central nervous system. Of the *Sox10* regulatory regions identified so far, only one exhibits activity in oligodendroglia. To better understand oligodendroglial Sox10 expression the activity and regulation of this evolutionary conserved U2 enhancer located in the 5' flanking region of the *Sox10* gene was analyzed both *in vitro* and in transgenic mice. We found that U2 was active in oligodendrocyte precursors, but not in differentiating oligodendrocytes. U2 activity also failed to account for initial Sox10 induction in oligodendrocyte precursors immediately after their specification pointing to the existence of additional regulatory regions which extend Sox10 expression in oligodendroglia beyond U2 activity. In oligodendrocyte precursors, U2 activity was strictly dependent on the bHLH transcription factor Olig2. In fact, Olig2 bound U2 through a highly conserved site in the part of its sequence that displayed the highest sequence conservation, and activated the enhancer directly. The closely related bHLH transcription factor Olig1 by contrast showed little binding and was a much less efficient activator of the U2 enhancer. Additionally, U2 activity was repressed by the homeodomain transcription factor Nkx6.2 in a manner that did not require site-specific binding of Nkx6.2 to U2 and probably involved sequestration of U2 activators including Olig2. Considering the altered ratio of Olig1 versus Olig2 and the onset of Nkx6.2 expression in differentiating oligodendrocytes, both factors likely contribute to the downregulation of U2 activity during terminal differentiation in this glial cell type.

ANTI-NEURONAL , ANTI-GLIAL AUTOANTIBODIES AND NEUROINFLAMMATION IN EXPERIMENTAL SPINOCEREBELLAR ATAXIA

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Spinocerebellar ataxia type 1 (SCA1) is a debilitating neurodegenerative disorder caused by an expanded CAG trinucleotide repeats within the coding sequence of the ataxin-1 protein. There are many evidences of inflammatory mechanisms of such disease. In order to prove this theory we the levels of different types of antibodies directed against neuronal and glial structures within SCA1 mice CNS. These mice considered as a most close models of SCA in humans. Autoantibodies (AAB) against Purkinje cells (PC), AAB against GAD, which is glutamic acid decarboxylase, an enzyme that catalyzes the decarboxylation of glutamate to GABA and CO2 , and , finally, proper anti-GABA (gamma aminobutiric axid) B receptor have been studied in the serum and cerebrospinal fluid (CSF) of 24 SCA1 mice. Control group consisted of 56 intact mice without any signs of ataxia or any other movement deficiency. We used Western blot analysis and ELISA and immunohistochemical studies, which revealed on mouse 2 μm brain sections of cerebellum, brain stem, hypothalamus and thalamic areas binding of high titer (up to 1:5.000) IgG AAB to the cerebellar molecular layer and white matter. Highly specific antibodies for PC were found binding for axons, dendrites and both sides of PC cell's membrane. These AAB have been produced by B-cell clones within the CNS, which we found by staining these brain slices with immunofluorscent antibodies. It was discovered significant neuronal (mostly PC) loss in the cerebellar cortex with infiltratinf C8+ B cells. Reactive astrogliosis and microglial activation have been detected in terms of increasing of Bergmann glia and considerable microglial proliferation. Markedly increased levels of anti-GAD and anti-GABA B receptor AAB were obtained both in the serum and CSF in experimental animals in comparison with the control mice. Our findings suggest the presence of inflammation in PC revealing by astroglial and microglial activation. Western blotting of SCA mice1 cerebellum extract showed binding of CSF and serum IgG to a 76-84 kDa protein that presented exactly in the PC. On the other hand we couldn't rule out a possible role of neoplastic cells in producing GAD AAB, which could be considered as a paraneoplastic marker in an experimental SCA1. Another approach emerged when anti-GABA B receptor AAB were revealed in the serum and CSF of SCA1 mice. Since this mice biology has been associated with signs of neuroinflammation with an active role of glial elements, the next step would include injection of anti- GABA B receptor AAB intratecally into cistern magna of SCA1 and intact mice for comparison of movement activity and neuroimmunological properties in brain morphology and future immunochemical studies.

ESTROGEN REVERSES MANGANESE-INDUCED IMPAIRMENT OF ASTROCYTIC GLUTAMATE TRANSPORTERS BY THE ENHANCEMENT OF TGF-A SIGNALING VIA THE GPR30 PATHWAY

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Chronic manganese (Mn) exposure causes manganism, a neurological disease resembling idiopathic Parkinson's disease. While the mechanism of Mn-induced neurotoxicity has not been completely established, glial cells play a crucial role in mediating its neurotoxicity as Mn induces impairment of astrocytic glutamate transporters. Two astrocyte-encoded glutamate transporters, GLAST and GLT-1, are primarily responsible for the removal of excess glutamate from the extracellular space and thus play a central role in preventing excitotoxic neuronal death. Numerous studies have reported that 17β-Estradiol (E2) and tamoxifen (TX), a selective estrogen receptor modulator (SERM) exert neuroprotective effects in various animal and cell models. Interestingly, many experimental results illustrate that astrocytes are the main target of E2/TX action. Therefore, we hypothesized that E2 and TX protect against Mn-induced neurotoxicity by enhancing astrocytic glutamate transporter function via the transforming growth factor-α (TGF-α) pathway. Studies in rat neonatal primary astrocyte cultures revealed that Mn decreased GLT-1 levels (mRNA and protein), while E2 and TX treatment increased its expression and reversed the Mn-induced reduction in GLT-1 expression and glutamate uptake. The effects of Mn and E2/TX on GLT-1 were parallel to those with TGF-α; namely, Mn decreased, whereas E2/TX increased and reversed TGF-α expression. TGF-α increased GLT-1 expression as well as glutamate uptake and reversed the astrocytic Mn-induced glutamate uptake inhibition. Moreover, blockade of the epidermal growth factor receptor (EGFR), the receptor for TGF-α with AG1478 completely abolished the effect of E2 and TX on GLT-1 expression, providing additional evidence that TGF-α mediates E2/TX-induced GLT-1 expression. To determine the ER subtypes responsible for these E2 and TX effects, the selective agonists for each ER subtype, propyl pyrazole triol (PPT) for the ER-α, diarylpropionitrile (DPN) for the ER-β and G1 for GPR30, were tested in relationship to GLT-1 expression. Among the three ER subtypes, GPR30 appears to play the most critical role in the enhancement of astrocytic glutamate transporter function, because G1 induced the highest GLT-1 expression and glutamate uptake activity. Taken together, these results suggest that E2 and TX reverse Mn-induced impairment of GLT-1 expression and glutamate uptake activity, at least in part, by the enhancement of TGF-α signaling via the GPR30 pathway in astrocytes.

DENDRITIC CELLS ARE EARLY RESPONDERS TO RETINAL INJURY

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The presence and activity of dendritic cells (DC) in retina is controversial, as their limited numbers make them difficult to identify. Transgenic mice (CD11c-DTR Ly5.2) that express green fluorescent protein (GFP) on the CD11c promoter to label DC allowed the visualization and quantification of retinal DC. Retinal whole mounts were stained for GFP+ cells and microglia (GFP-CD11b+ MG), and analyzed by confocal microscopy. A retina injury model, the optic nerve crush (ONC), was used to study their injury response. Many GFP+ DC were recruited to the ganglion cell layer/nerve fiber layer (GCL/NFL) following an ONC; many GFP+ cells were tightly associated with retinal ganglion cell (RGC) nerve fibers. Conversely, very few MG were found in close contact. All of the GFP+ DC were CD11b+, suggesting a myeloid origin. In addition, the GFP+ DC upregulated expression of MHC class II after injury, while the MG did not. To study the origin of retinal GFP+ DC, radiation bone marrow (BM) chimeras [CD11c-DTR Ly5.2 recipients of Ly5.1 BM, and vice versa] were used. Retinal GFP+ cells were depleted by injecting diphtheria toxin (DTx) into the anterior chamber. Analysis of retinas from Ly5.1 recipients of CD11c-DTR BM, in the absence of injury, showed a slow increase in GFP+ cells. This suggests minimal turnover from grafted cells up to six months, even though the mice were irradiated. Retinas from Ly5.1 recipients of CD11c-DTR BM that received an ONC prior to harvesting had many donor GFP+ cells in the crushed eyes around the optic disc and in the periphery. These GFP+ cells appeared to be derived from circulating precursors. The persistence of retinal GFP+ cells in CD11c-DTR recipients of Ly5.1 BM up to 7 months pointed to a precursor that must come from a non-circulating source, possibly MG or local progenitor cells. The number of MG was found to be unchanged after the retinas of these mice were repeatedly treated with DTx. Thus, MG are unlikely to be contributors of GFP+ cells. The injection of DTx not only led to the depletion of GFP+ cells; it also seemed to deplete their local progenitor cells, as no GFP+ cells were found after the retinas had received an additional stimulus to recruit more GFP+ cells. These results suggest that MG do not contribute to the GFP+ cells. Use of the radiation BM chimeras together with DTx treatment showed that GFP+ cells were recruited, in part, from local precursors, and can also be recruited from circulating precursors. Overall, this study showed that DC were found in the retina and that they rapidly responded to neural injury. We propose that they are a previously overlooked population, distinct from MG, and may be important in the injury response.

GENETIC MOSAIC ANALYSIS REVEALS A CENTRAL ROLE OF OLIGODENDROCYTE PRECURSOR CELLS IN GLIOMAGENESIS

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Pinpointing the identity of tumor propagating cells (TPCs) in gliomas is critical for designing targeted therapies. Previous studies have postulated the neural stem cell (NSC) nature of TPCs based on their abilities to self-renew and to differntiate into mutiple cell types in cell culture. However, the sole dependence of end-point analysis could be misleading since late-stage tumor cells could acquire aberrant features during malignant transformation. An ideal approach to fully understand TPCs would be to analyze mutant cells throughout the tumorigenic process, with a greater focus on stages piror to the malignant transformation. To overcome the difficulty in identifying pre-transformed mutant cells free of identifiable pathological features, we used a mouse genetic mosaic model termed MADM to generate sporadic GFP-labeled mutant cells in an otherwise colorless normal mouse, enabling mutant cell visualization upon their generation. By inducing co-LOH of p53 and NF1 from embryonic NSCs, MADM model effectively induced glioma formation with almost full penetrance within 4-6 months. Genomic fingerprinting confirmed the close relevance of this model to the proneural subtype of human GBMs based on recent TCGA classification. Taking advantage of the unambiguous GFP labeling of mutant cells, we analyzed the extent of cell expansion in each and every CNS lineages in 2-month-old mice, prior to any signs of maligancy. Surprisingly, although mutations were induced in NSCs, there was no detectable over-proliferation of mutant NSCs compared to WT ones. Among all neuroglial lineages, the oligodendrocyte precursor cell (OPC) lineage was the only one showing drastic over-expansion. Upon tumor formation, marker staining and microarray analysis again revealed that actively proliferating tumor cells share many OPC features. Interestingly, purified OPC-like tumor cells could self-renew in culture, differentiate into multiple cell types upon induction, and initiate secondary tumors by orthograft assay. In conclusion, MADM enabled us to study TPC identity as a developmental process. Our findings suggest that stem cell features of TPCs may not necessarily be an indication of their NSC nature, rather these features could be acquired by mutant OPCs during malignant transformation.

CRITICAL ROLE OF HISTONE 3 LYSINE 9 METHYLATION IN OLIGODENDROCYTE PROGENITOR DIFFERENTIATION

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Demyelination and failure to remyelinate after demyelinating lesions contribute to the neuropathological changes observed in neurological disorders, such as multiple sclerosis. Oligodendrocytes are the myelin-forming cells in the central nervous system. We have previously demonstrated that the differentiation of oligodendrocyte progenitor cells (OPC) requires histone deacetylation, to decrease the transcript levels of oligodendrocyte differentiation inhibitors and neural stem cell markers. However histone deacetylation is a transient modification which is followed by histone methylation. Therefore we studied the role of repressive histone 3 lysine 9 methylation in OPC differentiation. We showed increasing levels of tri-methylated histone 3 lysine 9 (me3H3K9) as OPCs mature into myelinating oligodendrocytes and in the developing corpus callosum in mice. This was consistent with increased expression of the histone methyltransferases responsible for this modification, in the oligodendrocyte lineage. Inhibition of H3K9 methylation led to decreased expression of myelin genes and decreased the number of mature oligodendrocytes in primary cultures. Together, these data suggest a critical role of H3K9 methylation in OPC differentiation and myelination.

THE EXTRACELLULAR MATRIX MOLECULE, SC1, LOCALIZES TO DIFFERENT POPULATIONS OF REACTIVE ASTROCYTES FOLLOWING TRANSIENT FOCAL ISCHEMIA

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SC1 is an anti-adhesive molecule that contributes to brain plasticity. In rodents, it is expressed during CNS development, and its cellular distribution in adults is altered following status epilepticus. Here, we assessed SC1 expression and cellular distribution for the first time after stroke. A transient focal ischemic stroke was evoked by injecting the vasoconstrictor peptide, endothelin-1 (Et-1), into the striatum of adult Sprague-Dawley rats. Changes in cellular distribution of SC1 protein were examined *in vivo* after stroke, as well as in cultured primary astrocytes after Et-1 treatment. Three days after the stroke, SC1 increased in the cell bodies of GFAP-labeled hypertrophied astrocytes that formed a glial limitans, which became prominent at 7 days. This up-regulation resulted from the stroke, because SC1 was unchanged in cultured astrocytes treated with Et-1. Interestingly, at one day after stroke, SC1 was detected in the cell bodies but also along the processes of 'starburst'-shaped astrocytes bordering the lesion, which were no longer present by 7 days. These cells were positive for GFAP, but not vimentin, nestin, NG2 or CD11b. The subcellular distribution of SC1 differs among reactive astrocyte sub-types following ischemia, which might reflect different roles of these cells and SC1 in astrogliosis. The 'starburst' astrocyte might represent a novel subclass of reactive astrocyte that responds to neural insults earlier than the classical hypertrophied astrocyte. This work has broader implications because astrogliosis occurs following many forms of brain damage and neural injury.

MICRORNA-MEDIATED CONTROL OF OLIGODENDROCYTE DIFFERENTIATION

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MicroRNAs (miRNAs) regulate various biological processes, but evidence for miRNAs that control the differentiation program of specific neural cell types has been elusive. To determine the role of miRNAs in the formation of myelinating oligodendrocytes, we selectively deleted a miRNA-processing enzyme, Dicer1, in oligodendrocyte lineage cells. Mice lacking Dicer1 display severe myelinating deficits despite an expansion of the oligodendrocyte progenitor pool. To search for miRNAs responsible for the induction of oligodendrocyte maturation, we identified miR-219 and miR-338 as oligodendrocyte-specific miRNAs in spinal cord. Overexpression of these miRNAs is sufficient to promote oligodendrocyte differentiation. Additionally, blockage of these miRNA activities in oligodendrocyte precursor culture and knockdown of miR-219 in zebrafish inhibit oligodendrocyte maturation. miR-219 and miR-338 function in part by directly repressing negative regulators of oligodendrocyte differentiation, including transcription factors Sox6 and Hes5. These findings suggest that these miRNAs are important promoters for oligodendrocyte differentiation while inhibiting oligodendrocyte precursor proliferation, and that they are potential targets for myelin repair.

ROLE OF NECL-4 COMPLEX IN PNS MYELINATION

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Interactions between axons and Schwann cells in the peripheral nervous system (PNS) induce myelination and result in the organization of axons into distinct domains. The largest domain by far is the internode, the portion of the axon located under the compact myelin sheath. The molecules that mediate the functional relationship between axon and myelinating glial cell along the internode are still incompletely characterized. We and others reported that DRG neurons and Schwann cells express distinct Nectin-like (Necl) cell adhesion proteins [Maurel et al, JCB, 2007: 178(5) and Spiegel et al, Nat Neurosci, 2007 Jul:10(7)]. A member of this family of proteins, Nectin-like 4 (Necl-4) is expressed on myelinating Schwann cells and binds specifically to Necl-1 expressed on axons. These proteins were localized to the internode and mediate heterophilic interactions. Knock down of Necl-4 inhibits Schwann cell differentiation and subsequent myelination. These results demonstrate that Necl-4 is needed to initiate PNS myelination and implicate Necl proteins as mediators of internodal interactions.

A key question is how Necl-4 promotes myelination. Its structural features provide important clues. Necl-4 mediates cell adhesion through its extracellular, N-terminal Ig-like domains and has been proposed to interact with PDZ-domain and cytoskeletal proteins through different segments of its cytoplasmic domain. Par-3, a PDZ-domain containing protein of the Par-aPKC polarity complex, was previously reported to be required for Schwann cell myelination [Chan et al, Science, 2006: 314(5800)]. Our data indicates that Necl-4, through its PDZ-binding domain, interacts with Par-3 directly. Necl-4 also interacts with members of the 4.1 family of cytoskeletal proteins via its FERM-binding domain. Together these findings suggest a model where Par-3 is recruited by Necl-4 to the adaxonal membrane of the Schwann cell upon axon-glial contact. The localization of Par-3 to this site in turn may target and activate aPKC/Par-6, and promote formation of a leading edge in the Schwann cell that drives spiral wrapping of the axon. This Necl-4/Par complex is then stabilized through interactions between Necl-4 and protein 4.1G and its association with spectrin and the actin cytoskeleton, thereby promoting long-term stability of the myelin sheath.

HOXA2 REPRESSES ROSTRAL HINDBRAIN OLIGODENDROGENESIS

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Oligodendrogenesis does not develop simultaneously nor with similar spatial patterns in all regions of the CNS. Very little is known about the control of this diversity along the rostro-caudal axis. *Hox* genes encode homeodomain transcription factors which display spatially restricted rostro-caudal patterns of expression and control the regional patterning of neural progenitors in the hindbrain and spinal cord. Here, we explored the involvement of the *Hox* genes in the rostro-caudal patterning of oligodendrocyte precursor cells in the mouse hindbrain, which is subdivided in neuroepithelial compartments known as rhombomeres (r).
Hindbrain oligodendrogenesis showed a rhombomere-specific timing, with a marked delay in r2-r3. *Hoxa2* is the only *Hox* gene expressed in r2, whereas it is only co-expressed with its paralogue *Hoxb2* in r3, suggesting that the paralogue group 2 *Hox* genes could control oligodendrogenesis at this level of the CNS. We examined oligodendrogenesis in the rostral hindbrain of homozygous *Hoxa2*$^{flox/flox}$ embryos, in which *Hoxa2* deletion was induced at E10.5 by tamoxifen administration to the pregnant mother. At E13.5, oligodendrogenesis was increased two-fold in the r2-r3 derived territories of conditional *Hoxa2* deficient embryos. Additional alterations of Pax6 and Nkx2.2 expression, which were down-regulated and up-regulated, respectively, were associated. Moreover, ventral progenitor expression of phosphoErk1/2, a downstream signal of FGFR activation, was increased. Therefore, *Hoxa2* appears to act as a negative regulator of oligodendrogenesis in the rostral hindbrain, by local control of Pax6 and Nkx2.2 expression and modulation of progenitor response to the pro-oligodendrogenic FGF signal.

IDENTIFICATION OF GLIAL CELLS EXPRESSING THE P75 NEUROTROPHIN RECEPTOR IN THE CONTROL AND REGENERATING OPTIC NERVE OF THE LIZARD GALLOTIA GALLOTI.

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The p75 neurotrophin receptor (p75NTR) is expressed in neurons and in a wide variety of glial cells. The aim of this study is to characterize the glial cells expressing p75NTR in the optic nerve (ON) of adult lizards and to analyze their response after optic nerve transection. Using immunohistochemical methods, we detected p75NTR+ cells co-expressing astrocyte markers as GFAP, glutamine synthetase and vimentin (Vim). After ON transection, reactive astrocytes upregulated GFAP and Vim but not p75NTR. Moreover, a subpopulation of reactive tomato-lectin+/p75NTR+ microglia/macrophages were observed in the lesion site. These data indicate that the p75NTR is expressed in astrocytes, and in a subpopulation of reactive microglia/macrophages although it does not seem to be involved in reactive astrogliosis unlike occurs in mammals. Finally, p75NTR+ endothelial cells were observed in the lesioned ON suggesting a possible role of this receptor in the maintenance of the blood-brain barrier integrity. This study was supported by the Spanish Ministry of Education (Research Project BFU2007-67139) and the Regional Canary Island Government (ACIISI, Research Projects SolSubC200801000281 and ULPAPD-08/01-4).

A DUAL ROLE FOR THE TRANSMEMBRANE SEMAPHORIN SEMA6A IN MYELINATION

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Myelination is a crucial step in brain development and maintenance. The identification of novel proteins selectively expressed by developing oligodendrocytes should help understanding this complex process. Semaphorin is a family of cell-cell signaling proteins that have been involved in axon guidance. We found that the transmembrane semaphorin 6A is expressed by myelinating oligodendrocytes in the postnatal mouse brain. We are studying the role of Sema6A in oligodendrocytes, in normal and pathological condition using *Sema6A* deficient mice. To determine if the number of oligodendrocytes and the time-course of myelination were normal in Sema6A-deficient mice, we have quantified the number of cells expressing specific oligodendrocyte lineage markers in major forebrain axonal tracts. This revealed a significant but transient reduction of the density of PLP and MAG expressing cells in the anterior commissure, while the density of Olig2 cells was comparable. We next studied the in vitro maturation of oligodendrocytes purified from *Sema6A*-/- newborn mice. We found that oligodendrocyte differentiation is delayed in oligodendrocytes lacking Sema6A and is not rescued by addition of exogenous Sema6A-Fc, suggesting a cell-autonomous function for Sema6A in myelination. We next developed myelinating co-culture of DRGs and oligodendrocytes and found that addition of Sema6A-Fc protein increased myelination in a dose dependent manner. This pro-myelinating activity appears to require plexin-A expression on myelinated axons, with Sema6A acting as a ligand on oligodendrocytes. Furthermore, a role of Sema6A has been analyzed in a pathophysiological context, in which demyelination is induced by cuprizone. There is a significant increase of oligodendrocytes expressing Sema6A, starting 3 weeks after the administration of cuprizone, peaking at 4 and then returning to the basal level of expression at 6. Altogether these results suggest that Sema6A play a role in myelination in the central nervous system. We are currently investigating the signalling pathways involved in this process.

RAF/MEK/ERK SIGNALLING CONTROLS PERIPHERAL NERVE REGENERATION SWITCHING SCHWANN CELL STATE IN VIVO.

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Peripheral Nervous System (PNS) injury induces myelinating Schwann cells to dedifferentiate to progenitor-like cells. These dedifferentiated Schwann cells and the recruitment of inflammatory cells are essential steps for successful nerve regeneration. The mechanism controlling this switch in cell state and the role of Schwann cells in orchestrating the inflammatory response are poorly understood.
We have previously shown the that Ras/Raf/Erk pathway is able to induce the dedifferentiation of Schwann cells *in vitro* (Harrisingh et al., Embo J, 2004). To address whether ERK activation is also sufficient to drive Schwann cell dedifferentiation *in vivo* and investigate the effect of this specific signal on the biology of the peripheral nerve, we have generated a transgenic mouse model in which Raf kinase can be activated in myelinating Schwann cells in the adult nerve. We found that activation of Raf in these mice drives demyelination of peripheral nerves *in vivo* and results in severe impairment of motor function. Moreover, we show that ERK activation also induces the break down of the nerve blood barrier and is consistently accompanied by an inflammatory response and macrophage infiltration along the entire nerve. Importantly, the phenotype of peripheral nerve degeneration is reversible with the period of dedifferentiation determined by the period of ERK activation. We conclude that Raf/MEK/ERK signalling can both initiate and sustain Wallerian-like degeneration in the absence of axonal injury.
This mouse model provides a powerful system to study the regulation of PNS degeneration and regeneration.

ROLE OF LYMPHOID CELL KINASE (LCK) SIGNALING IN SCHWANN CELL CYTOSKELETON ORGANIZATION AND MYELINATION

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Myelination in the peripheral nervous system requires complex signaling events between Schwann cells, axons, and the basal lamina. The signaling pathways involved in the myelination in the peripheral nervous system are not fully understood. Lymphoid cell kinase (Lck), a non-receptor tyrosine kinase Src family member, regulates proliferation in *M. leprae*-infected Schwann cells through a PKCξ/Lck dependent signaling pathway (Tapinos and Rambukkana, 2005). Here we show that Lck is also involved in laminin-induced signaling in rat Schwann cells and that inhibition of Lck negatively affects myelination in DRG co-cultures. Phosphorylation of the Lck active site can be detected after 10 min of laminin treatment in rat Schwann cells overexpressing Lck. In non-transfected rat Schwann cells, small molecule inhibitors of Lck significantly inhibit radial lamellipodia formation on a laminin substrate, but do not affect the extension of axial lamellipodia. Live imaging of rat Schwann cells plated on laminin shows a reduction in the number and rate of radial lamellipodia formation when Schwann cells are treated with Lck inhibitors over the course of two hours. Inhibition of Lck signaling in Schwann cell/DRG co-cultures reduces the average length of myelin internodes, but does not affect the total number of myelin segments formed. Lck inhibition does not affect the alignment of Schwann cells with axons. Reduced internode lengths are seen if the Lck inhibitor is introduced at the time of Schwann cell addition to the DRG cultures or during ascorbate treatment only. The data suggest that Lck is a critical component of laminin signaling and the formation of radial lamellipodia during myelination in the peripheral nervous system.

PATCHED, DISPATCHED AND THE NEMO-LIKE KINASE MODULE INTERACT TO REGULATE GLIAL ENSHEATHMENT OF NEURONS IN *C. ELEGANS*

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Glia ensheath neurons to establish a niche that facilitates neuronal morphogenesis and function. This architecture is evident in the amphid, the main sensory organ of *C. elegans*, where glia form a tube through which the cilia of sensory dendrites gain access to the environment. We have been studying amphid development as a model system for neuron-glia interactions.

We previously showed that *daf-6*/Patched is required within glia for the normal morphogenesis of the amphid channel. In *daf-6* animals the channel is bloated and the sensory cilia become trapped within the glia. DAF-6 lines most luminal surfaces, and *daf-6* interacts with *che-14*/Dispatched in regulating tubulogenesis in many organs, thus providing a molecular link between the processes of tubulogenesis and glial ensheathment.

To further understand neuron-glia interactions in *C. elegans*, we performed a screen for suppressors of *daf-6*/Patched. One suppressor is an allele of the Nemo-like kinase homolog *lit-1*. *lit-1* is part of a MAPK module that has been previously shown to guide cell fate decisions by regulating Wnt signaling. However, mutations in *lit-1* do not affect glia specification. Rather, *lit-1*, which is expressed in many glial cells, acts within amphid glia to regulate amphid tubulogenesis. In contrast to the bloated channel seen in *daf-6* mutants, *lit-1* animals often have channels that are too small or fail to form altogether, suggesting opposing activities for *daf-6* and *lit-1* in regulating the size of the amphid channel, in accordance with the genetic suppression of *daf-6* by *lit-1*.

A GFP::LIT-1 fusion lines the amphid channel, similar to DAF-6::GFP, with the carboxy-terminal domain of LIT-1 being necessary and sufficient for this localization. LIT-1 localization is disturbed in mutants that fail to form sensory cilia, hinting at neuron-glia crosstalk in establishing the glial lumen. In a Y2H screen, the carboxyterminal domain of LIT-1 was found to interact with actin and WASP, suggesting that LIT-1 could direct glial tubulogenesis through the regulation of cytoskeleton dynamics. In accordance with this hypothesis, GFP::ACT-4 also lines the amphid channel and mutation of *wsp-1* suppresses *daf-6*.

LIT-1 is part of a MAPK module that regulates Wnt signaling in *C. elegans*, Drosophila and vertebrates, but Wnt pathway mutants do not affect glial tubulogenesis. However, the two other components of the MAPK module, *wrm-1*/beta-catenin and *mom-4*/MAP3K, suppress *daf-6*, and both WRM-1 and MOM-4 localize to the amphid channel, suggesting a new function for this conserved module.

Our results suggest a novel interaction between Patched, Dispatched and the LIT-1/MAPK module in directing the ensheathment of neurons by glia in *C. elegans*.

REGULATION OF DIFFERENTIATION AND PROLIFERATION IN MERLIN NULL SCHWANNOMA CELLS

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Our research focusses on trying to understand how Merlin loss leads to the development of Schwannoma tumours in Neurofibromatosis type 2 (NF2). Schwannoma cells lacking Merlin appear undifferentiated in vivo showing decreased expression of the compact myelin proteins P0, Peripheral Myelin Protein 22 (PMP22) and Myelin Basic Protein (MBP). Our in vitro experiments on primary human cell cultures have also shown that Schwannoma cells show enhanced proliferation compared to wild-type Schwann cells, mediated through increased activation of both platelet-derived growth factor (PDGF) and insulin-like growth factor (IGF) signalling.

We want to understand the process that follows Merlin loss and the mechanisms that cause the abnormal de-differentiation and increased proliferation of Schwann cells in these tumours. We have previously shown that Krox-20 acts as a key regulator of Schwann cell behaviour, controlling cell differentiation, proliferation and survival. Using an in vitro model we have investigated both the upstream control of Krox-20 expression and the downstream effect of enforcing Krox-20 in Schwannoma cells. Critically we found that Schwannoma cells were unable to induce either Krox-20 or the myelin marker periaxin in response to the in vitro myelination signal cyclic AMP. During myelination in vivo Krox-20 expression is controlled from the Myelinating Schwann cell Enhancer (MSE) element through binding of the transcription factors Oct-6 and Sox-10. We have found that the induction of Oct-6 in response to cAMP is also reduced in Schwannoma cells. Potentially the failure to induce Krox-20 and Oct-6 may result from the decreased expression of Sox-10 we have observed in these cells. Despite these defects, enforced expression of Krox-20 can effectively drive Schwannoma cell differentiation through up-regulation of the myelin protein P0 and the myelin marker periaxin. Importantly staining for the cell proliferation marker Ki-67 showed that expression of Krox-20 also halts Schwannoma cell proliferation in response to mitogenic signals such as Neuregulin-1 (NRG), PDGF and IGF-1. This data suggests that the abnormal proliferation and dedifferentiation of Schwannoma cells may be due to the impaired induction and maintenance of Krox-20. Furthermore, the key to a future treatment of these tumours may lie in understanding the relationship between Merlin and Krox-20.

DROSOPHILA GLIAL GLUTAMATE TRANSPORTER EAAT1 IS REGULATED BY FRINGE-MEDIATED NOTCH SIGNALING AND IS ESSENTIAL FOR LARVAL LOCOMOTION

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In the mammalian central nervous system (CNS), glial cells expressing Excitatory Amino Acid Transporters (EAATs) tightly regulate extracellular glutamate levels to control neurotransmission and protect neurons from excitotoxic damage. Dysregulated EAAT expression is associated with several CNS pathologies in humans, yet mechanisms of EAAT regulation and the importance of glutamate transport for CNS development and function *in vivo* remain incompletely understood. *Drosophila* is an advanced genetic model with only a single high-affinity glutamate transporter termed Eaat1. We found that Eaat1 expression in CNS glia is regulated by the glycosyltransferase Fringe, which promotes neuron-to-glia signaling through the Delta-Notch ligand-receptor pair during embryogenesis. We made *Eaat1* loss-of-function mutations and found that homozygous larvae could not perform the rhythmic peristaltic contractions required for crawling. We found no evidence for excitotoxic cell death or overt defects in the development of neurons and glia, and the crawling defect could be induced by post-embryonic inactivation of *Eaat1*. Eaat1 fully rescued locomotor activity when expressed in only a limited subpopulation of glial cells situated near potential glutamatergic synapses within the CNS neuropil. *Eaat1* mutants had deficits in the frequency, amplitude and kinetics of synaptic currents in motor neurons whose rhythmic patterns of activity may be regulated by glutamatergic neurotransmission among pre-motor interneurons; similar results were seen with pharmacological manipulations of glutamate transport. Our findings indicate that Eaat1 expression is promoted by Fringe-mediated neuron-glial communication during development, and suggest that Eaat1 plays an essential role to regulate CNS neural circuits controlling locomotion in *Drosophila*.

ACUTELY GRAFTED EMBRYONIC PROGENITORS RESULT IN MORE EXTENSIVE OLIGODENDROGLIAL PRODUCTION AND MYELINATION IN *SHIVERER* FOREBRAIN COMPARED TO PURIFIED AND EXPANDED EMBRYONIC OLIGODENDROCYTE PRECURSOR CELLS.

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Recent pre-clinical experiments in mouse leukodystrophy models have utilized exogenous cell-based transplantation approaches in an effort to restore myelin and improve functional outcome. The aim of our study was to determine whether cultured and expanded embryonic oligodendroglial precursor cells are as effective at engrafting and myelinating as acutely harvested and transplanted embryonic progenitors. The medial ganglionic eminence (MGE) and anterior entopeduncular area (AEP) is a source of embryonic oligodendrocyte precursor cells. We harvested cells from the E15.5 MGE/AEP progenitor zones, and acutely transplanted them into hypomyelinated newborn shiverer mice. In a parallel set of experiments, we purified and expanded proliferating PDGFRα-expressing oligodendrocyte precursor cells (OPCs) from E15.5 ventral embryonic forebrain before transplantation into newborn shiverer mice. We found that acutely transplanted progenitors resulted in robust engraftment in shiverer recipient forebrains and led to production of numerous oligodendroglia within white matter tracts, as well as some cortical interneurons and few astrocytes. In contrast, oligodendrocyte production and myelination was less extensive in shiverer mice transplanted with equivalent number of purified and expanded OPCs. These results suggest that cultured OPCs are less capable then progenitors of engraftment and myelination upon transplantation. Next, we investigated whether OPCs that have been genetically modified might display an improved ability to engraft and myelinate. We have shown previously that ~7 fold more OPCs are generated in the MGE/AEP of Dlx1&2 mutant mice compared to controls, and that these OPCs precociously express markers of maturing oligodendrocytes. We transplanted either acutely dissociated progenitors from the MGE/AEP of Dlx1&2-mutants or purified and expanded embryonic OPCs from Dlx1&2 mutant forebrain. Transplantation of Dlx1&2-mutant progenitors resulted in greater engraftment and myelination within white matter tracts with no neurons and only rare astrocytes generated compared to wildtype progenitors. In contrast, we did not observe obvious differences in engraftment and myelination between purified Dlx1&2 mutant and wildtype OPCs. Thus, we conclude that acutely-grafted, uncommitted progenitors from ventral telencephalic regions that give rise to oligodendrocytes during normal embryonic development are superior at incorporating and myelinating white matter in the hypomyelinated brain than cultured, purified oligodendroglial precursors. Our results suggest that cultured cells, which are under clinical investigation for leukodystrophies, may be significantly less capable than freshly harvested progenitors at engraftment and myelination.

NOGO-A ESTABLISHES SPATIAL SEGREGATION AND EXTENT OF MYELINATION DURING DEVELOPMENT

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A requisite component of nervous system development is the achievement of cellular recognition and spatial segregation through competition-based refinement mechanisms. Competition for available axon space by myelinating oligodendrocytes ensures that all relevant CNS axons are myelinated properly. To ascertain the nature of this competition, we generated a transgenic mouse with sparsely labeled oligodendrocytes and establish that individual oligodendrocytes occupying similar axon tracts can greatly vary the number and lengths of their myelin internodes. Here we show that intercellular interactions between competing oligodendroglia influence the number and length of myelin internodes and identify the amino-terminal region of Nogo-A, expressed by oligodendroglia, as necessary and sufficient to inhibit this myelinogenic potential. Additionally, the formation of exuberant myelin in the absence of Nogo suggests that spatial segregation and the extent of myelination is a consequence of the microenvironmental inhibition of myelinogenic potential. We demonstrate a novel physiological role for Nogo-A in the precise myelination of the developing CNS. Maximizing the myelinogenic potential of oligodendrocytes may offer an effective strategy for repair in future therapies for demyelination.

SCHWANN CELL MITOCHONDRIAL DYSFUNCTION DISRUPTS AXO-GLIAL INTERACTIONS WITHOUT INTERFERING WITH SCHWANN CELL SURVIVAL

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Schwann cells (SCs) are the primary glial cell type in the peripheral nervous system and play a central role in axonal integrity and peripheral nerve function. Mitochondrial dysfunction is a common cause of peripheral neuropathy; however, whether SC mitochondrial dysfunction contributes to axonal degeneration or demyelination is unknown.
With the goal of understanding how SC mitochondrial abnormalities affect SCs and their ability to support axonal integrity, we generated a mouse with impaired mitochondrial function exclusively in SCs. These Tfam-SCKO mice were generated through the tissue-specific deletion (via MPZ-Cre) of the mitochondrial transcription factor A gene (*Tfam*), an essential regulator of mitochondrial DNA transcription and replication. Tfam-SCKOs were viable and developed normally for the first few weeks of life. As they aged, however, Tfam-SCKOs developed a progressive peripheral neuropathy characterized by nerve conduction abnormalities as well as extensive muscle denervation. Morphological examination of Tfam-SCKO nerves revealed that pathological changes due to SC mitochondrial dysfunction first appeared in small unmyelinated fibers. Consistent with the degeneration of these small fibers, Tfam-SCKOs displayed early skin denervation and sensory deficits. By 3 months, pathology was also observed in large myelinated axons. Extensive axonal loss as well as demyelination became prominent by 4-6 months, with accompanying motor deficits and muscle weakness. Remarkably, the severe mitochondrial DNA depletion and respiratory chain abnormalities in Tfam-SCKO mice did not affect SC survival.
Normal mitochondrial function in SCs is, therefore, essential for maintenance of SC-axonal interactions and axonal survival, suggesting that SC mitochondrial dysfunction contributes to human peripheral neuropathies.

SCHWANN CELLS REMODEL BASEMENT MEMBRANES TO LOCALIZE PERIPHERAL NERVES

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Schwann cells play well known roles in myelinating and supporting axons. Recently, new roles for Schwann cells have emerged, including regulating the differentiation of sensory organs and preventing oligodendrocytes from exiting the spinal cord. We describe a new role for Schwann cells in relocating peripheral nerves across basement membranes. We find that the posterior lateral line nerve of the zebrafish initially grows in the epidermis and then rapidly transitions across the epidermal basement membrane into the subepidermal space. In mutants lacking Schwann cells, the nerve is improperly localized within the epidermis. Transplantation of wildtype Schwann cells into these mutants rescues the position of the nerve, such that it is correctly located below the epidermal basement membrane. We postulate that relocating the posterior lateral line nerve into the subepidermal space serves a protective function, since the mislocalization of the nerve in mutants lacking Schwann cells results in significant disorganization of the nerve. These results define a new role for Schwann cells and a new mechanism for axon localization that is distinct from growth cone pathfinding.

HOW SCHWANN CELLS CONTROL THE CLUSTERING OF SODIUM CHANNELS AT NODES OF RANVIER

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Although myelin is an excellent insulator that allows axons to conduct at faster speed than achievable without it, action potentials would decay over a few millimeters and thus must be regenerated at regular intervals. Nodes of Ranvier are gaps in the myelin that are exquisitely designed for just this purpose, equipped with high density of voltage-gated sodium channels. The nodal region is organized into several distinct domains, each of which contains a unique set of ion channels, cell adhesion molecules and cytoplasmic adaptor proteins. This local differentiation of myelinated axons is tightly regulated by myelinating Schwann cells. Two cell recognition molecules, Caspr and Caspr2 mediate axoglial interactions around the nodes and play an important role in the organization of the nodal environs by two different mechanisms: Caspr participate in the generation of a barrier-like structure at the paranodal junction formed at both sides of the nodes, whereas Caspr2 serves as a scaffold that maintains potassium channels at the nearby juxtaparanodal region. Initial clustering of sodium channels in heminodes that border each myelin segment requires gliomedin, NrCAM and neurofascin 186 (NF186), three cell adhesion molecules (CAMs) that mediate the interaction between Schwann cells and the axon. We further identify NrCAM as a glial component of PNS nodes, which cooperates with gliomedin in the capturing of Na+ channels at heminodes by binding to NF186. Heminodal clustering coincides with a second, paranodal junction (PNJ)-dependent mechanism that allows Na+ channels to accumulate in mature nodes by restricting their distribution between two growing myelin segments. We propose that Schwann cells govern the assembly of nodes in the PNS by two independent adhesion systems that provide reciprocal backup capacities.

DIFFERENTIAL MECHANISMS INITIATE AND MAINTAIN ASSEMBLY OF NODES OF RANVIER

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Axons reorganize into distinct domains during myelination, centered around nodes of Ranvier; the mechanisms which underlie this reorganization are poorly understood. We now show that nodes assemble sequentially from distinct sources of proteins: existing pools of adhesion molecules on the axon surface redistribute to initiate node, and paranode formation whereas ion channels and cytoskeletal proteins are recruited to the node from transport-dependent, internal stores. NF186, a key adhesion molecule, is targeted to nascent nodes via extracellular interactions. In contrast, NF186 is targeted to mature nodes, where it turns over slowly, via intracellular interactions with ankyrin G, aided by paranodal interactions. These results support a model in which domain formation is initiated by redistribution of axon adhesion molecules which concentrate at the node via diffusion trapping with cognate Schwann cell receptors whereas ion channels, cytoskeletal proteins and replenished domain components are recruited from internal stores transported to these sites.

CONTROL OF *DROSOPHILA* MIDLINE GLIAL SUBTYPE SPECIFICATION AND AXON-GLIAL INTERACTIONS

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The *Drosophila* midline glia (MG) perform a variety of functions in controlling and supporting neural development and function. MG-derived factors guide and provide trophic support for commissural axons and neurons. There are 2 distinct types of MG, anterior (AMG) and posterior (PMG). AMG contact, migrate around and ensheath bundles of commissural axons. The function of PMG is currently unknown and all undergo apoptosis during embryogenesis. We are interested in understanding the molecular mechanisms that govern the formation of MG types and the regulation of MG migration and MG-axon interactions. To date, we have identified 56 genes (15 transcription factors) expressed in MG including genes differentially expressed in AMG and PMG. These include the transcription factors *runt* and *engrailed* (*en*) that are expressed throughout development in AMG and PMG, respectively. Early in development, Notch signaling mediates the decision of bipotential MG-neuronal precursors to activate MG gene expression, but does not control AMG or PMG-specific gene expression. In contrast, the Hedgehog morphogen directs *en* expression as *hh* mutants showed loss of En and *hh* overexpression showed excess En. In addition, *runt* expression was expanded to all MG in *hh* mutants and was absent in *hh* overexpression suggesting that *hh* repressed *runt* expression possibly via En repression. Misexpression of *runt* showed that Runt activated AMG gene expression and repressed *en* and other PMG gene expression in AMG. In *en* misexpression PMG gene expression was activated and *runt* and AMG gene expression was repressed. The mutual repression between Runt and En reinforced the distinction between MG types leading to different expression profiles and functions. The membrane protein Wrapper is found at high-levels in AMG and low-levels in PMG, its expression is regulated by *hh-runt-en*, and it is required for AMG migration and commissural ensheathment through its interaction with Neurexin IV on axons. Thus, one functional consequence of *hh-runt-en* regulation is restricting MG:neuron interactions only to AMG. Current work uses live imaging and genetic analyses to uncover additional regulatory proteins

STATISFACTORY CLEARANCE OF AXONAL DEBRIS: STAT92E GOVERNS PHAGOCYTIC COMPETENCE OF GLIAL CELLS

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One critical and conserved feature of glial cells is their ability to sense and respond to neuronal injury. During injury responses, glia exhibit robust changes in gene expression, migrate to sites of trauma, and phagocytose degenerating neuronal debris. Glial responses to neurodegeneration are thought to help minimize brain damage however the molecules and signaling pathways mediating neuron-glia communication after injury remain poorly defined. We use the adult *Drosophila* olfactory system as a powerful *in vivo* genetic model to explore the molecular mechanisms that govern glial recognition and clearance of degenerating axons. Previously, we showed that glia respond robustly to axotomy of olfactory receptor neurons (ORNs) by upregulating the phagocytic receptor Draper, extending membranes to severed axons, and clearing degenerating axons from the CNS. We recently performed a large-scale, *in vivo*, RNAi screen in an effort to identify novel genes required for glial responses to axon injury. The STAT (Signal Transducer and Activator of Transcription) pathway is a highly conserved signaling cascade which results in STAT-dependent transcriptional activation of target genes in response to cytokine signaling. Here we describe a novel role for STAT signaling in establishing glial competence to respond to injury and modulating injury-induced upregulation of engulfment genes in phagocytic glial cell types.

We found that RNAi knockdown of STAT in glia completely suppressed the recruitment of glial membranes to severed axons, and the glial engulfment of axonal debris. Loss of STAT function in glia (by RNAi knockdown, or in mutant clones) eliminates basal levels of glial Draper expression, as well as the injury-induced increases in Draper expression observed in control animals. Using GFP reporters that reflect STAT activity, we detect a basal level of glial STAT activity in uninjured animals as well as a transient burst in glial STAT activation following injury. These data raise the intriguing possibility that STAT directly regulates Draper expression, and thereby competence of glia to respond to injury. The Drosophila genome contains only one STAT (*stat92E*), a single JAK kinase (*hopscotch*), and a single STAT-activating receptor (*domeless*). Surprisingly, genetic perturbation of these STAT signaling effectors had no effect on glial clearance of degenerating axons or Draper expression levels. Our data suggest that STAT92E signals through a non-canonical signaling cascade to regulate constitutive as well as injury-induced transcription of the *draper* gene and may reveal a novel STAT-activating signaling pathway activated within glia in response to neurodegeneration.

GLIAL CELLS INITIATE SPONTANEOUS ACTIVITY IN THE DEVELOPING AUDITORY SYSTEM

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Developing sensory systems rely on intrinsically-generated electrical activity to guide the maturation of pathways required for processing sensory information. In all sensory modalities that have been examined, this spontaneous activity occurs in the form of discrete bursts of action potentials (APs) separated by long periods of quiescence. In the developing auditory system, bursts of action potentials occur in primary afferent neurons prior to the onset of hearing, yet little is known about how these patterns are generated or how they influence the formation of developing circuits. We found that a group of glial-like "supporting" cells in the cochlea spontaneously release adenosine 5'-triphosphate (ATP) during this period. ATP then activates purinergic autoreceptors, induces a rise in intracellular Ca^{2+} and depolarization, and eventually forces crenation of these progenitors. In parallel, this paracrine release of ATP activates purinergic receptors on adjacent inner hair cells, triggering Ca^{2+} spikes, and inducing release of glutamate onto primary afferent dendrites, ultimately leading to bursts of action potentials in primary auditory neurons. *In vivo* recordings from principal neurons in two auditory nuclei in prehearing rats revealed that the firing patterns initiated by ATP in the developing cochlea propagate to auditory centers of the brain. Spontaneous purinergic signaling in the cochlea rapidly subsides after the onset of hearing, and is sufficient to initiate synchronous activity in hair cells that will eventually exhibit similar characteristic frequencies. These results suggest that glial cells in the cochlea play a crucial role in the maturation of this peripheral sensory organ as well as auditory circuits within the brain. Preliminary studies suggest that ATP is released from these supporting cells through connexin hemichannels. As many congenital forms of deafness arise from mutations in connexins (e.g Cx26) that are expressed exclusively by supporting cells, it is possible that these forms of hearing loss arise from alterations in purinergic signaling during this crucial period of development. Supported by NIH (DC009464, DC008860).

IDENTIFICATION OF AN ASTROCYTE-SECRETED PROTEIN THAT IS SUFFICIENT TO INDUCE FULLY FUNCTIONAL SYNAPSE FORMATION

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Synapses are specialized cell adhesions that are the fundamental functional units of the nervous system, but the extracellular signals that induce CNS synapse formation and function are poorly understood. We have been investigating the role of astrocytes in the formation and function of excitatory synapses *in vitro* and *in vivo*. Using retinal ganglion cells (RGCs) as a model CNS neuron, we previously found that astrocytes release several factors that strongly enhance the formation and function of excitatory synapses onto RGCs. Biotinylation and Western blotting experiments showed that astrocytes increase the surface levels of all 4 AMPA glutamate receptor subunits in RGCs by 3-fold, and immunostaining for surface GluR1 subunits demonstrated that astrocytes induce a clustering of AMPA receptors in synaptic sites. We identified thrombospondins as astrocyte-derived proteins that normally promote CNS synaptogenesis *in vivo* and are sufficient to induce ultrastructurally normal synapses *in vitro*, however thrombospondin-induced synapses are post-synaptically silent due to the absence of AMPA glutamate receptors. We therefore took a biochemical approach to identity the astrocyte-secreted signal that increases the number of synaptic AMPA receptors. We used a combination of chromatography columns to fractionate the hundreds of proteins present in astrocyte conditioned medium (ACM), and obtained a fraction that contained 1% of the starting proteins and was 5-fold enriched for functional activity, as assayed by adding this fraction to RGCs and subsequent electrophysiological recording of synaptic activity. Analysis of this fraction by mass spectrometry identified approximately 20 proteins as being present, and using an over-expression system we identified one of these proteins as being sufficient to induce an increase in synaptic activity in RGCs. This protein was sufficient to significantly increase mEPSC amplitude and frequency in RGCs *in vitro* to the same level as astrocytes, and also increased surface levels of GluR1 subunits and clustering of GluR1 at synaptic sites. Expression profiling of acutely isolated astrocytes and neurons showed that the mRNA for this protein is enriched in astrocytes *in vivo* and present during synaptogenic periods. We are currently characterizing mice deficient in this protein to determine its role in synapse formation and function *in vivo*.

TNFα, GLIA AND SYNAPTIC FUNCTION

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Glia can regulate the development and function of the nervous system. We have previously demonstrated the the glial release of the pro-inflammatory cytokine tumor necrosis factor alpha (TNFα) is an important modulator of synaptic function. Through activation of neuronal TNF-R1 receptors, TNFα causes the exocytosis of GluR2-lacking AMPA receptors and results in an increase of excitatory synaptic strength. Concurrently, TNFα application leads to an endocytosis of GABA-A receptors, and a decrease in inhibitory synaptic strength. The release of TNFα is increased when neural activity is reduced and mediates homeostatic synaptic plasticity, where the nervous system compensates for a long-term reduction in neural activity levels by increasing the excitation and reducing the inhibition in the neural circuit. These compensatory changes are usually assumed to occur proportionally across all the synapses on a given neuron. Here we present data that the local application of TNFα results in a local up-regulation of AMPA receptors, suggesting that homeostatic plasticity does not necessarily in the multiplicative scaling of all synapses. It is possible that glia regulate synapses in a very local manner. Further, we have evidence that TNFα-mediated synaptic plasticity is occurring in vivo, and contributes to the slow modification of neuronal circuit function. These changes can be adaptive, such as during the development of dykinesia, or mal-adaptive, such as the increase in excitotoxicity following neuronal insults.

SUPPORTING CELLS REGULATE SYNAPSES FORMATION IN THE VESTIBULAR EPITHELIUM

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Supporting cells of the vestibular epithelium, non–neuronal cells with many similarities to glia, are closely associated with hair cells and afferent nerve terminals, making them potential contributors to vestibular development and function. Vestibular supporting cells express erbB receptors and vestibular sensory neurons express the erbB ligand neuregulin 1. We tested the potential roles of this pathway in the vestibular system using a transgenic mouse line in which erbB receptor signaling in vestibular supporting cells is blocked by expression of a dominant-negative erbB receptor (DN-erbB4).

Adult DN-erbB4 mice display behaviors consistent with vestibular dysfunction (ataxia, spinning behavior, inability to swim). Evoked potential recordings showed that vestibular function is severely affected by P21, even though macular epithelia are normal in size and general structure and afferent nerve terminal appear unaffected. FM1-43 dye uptake and neurofilament staining are normal in mutant mice, indicating that hair cell mechano-transduction is unaffected as well. In contrast, synaptic site numbers (defined as the colocalization of RIBEYE and GluR2/3 staining) are dramatically reduced, suggesting a synaptic defect. Analysis of synapse numbers at different postnatal ages showed that the number of synaptic puncta increases by 5 fold between birth and P21 in wild types, but this does not occur in the mutants. The phenotype produced by loss of erbB signaling correlates with reduced BDNF expression in supporting cells and is rescued by re-expression of BDNF in these glia. Furthermore, knock-down of BDNF expression in supporting cells postnatally phenocopies the loss of erbB signaling. Together these results indicate that vestibular supporting cells contribute to the formation/maturation of synapses in the postnatal vestibular maculae and that this is mediated by NRG1-erbB and BDNF-TrkB signaling.

EXCITABILITY AND SYNAPTIC COMMUNICATION WITHIN THE OLIGODENDROCYTE LINEAGE

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The mammalian CNS contains an abundant, widely distributed population of glial cells that serve as oligodendrocyte progenitors. It has been reported that these NG2-immunoreactive cells ($NG2^+$ cells) form synapses and generate action potentials, suggesting that neural-evoked excitation of these progenitors may regulate oligodendrogenesis. However, recent studies also suggest that $NG2^+$ cells may be comprised of functionally distinct groups that differ in their ability to respond to neuronal activity, undergo differentiation, and experience injury following ischemia. To better define the physiological properties of $NG2^+$ cells, we used transgenic mice that allowed an unbiased sampling of this population and unambiguous identification of cells in discrete states of differentiation. Using acute brain slices prepared from developing (P5-8, P12-15, P22-26) and mature (P40-45) mice, we defined the physiological properties of $NG2^+$ cells in two white matter regions (corpus callosum and cerebellar white matter) and two grey matter regions (CA1 hippocampus and cerebellar molecular layer). $NG2^+$ cells in these diverse brain regions shared a core set of physiological properties, including expression of voltage-gated Na^+ (NaV) channels and ionotropic glutamate receptors, and formation of synapses with glutamatergic neurons. Although small amplitude Na^+ spikes could be elicited in some $NG2^+$ cells (4/18 cells) during the first postnatal week, these cells were not capable of generating action potentials nor did they exhibit spontaneous spiking. Na^+ spikes were not observed at any later developmental time points (0/76 cells P12-45), indicating that this rudimentary form of excitability is not a prominent or lasting feature of these cells. Transition of $NG2^+$ progenitors to the pre-myelinating stage was accompanied by the rapid removal of synaptic input, as well as down regulation of AMPA and NMDA receptors and NaV channels. Thus, prior reports of physiological heterogeneity among $NG2^+$ cells may reflect analysis of cells in later stages of maturation. These results suggest that $NG2^+$ cells are uniquely positioned within the oligodendrocyte lineage to monitor the firing patterns of surrounding neurons.

ASTROCYTIC MODULATION OF MGLUR5 ENHANCES LONG-TERM POTENTIATION AND LEARNING

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Metabotropic glutamate receptor 5 (mGluR5) is receiving attention as a therapeutic target in schizophrenia since positive allosteric modulators (PAMs) of this receptor augment synaptic plasticity and learning. Because synaptic stimuli activate astrocytic Ca^{2+} signals which can in turn lead to the release of glutamate, we have developed a line of mice in which we attenuated astrocytic IP_3/Ca^{2+} signaling and asked the role of this glial pathway in contributing to effects of mGluR5 PAMs at hippocampal CA1 synapses and learning. We have identified three distinct stimulus-dependent conditions in which astrocytes play differing roles in induction of long-term potentiation (LTP) of synaptic transmission: with weak neuronal stimulation astrocytes are not sufficiently activated to contribute to LTP. However, under this condition the extrinsic activation of astrocytic Gq/Ca^{2+} signaling can cause an mGluR5-dependent enhancement of LTP induction. Under intermediate stimulus conditions the activity of neuronal afferents substantially recruits astrocytic IP_3/Ca^{2+} signaling which contributes significantly to the modulation of the magnitude of LTP. Under this stimulus condition LTP is sensitive to mGluR5 inhibition. Finally, under intense neuronal stimulus conditions astrocytic IP_3/Ca^{2+} signaling does not significantly contribute to LTP induction, demonstrating that glial IP_3/Ca^{2+} signals modulate synaptic plasticity by causing an mGluR5-dependent enhancement of LTP. Furthermore, we show that the ability of mGluR5 PAMs to enhance LTP and learning requires astrocytic Ca^{2+} signals, raising the potential for novel astrocytic targets for cognitive enhancement in disorders such as schizophrenia.

HIPPOCAMPAL SHORT- AND LONG-TERM PLASTICITY ARE NOT MODULATED BY ASTROCYTE CA2+ SIGNALING

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The concept that astrocytes release neuroactive molecules (gliotransmitters) to affect synaptic transmission is one of the most important paradigm shifts in neuroscience over the past decade. This concept suggests that astrocytes together with pre- and postsynaptic neuronal elements comprise a functional synapse. Astrocyte release of gliotransmitters (e.g., glutamate, ATP, D-serine) is generally accepted to be a G_q GPCR $Ca2^+$ dependent process. We used two mouse lines to either selectively increase or obliterate astrocytic G_q GPCR $Ca2^+$ signaling to further test the hypothesis that astrocytes release gliotransmitters in a $Ca2^+$-dependent manner to affect synaptic transmission. Neither increasing nor obliterating astrocytic $Ca2^+$ fluxes affects spontaneous and evoked excitatory synaptic transmission or synaptic plasticity. Our findings suggest that, at least in the hippocampus, the mechanisms of gliotransmission need to be reconsidered.

MUTATION OF A GLIAL-SPECIFIC NCKX CAUSES DEFECTS IN GLIAL CALCIUM SIGNALING AND NEURONAL HYPEREXCITABILITY

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Glia exhibit large fluctuations in intracellular calcium both spontaneously and in response to neuronal activity, although the influence of glial calcium transients on neuronal signaling is not well understood. By screening for temperature-sensitive seizure mutations in *Drosophila* we identified the *zydeco* mutation, which disrupts a glial-specific K^+-dependent, Na^+/Ca^{2+} exchanger (NCKX) that utilizes Na^+ and K^+ gradients to export intracellular calcium. *Zydeco* mutant seizures can be rescued by expression of a *zydeco* transgene in cortex glia and astrocyte-like glia, which are cell types closely associated with neuronal cell bodies and neuropil, respectively. Knockdown of *zydeco* by RNAi in cortex glia and astrocyte-like glia also causes temperature-sensitive seizures in adults, indicating that *zydeco* expression in glia is both necessary and sufficient to regulate neuronal excitability. To determine the temporal requirement for NCKX function in glia, we conditionally expressed a *zydeco* transgene using a heat-shock inducible Gal4 driver. We find that adult specific expression of *zydeco* is sufficient to rescue the neuronal seizure phenotype, supporting an acute rather than developmental role for *zydeco* in the nervous system. We next investigated the contribution of *zydeco* to glial calcium signaling by imaging glial calcium waves *in vivo* using a genetically encoded calcium sensitive GFP (GCamp3). *Zydeco* mutant and RNAi knockdown animals exhibit glial calcium waves of reduced frequency compared to wild-type. We verified the importance of glial calcium signaling in the *Drosophila* nervous system by expressing a heat sensitive UAS-Trp channel in glia, which at elevated temperature allows influx of cations, including calcium. Activation of UAS-Trp in cortex glia and astrocyte-like glia causes rapid paralysis in adult flies, indicating glial calcium signaling is acutely required for nervous system function. Our results indicate that a NCKX regulates glial calcium waves and that acute disruption of glial calcium signaling alters neuronal excitability and leads to seizures.

MICROGLIAL ACTIVATION RAPIDLY MODULATES SYNAPTIC ACTIVITY

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Fine control of neuronal activity is crucial to rapidly adjust to subtle changes of the environment. This fine-tuning was thought to be purely neuronal until the finding that glial cells could also modulate neuronal activity. Yet, the regulation of synaptic and neuronal activity by astrocytes is well documented but the contribution of microglia has to be explored. Microglial cells display several features of putative functional partners of synapses. They are present at high density in the brain, they rapidly activate upon stimulation by releasing various neuro-active factors. Finally, pathological activation of microglia, which is the primary stage of inflammation, and synaptic alterations are common early features of most neuropathies. We have now established a functional link between microglial activation and synaptic function. We have shown that activation of microglia induced a rapid increase of spontaneous AMPAergic synaptic events frequency. We further demonstrated that upon activation, microglia rapidly releases ATP, which recruits astrocytes through purinergic receptors. ATP stimulation of astrocytes induces a neuronal mGluR response leading to the modulation of AMPAergic transmission. These results depict microglia as a novel actor in the regulation of neurotransmission and provide a basis to understand synaptic dysfunctions in neuronal diseases.

SYNAPTIC PRUNING IN THE CNS: THE ROLE OF MICROGLIA AND THE COMPLEMENT SYSTEM

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During development, neural circuitry undergoes a remodeling process in which excess synapses are eliminated or pruned and the remaining synapses are strengthened. While it is clear that this is an activity-dependent process, the precise molecular mechanisms have not been elucidated. Stevens et al. (Cell, 2007) discovered that components of the classical complement cascade (C1q and C3), traditionally associated with innate immune system function, are necessary for synapse elimination in the normal, developing CNS. Furthermore, complement proteins were found to localize to synapses in the developing brain and during early stages of neurodegenerative disease. One of the major questions arising from these findings and main objective of the current study is to determine by what mechanism complement is mediating synapse removal. In the innate immune system, complement proteins coat or opsonize debris for removal. A common pathway for complement-opsonized debris removal is through phagocytosis by macrophages. Because microglia are the resident phagocytic cell of the CNS, we suggest that this cannonical immune system mechanism may be involved in the normal, developing CNS. We hypothesize that weak or inappropriate synapses may be tagged by activated C3 and subsequently phagocytosed by microglia. Using high resolution imaging and assesment of mice deficient in complement-related proteins, we provide evidence that microglia actively participate in synaptic pruning by phagocytosing neuronal processes. Furthermore, our data suggest that this is a complement-dependent process. Taken together, our data demonstrate new roles for microglia in the developing CNS and provide a mechanism underlying complement-dependent synaptic pruning.

MICROGLIA SHAPE ADULT HIPPOCAMPAL NEUROGENESIS THROUGH APOPTOSIS-COUPLED PHAGOCYTOSIS

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In the adult hippocampus, neuroprogenitor cells in the subgranular zone (SGZ) of the dentate gyrus give rise to newborn neuroblasts. However, only a small subset these cells integrate into the hippocampal circuitry as mature neurons at the end of a four-week period. Here, we show that the majority of the newborn cells undergo death by apoptosis in the first one to four days of life, during the transition from amplifying neuroprogenitors to neuroblasts. These apoptotic newborn cells are rapidly cleared out through phagocytosis by unchallenged microglia present in the adult SGZ niche. Phagocytosis by the microglia is efficient and undeterred by aging or inflammatory challenge. Our results suggest that the critical period of newborn cell survival occurs within a few days of birth and reveal a new role for microglia in maintaining the homeostasis of the baseline neurogenic cascade.

POST-NATAL REGULATION OF TOLERATED CNS INFLAMMATION IN RESPONSE TO SYSTEMIC IMMUNE CHALLENGE

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Neuroinflammation triggered by systemic inflammation and occurring during critical periods of CNS development is hypothesized to contribute to the development and/or pathogenesis of many neurodevelopmental disorders including cerebral palsy, schizophrenia and autism. In the adult CNS, the type and magnitude of microglial activation and macrophage influx triggered by systemic inflammation are well characterized. As yet, little is known about if and how microglial phenotype changes during critical periods of CNS development associated with synaptogenesis and oligodendrocyte development. Here, using flow cytometry and dual in situ hybridization/immunohistochemistry we show that in the early postnatal murine brain, microglia display an activated phenotype that is not polarized toward either “classic” proinflammatory or “alternative” anti-inflammatory activation states. Furthermore, we find that at all ages examined, systemic inflammation induced by an intraperitoneal injection of LPS leads to widespread activation of microglia and a transient influx of peripheral macrophages into the murine CNS. However, the magnitude of macrophage influx into the CNS is developmentally modulated. Systemic inflammation triggers the highest level of macrophage influx into the CNS between days 7 and 14. In addition, CNS-infiltrating macrophages display a highly polarized pro-inflammatory phenotype. During this same developmental period, microglial activation is associated with high level expression of multiple alternative activation markers and induction of TREM2. We speculate that these microglial-specific forms of activation in part serve to compensate for the higher levels of pro-inflammatory macrophages that infiltrate the CNS during early development. Altogether our data contribute to the growing literature demonstrating that the brain may have age-specific susceptibilities to insults associated with the development and treatment of neurodevelopmental disorders.

ROLE OF COMPLEMENT IN MODULATING GLIAL CELL ACTIVATION IN AMYOTROPHIC LATERAL SCLEROSIS

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In the neuro-degenerative disease Amyotrophic Lateral Sclerosis (ALS), death of motor neurons is accompanied by robust glial cell activation. In the mutant superoxide dismutase 1 (SOD1) transgenic model of ALS, non-neuronal cells including microglia and astrocytes have been found to contribute significantly to disease progression. However, the molecular mechanisms governing neuron-glia interactions in ALS are not well characterized. The humoral complement system mediates synapse elimination during CNS development and glia express receptors for complement. In mSOD1 transgenic mice, we found that several components of complement were up-regulated in spinal cord and peripheral nerves during disease progression. In the degenerating spinal cord, white and gray matter GFAP+ astrocytes expressed C3, while motor neurons stained for C1q. We bred the mSOD1 transgene onto a complement C4-/- background to ablate classical and lectin pathways of complement. In mSOD1C4-/- mice, astrocyte and microglia activation were significantly decreased compared to mSOD1C4+/+ mice. FACS analysis of acutely isolated microglia from mSOD1C4-/- spinal cord showed stunted up-regulation of dendritic cell markers CD86 and CD11c. However, C3 deposition in ventral horns remained elevated in the absence of C4, indicating activation of the alternative complement pathway. Thus, complement plays a role in modulating the glial response in ALS. Further investigation of complement induction and deposition may produce insights into spinal cord gliosis and potential therapeutic avenues for motor neuron disease.

SELECTIVE TRANSFER OF MEMBRANE VESICLES CONTAINING ENCEPHALITOGENIC ANTIGENS FROM OLIGODENDROCYTES TO MICROGLIA

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The transfer of antigens from oligodendrocytes to immune cells has been implicated in the pathogenesis of autoimmune diseases. Here, we show that oligodendrocytes secrete small membrane vesicles, exosomes, containing encephalitogenic antigens to the extracellular space. These exosomes are specifically and efficiently taken up by microglia both *in vitro* and *in vivo*. Internalization of exosomes occurs by a macropinocytotic mechanism without inducing a concomitant inflammatory response. After stimulation of microglia with interferon-γ, we observe an upregulation of MHC II in a subpopulation of microglia. However, exosomes are preferentially internalized in microglia that do not seem to have antigen-presenting capacity. We propose that the constitutive macropinocytotic clearance of exosomes by a subset of microglia represents an important mechanism through which microglia participate in the degradation of oligodendroglial membrane in an immunologically "silent" manner. By designating the capacity for macropinocytosis and antigen presentation to distinct cells, degradation and immune function might be assigned to different subtypes of microglia.

OLIGODENDROCYTE (OL) AND PROGENITOR CELL (OPC) RESPONSES TO INJURY: EFFECTS OF AXONAL DAMAGE AND OXIDATIVE STRESS

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The role of myelin loss and repair in demyelinating diseases (e.g. MS) is well established although axon loss complicates the picture. The role of demyelination after spinal cord injury (SCI) is less clear. Numerous studies have shown that oligodendrocytes (OL) undergo secondary apoptosis after SCI, and this loss has been correlated with the presence of axonal degeneration and microglial activation (reviewed in Beattie, 2004). However, even if substantial demyelination occurs in long tracts or at the site of injury, spared axons may be re-myelinated by resident OPCs. Thus, the myelination status of spared axons after SCI depends upon both cell loss and the reparative response of OPCs.

Thoracic contusion SCI in rats produced delayed OL apoptosis and cell loss in the dorsal columns (DC) rostral to the lesion associated with both axonal degeneration and morphological features of microglial activation. OL apoptosis peaked at 8 days after injury, and was preceded by evidence of early intracellular oxidation of RNA beginning before 24 hrs post injury (Sun et al, in press). In contrast, massive axonal degeneration induced by dorsal root section outside of the CNS produced similar evidence for microglial activation, but failed to induce either OL RNA oxidation or apoptosis. Both types of injury, however, stimulated OPC (NG2+) proliferation and differentiation in the DC, and neither resulted in demyelination of intact axons adjacent to degenerating tracts. Parallel studies of OPCs isolated from neonatal rat cortex confirmed that oxidative stress induced by activated microglia (Miller et al, 2007) or t-buOOH accelerated OPC apoptosis. OPCs from transgenic rats over-expressing SOD-1 were protected. Further, OPCs from SOD-1 over-expressers proliferated and differentiated more rapidly than wild type OPCs in vitro. In vivo, SOD-1 over-expressing rats have more OLs in the white matter after SCI. Together, these studies suggest that axonal injury and microglial activation are associated with both OL death and repair, and that their effects may be modulated by oxidative stress.

Beattie MS (2004) Trends in Molecular Medicine, 10: 580-583.
Miller B et al (2007) J. Neuroinflammation, 4:28.
Sun F et al (2010) Glia, in press

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A MILD VASCULAR CHALLENGE CAUSES A RAPID DISRUPTION OF AXON-GLIAL INTEGRITY AND FUNCTION.

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Ageing of the brain can lead to cognitive decline which causes severe personal and social problems. Studies have shown that with advancing age there is a decline in white matter integrity and changes in myelin structure and function are suggested to contribute to cognitive decline. The occurrence of changes in white matter are often linked with chronic cerebral hypoperfusion (modest reductions in cerebral blood flow). In humans investigating the causes of these changes is strictly limited to brain imaging and in some cases post-mortem tissue analysis. Thus the underlying morphological changes and cellular mechanisms in white matter remain largely unclear in the ageing brain.
A mouse model of chronic cerebral hypoperfusion has been developed, whereby hypoperfusion is induced by application of microcoils to both common carotid arteries (BCAS). In this BCAS model, incredibly modest reductions in blood flow results in selective damage to the white matter and mimics aspects of cerebrovascular white matter lesions. This model permits insight to mechanisms that occur early in response to hypoperfusion that are prohibited in human brain.
Here we investigated the effects of chronic cerebral hypoperfusion on the integrity of the white matter at the cellular level using detailed confocal imaging. Key proteins were analysed at the axon-glial junction after chronic cerebral hypoperfusion. We found that the axon-glial connection is disrupted early in response to BCAS (within 3 days) in the absence of major changes to the myelin and axon. Furthermore, at later times after BCAS (1 month) these white matter changes were associated with a cognitive impairment, specifically working memory. Together the results highlight the vulnerability of key components of white matter to modest reductions in blood flow which is sufficient to impair cognitive abilities.

This work was conducted as part of the Disconnected Mind program, supported by Age Concern.

LAMININ REGULATES OLIGODENDROGENESIS IN THE DEVELOPING POSTNATAL BRAIN.

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Brain size, organization, and function are abnormal in children with genetic deficiencies in the extracellular matrix protein laminin. During mouse embryogenesis, laminin loss-of-function leads to detachment and misplacement of radial glia, which in turn causes disturbances in neuronal progeny and cerebral cortical development. Members of the laminin family are found throughout the cortical plate during corticogenesis and are also highly enriched at the ventricular and pial surfaces, i.e. sites of radial glial attachment; later, laminins adopt a more restricted distribution in the adult brain where laminin-containing fractone structures are found in the neural stem cell niche of the subventricular zones. It is not known, however, if laminins regulate the wave of gliogenesis that originates from the subventricular zones in the neonatal and postnatal brain. Here we report that laminin alpha 2 (LAMA2) regulates postnatal oligodendrogenesis. During early postnatal development, $LAMA2^{-/-}$ mice possess significantly fewer oligodendrocyte progenitor cells in a future white matter tract, the corpus callosum. Preliminary evidence suggests that during this early oligodendrogenesis, the subventricular zones of LAMA2 $^{-/-}$ mice have disturbed cellular organization in conjunction with higher numbers of $Olig2^{+}$ and $Sox2^{+}$ progenitor cell populations. Later, the development of the LAMA2 null corpus callosum continues to lag behind, developing fewer mature oligodendrocytes in conjunction with what are now elevated levels of oligodendrocyte progenitors relative to those in wildtype littermates. These deficits lead to delayed myelination in the developing cerebral cortex by postnatal day 21. And, while fewer mature oligodendrocytes were observed in the developing corpus callosum of $LAMA2^{-/-}$ mice, these cells did not exhibit increased cell death. Together these data suggest that laminin-cell interactions in the developing postnatal brain regulate the timing or ability of oligodendrogenesis to occur appropriately.

TWO-PHOTON IMAGING REVEALS ASTROGLIAL INJURY ALONGSIDE NEURONAL DAMAGE DURING STROKE-INDUCED ISCHEMIC DEPOLARIZATIONS.

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Two-photon laser scanning microscopy (2PLSM) enables real-time visualization of functioning cells expressing Green Fluorescent Protein (GFP) deep within living neocortex in vivo. Using 2PLSM we have previously shown that cardiac arrest-induced global ischemia elicits astroglial soma and process swelling, indicating acute damage. We also have evidence that spreading depolarizations (SDs) cause acute damage to neuronal dendrites and spines in the ischemic penumbra. Here we used 2PLSM to monitor changes in astroglial volume concurrently with signs of neuronal injury in two different models of ischemic stroke. The first method was transient bilateral common carotid artery occlusion (CCAO), which allowed for the induction of global ischemia and subsequent reperfusion. The second method was modified photothrombotic microcirculatory occlusion, in which a square-shaped ischemic lesion was made to surround a penumbra-like “area at risk” with SDs recurring for minutes to hours following photothrombosis. With both methods, dendrites showed a relatively high rate of recovery for several hours after insult. However, astroglial recovery was much more variable, with many somata and processes remaining swollen well after the induction of ischemia. Thus the fate of astrocytes may be a more critical determinant of the final outcome of ischemic stroke than was previously thought.

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EXPRESSION OF THE VESICULAR GLUTAMATE TRANSPORTER 1 (VGLUT1) AND THE GLUTAMINE SYNTHETASE (GS) IN THE CONTROL AND REGENERATING LIZARD VISUAL SYSTEM.

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Our purpose is to study the distribution of the VGLUT1 and GS in the retina and optic nerve of the adult lizard Gallotia galloti. In the retina, VGLUT1+ ganglion and amacrine cells were identified. The latter also displayed dendrite-like processes into the inner plexiform layer (IPL). Punctate staining of VGLUT1 was confined to a main horizontal sublamina in the IPL. Strong GS staining was detected in the Müller glia processes and endfeet. Interestingly, VGLUT1+/GS+ and VGLUT1-/GS+ glial cells were abundantly distributed in the optic nerve. After unilateral optic nerve transection, no relevant changes in VGLUT1 and GS staining were detected in the experimental retina. In the lesioned optic nerve, VGLUT1 labeling was apparently unmodified whereas down-regulation of GS was exclusively observed at the lesion site at the different postlesion time points studied. We conclude that the GS is expressed in a glutamatergic glial network in the lizard ON and may contribute to homeostasis under control and regeneration conditions. This work was supported by the Spanish Ministry of Education (Research Project BFU2007-67139) and the Regional Canary Island Government (ACIISI, Research Projects SolSubC200801000281 and ULPAPD-08/01-4).

ACETATE SUPPLEMENTATION REDUCES NEUROGLIA ACTIVATION AND CHOLINERGIC CELL LOSS WHILE INCREASING BRAIN ENERGY RESERVES AND LIPID DEPOSITION IN A RAT MODEL OF NEUROINFLAMMATION

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Glyceryl triacetate (GTA) a compound effective in reducing myelin loss in a mouse model of Canavan disease was used to treat rats subjected to chronic neuroinflammation. To induce neuroinflammation, rats were infused directly into the 4th ventricle for 28 days with either artificial cerebral spinal fluid (aCSF) or bacterial lipopolysaccharide (LPS) dissolved in aCSF (5.0 ng/hour). During this period rats were given a daily treatment of either water or GTA at doses of 6g/kg by oral gavage. Brain neuroglia activation was measured by quantifying morphologic changes in CD11b-positive microglia and GFAP-positive astrocytes. Cholinergic cell loss was measure by counting choline acetyltransferase (ChAT)-positive cells in the basal forebrain. Immunohistochemical analysis demonstrated that treatment significantly reduced the percentage of activated astrocytes and microglia by 40-50% compared to controls. Further, treatment significantly increased the number of choline acetyltransferase positive cells by 40% in the basal forebrain. To begin to determine the mechanism(s) involved in the anti-inflammatory and neuroprotective effect of acetate supplementation we measured changes in lipid content, acetyl-CoA, nucleotide, phosphocreatine, and creatine levels in treated rats subjected to head-focused microwave irradiation to stop metabolism. These studies show that acetate supplementation significantly increased plasma acetate levels by 15 min and remained elevated for up to 4 hr resulting in a 4-fold increase in brain acetyl-CoA by 30 min which also remained elevated up to 4 hr. Treatment did not alter brain energy supplies; individual brain nucleotide levels or the energy charge ratio [ECR, (ATP+0.5ADP)/(ATP+ADP+AMP)] when compared to controls. However, treatment did significantly increase by 0.4-fold ($p < 0.001$) the phosphocreatine to creatine ratio (PCr/Cr) and by 0.9-fold ($p < 0.01$) the energy potential ratio [EPR, (PCr/Cr) x (ATP/ADP)] by 4 hr. Further, treatment significantly increased the levels of ethanolamine and choline plasmalogen by 2- and 4-fold respectively without altering the concentrations of other brain phospholipid. These data suggests that acetate supplementation alters brain energy reserves and lipid metabolism and provides insight into the anti-inflammatory and neuroprotective mechanism(s) of acetate supplementation.

MYOSIN II IS A NEGATIVE REGULATOR OF OLIGODENDROCYTE MORPHOLOGICAL DIFFERENTIATION.

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The myelinating cell of the central nervous system, the oligodendrocyte (OL), undergoes a dramatic change in the organization of its cytoskeleton as it differentiates from an oligodendrocyte precursor cell (OPC) to a myelin forming OL. This change involves an increase in branching, which is required for the OL's ability to myelinate multiple axons.
We have shown that levels of non-muscle myosin II (MII), a regulator of cytoskeletal contractility, decrease as a function of differentiation and that inhibition of MII activity increases branching and myelination of OL in co-culture with neurons. Although several pathways have been implicated in OL morphogenesis, their specific contribution to regulation of myosin II activity and expression in OL has not being directly examined.
Previous work has shown that Fyn kinase a member of the src family kinase is upregulated during OL differentiation, and that overexpression of fyn or its downstream target p190RhoGAP decreases levels of active RhoA and induces branching in the OPC. We have tested the hypothesis that the activity of MII in OPC is negatively regulated by fyn through RhoA-ROCK and the phosphorylation status of its regulatory light chain (MLC). In support of this hyphotesis, we have found that pharmacological inhibition of fyn prevents the decrease in phospho-MLC levels normally observed during the differentiation of OPC in vitro. Furthermore, the inhibition of OPC branching induced by over-expression of a constitutively active form of RhoA can be reversed by pharmacological inhibition of MII.
To test the relevance of our findings to OL differentiation in vivo, we have started to characterize myelin formation by OPC isolated from MII KO mice. In agreement with our previous studies, we have found that OL maturation is accelerated in MII KO mice compared to wild type, as shown by a significant increase in the number of mature MBP+ in OL derived from KO cultures. Taken together our results confirm that downregulation of myosin II promotes OL branching and maturation and suggest that upstream activation of Fyn kinase acts as a negative regulator of myosin II activity in OL promoting active actin-remodeling and maturation.

IMAGING NEUROINFLAMMATION IN MAN: 13C MRS STUDY

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Introduction: Despite the critical role played by glia in neurological disorders, very little is known concerning glial function in the intact human brain. Using carbon-13 magnetic resonance spectroscopy (13C MRS) we demonstrate metabolic adaptation of neurons and glia when independently assayed in multiple regions of human brain. Specificity is provided by the selective use of a glial fuel, acetate in place of a neuronal fuel, glucose while regional selectivity is based upon MRI guided MRS voxel placement.

Methods: Technological improvements allow for human 13C MRS with safe examination of frontal brain structures, a brain region previously inaccessible to 13C MRS on grounds of RF safety. Using a routine clinical MR scanner (GE 1.5 Tesla) more than 150 human subjects have been examined during intra-venous infusions of 1 or 2-labelled 13C glucose or 1-labelled 13C acetate. Metabolic rates of neuronal or glial TCA cycle, glutamate-glutamine cycle and final oxidation product 13CO2 (as bicarbonate, H13CO3-) products identified within the brain as a function of time, provide rates of glutamate neurotransmission and substrate turnover.

Results: Cerebral uptake of 1-13C glucose and 2-13C glucose were comparable. Using 2-13C glucose, neuronal metabolic rate for 13C glucose was comparable in frontal and posterior regions of normal human brain. Cerebral uptake of 1-13C acetate and its conversion to C5 labeled glutamate and glutamine was confirmed, providing evidence of significant glial metabolism. Using the maximum rate of appearance of 13C bicarbonate, glial TCA cycle rate in the intact human brain was 0.5 μmoles/min/g, approximately 30% of the maximum rate of neuronal metabolism in the same brain region(s) determined with 13C glucose.
Metabolic adjustments of neuronal metabolism occur in patients with Alzheimer's disease (Lin et al. MAGMA,2003;(16):29-42) and ornithine transcarbamylase deficiency (Gropman et al. Radiology, 2009; 252(3): 833-41). 50% modification in glial metabolic rate was observed in subjects abstinent from prolonged abuse of methamphetamine (Sailasuta et al: Cerebral Blood Flow and Metabolism, 2010; 30: 950-960)

Conclusions: In vivo glial metabolic rate is modified significantly in response to a pathological state. We postulate that glial metabolic rate assay may be reflective of adaptation in microglia and therefore could provide a reliable non-invasive assay for the phenomenon of neuroinflammation. Future studies with 13C MRS after 1-13C acetate infusion could directly establish a role for neuroinflammation in the many human brain disorders in which this process has been implicated.

PTPRZ1 REGULATES OLIGODENDROCYTE DEVELOPMENT AND SCHIZOPHRENIA RELATED PHENOTYPES IN MICE

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Schizophrenia is a serious neurodevelopmental psychiatric disorder in which oligodendrocyte/myelin abnormalities have been implicated. The neuregulin (NRG) signaling system has been implicated in the etiology of schizophrenia by genetic studies and by animal models. PTPRZ1, receptor type protein tyrosine phosphatase Z, also been implicated in schizophrenia, can inhibit NRG/erbB4 signaling. In cultured rat oligodendrocyte precursor cells (OPCs), the expressions of Ptprz1 receptor isoforms (long and short) are downregulated during differentiation of OPCs in vitro. Inhibition of PTPRZ1 activity in OPCs by its ligands or shRNA treatment leads to acceleration of oligodendrocyte differentiation, whereas overexpression of PTPRZ1 leads to a delay of oligodendrocyte differentiation, supporting a role in oligodendrocyte development. In schizophrenia postmortem brains, *PTPRZ1* is upregulated. To test PTPRZ1 involvement in schizophrenia, we have developed BAC transgenic mice that contain one extra copy of *PTPRZ1* (*PTPRZ1*- BAC).
Quantitative polymerase chain reaction (QPCR) studies demonstrated a downregulation of the expression of oligodendrocyte/myelin genes in the young *PTPRZ1*-BAC mice. Immunohistochemical analyses showed an increased ratio of oligodendrocyte precursor cells to mature oligodendrocytes in *PTPRZ1*-BAC, indicating delayed oligodendrocyte differentiation. In addition, we found increased levels of dopamine and of the *Drd2* dopamine receptor in the striatum of *PTPRZ1*-BAC, indicating elevated dopamine signaling in these mice. Furthermore, glutamate related genes are downregulated in the hippocampus. Behavioral analysis showed increased spontaneous and drug-induced activity in the open field test and reduced prepulse inhibition, all considered to be behavioral changes relevant to schizophrenia in mice. These results are consistent with developmental hypothesis of schizophrenia, in which altered developmental processes can induce sustained effects on brain circuitry and behavior, leading to schizophrenia.
We propose a model in which increased expression of *PTPRZ1* leads to deficits in oligodendrocyte development, which can in turn contribute to aspects of the schizophrenia phenotype.
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REOVIRUS MYELITIS INCREASES GLIAL ACTIVATION AND EXPRESSION OF INTERFERON STIMULATED GENES

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A better understanding of the pathogenesis of virus-induced spinal cord injury (SCI) is essential for identification of novel targets for therapy. We have recently developed an experimental model of viral induced SCI. Neonatal mice are infected with 10^6 plaque forming units of a neurotropic reovirus (Type 3 Dearing) in the right hind limb. This leads to a progressive paralysis first of the ipsilateral hind limb and then of both hind limbs (paraparesis/paraplegia) by nine days post infection. Tissue was collected from paralyzed animals for use in western blot analysis, immuno-fluorescence analysis (IFA) with confocal microscopy, or RNA isolation for gene expression analysis using reverse transcriptase PCR – evaluating levels either on agarose gel or quantitatively by SYBR Green based real time PCR. Viral infection is associated with an increase in astrocytes (glial fibrillary acidic protein (GFAP) + cells) in proximity to infected neurons in the anterior horn of the lumbar spinal cord, as well as activation associated cell hypertrophy. Microglial activation is also evident as determined using IFA with antibodies against ionized calcium-binding adaptor molecule 1 (Iba1). Microglia show altered morphology consistent with activation with quantitative cell counts showing an 18 fold ($p<0.0001$) increase in activated microglia in SC from paralyzed as compared to control mice. Activated microglia are found throughout the lumbar spinal cord and in close association with infected neurons. Since both astrocytes and microglial contribute to host innate immune responses in the CNS through activation of cytokine and chemokine signaling pathways, we examined expression of genes encoding these molecules. We found that virus infection of the spinal cord resulted in increased expression of interferon (IFN) stimulated genes (ISG) encoding; (i) antiviral proteins, including 2'-5'oligoadenylate synthetase (OAS) and signal transducer and activator of transcription 1 (STAT1); (ii) inflammatory cytokines and chemokines, including tumor necrosis factor alpha (TNFα) and chemokine (C-C motif) ligand (CCL) 5; (iii) proteins which can sensitize cells to apoptotic death, including caspase 1 and interferon regulatory factor 1 (IRF1) and; (iv) pattern recognition receptors, including retinoic acid inducible gene-I (Rig-I) and toll like receptor 2 (TLR2). Activated glia may contribute to the increased expression of inflammatory mediators found in reovirus infected spinal cords, including inducible nitric oxide synthase and TNFα which are neurotoxic. Current studies are evaluating these responses to viral infection in IFN(α/β)R1 knockout mice, which show enhanced viral replication and accelerated mortality after reovirus infection.

IDENTIFICATION OF NEW GLIAL CELL SPECIFC GENE FUNCTIONS IN *DROSOPHILA*

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The main functional features of the nervous system have been conserved during evolution. Likewise the biology of neurons and glial cells is remarkable similar in different animal species. In all cases, glial cells exert a number of important supporting functions for the neuronal circuitry. These functions include the insulation and support of neurons, the facilitation and modulation of electrical conductance and synaptic transmission. To decipher these functions, the simple structured nervous system of Drosophila offers a number of advantages. In the embryo and the larval nervous system every segmental unit of the ventral nerve cord comprises only 65 glial cells. All of these cells are known and markers exist to label the different cell types in wild type and mutant backgrounds. Moreover, the Drosophila glia can be specifically manipulated by a set of cell type specific Gal4 drivers.

To get further insights into Drosophila glial cell biology we performed a glial cell specific RNAi Screen. So far we screened about 5,000 different genes for a cell autonomous requirement in all glia using the panglial driver line repoGal4. The knock down of about 14% of the tested genes leads to lethality. In about 1% of the cases we noted flies with reduced viability and with locomotion defects. To determine whether the function of these 800 candidate genes can be attributed to a specific glial cell class we silenced their expression using Gal4 driver lines specific to individual glial cell types (perineurial glia, subperineurial glia, wrapping glia and astrocytic glia). Here we focus on genes required in the wrapping glia.

The knock down of kinesin heavy chain (Khc) or tubulin severely affects viability and renders the flies hyperactive. Khc is a well known protein and has been thoroughly analyzed for its role in anterograde axonal transport. In glial cells a function of Khc has been recently described (Lyons et al., 2009). In Drosophila loss of Khc in glial cells disrupts transport of mitochondria and leads to a cell-autonomous differentiation defect during early larval stages. A model describing Khc function in Drosophila glia will be discussed.

SEARCHING FOR THE ROLE OF CLN5 IN GLIAL CELLS

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Neuronal Ceroid Lipofuscinoses (NCLs) comprise a group of children's inherited neurodegenerative disorders caused by mutations in at least 8 different genes. NCLs share many clinical and pathological features like visual loss, brain atrophy and ultimately lead to premature death. Mutations in the *CLN5* gene result in the Finnish variant late infantile form of NCL (vLINCLFin) and 27 disease-causing mutations have been reported (http://www.ucl.ac.uk/ncl). CLN5 shows no homology to other known proteins and the function of the protein is unknown.
The aim of our current research project is to analyze the impact of Cln5 deficiency in glial cells. Loss of myelin has been reported to be associated with the vLINCLFIN disease pathology [1, 2] and gene expression profiling of *Cln5* deficient mouse brains implicate myelination related changes [3, 4] . We have used qPCR as an approach to study developmental expression of *Cln5* in wt mice and to detect possible defects in the oligodendrocyte/myelin maturation in *Cln5* deficient mice. Our results show that *Cln5* gene expression is linearly rising in mouse brain during development (P3, P10, P20, P30, 3mo, 5mo) and that it is markedly higher in glial cells than in neurons. Preliminary analyses of the developmental expression of oligodendrocyte specific genes indicate that Cnp1 (2',3'-cyclic nucleotide phosphodiesterase) gene expression is reduced in Cln5 ko mouse brains. CNP1 protein plays an essential role in maintaining axonal survival [5] and we are currently investigating the CNP1 protein in *Cln5* deficient mouse brains and neuronal cultures. These findings suggest an important role of CLN5 in glial cells.

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GAS6 ENHANCES DEBRIS REMOVAL, REMYELINATION AND AXONAL SURVIVAL FOLLOWING CUPRIZONE TOXICITY

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Growth arrest-specific protein 6 (Gas6) activities are mediated through the Tyro3, Axl, and Mer (TAM) family of receptor tyrosine kinases. Gas6 is expressed and secreted by a wide variety of cell types, including cells in the central nervous system (CNS). Gas6 affects cell proliferation, differentiation, phagocytosis, cell survival and apoptosis. Previous studies showed that mice with deletion of Gas6 and Axl have delayed CNS recovery following cuprizone intoxication. In this study, we tested the hypothesis that administration of recombinant human Gas6 protein (rhGas6) directly into the CNS would improve recovery following cuprizone withdrawal. After a 4-week cuprizone diet (0.2 % w/w), cuprizone was removed, and PBS and rhGas6 (400 ng/ml, 4 µg/ml and 40 µg/ml) were delivered into the corpus callosum of C57Bl6 mice for 14 days. Analysis of the lipid-associated debris in the corpus callosum of 9 out of 11 (82 %) PBS-treated mice showed extensive Oil-Red-O positive deposits while mice treated independently with three rhGas6 doses, only 4 out of 19 (21 %) mice had low to moderate Oil-Red-O staining. In the corpus callosum of rhGas6-treated mice, SMI32 positive axonal spheroids and APP positive deposits were reduced in number relative to PBS-treated mice. Compared to PBS, rhGas6 enhanced remyelination detected by MBP immunostaining and by electron microscopy. The rhGas6-treated mice had more oligodendrocytes with Olig1-positive cytoplasmic localization, indicative of oligodendrocyte progenitor cell maturation. The number of Iba1-positive cells did not differ between the rhGas6- and PBS-treated groups. The data show that compared to PBS, rhGas6 treatment resulted in more efficient repair evidenced by clearance of cellular and myelin debris, greater maintenance of axonal integrity, enhanced oligodendrocyte progenitor cell maturation, all contributing to the observed enhanced remyelination.

HYPOXIA/REOXYGENATION DIFFERENTIALLY MODULATES ASTROGLIAL-SOLUBLE EPOXIDE HYDROLASE EXPRESSION

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More than 100,000 Americans are suffering from stroke or ischemic-brain injury annually and the rate of incidents is on the rise. Stroke, commonly called as 'brain attack' is the third leading cause of death in adults as well as in older people. Emerging evidence suggests that astroglial cells, a major glial cell population in brain, may play a role in hypoxia-ischemic brain injury. Our previous studies demonstrate that astroglial cells metabolize arachidonic acid by a cytochrome P450 epoxygenase pathway into four regioisomers of epoxides in addition to cyclooxygenase, lipoxygenase pathways. We have shown that these epoxides play a neuroprotective role causing vasodilation in the pial arterioles and their action is transient due to rapid hydrolysis by an epoxide hydrolase activity. Our recent studies show that normal rat brain astroglial cells express both microsomal (mEH) and soluble (sEH) epoxide hydrolases, and the latter is responsible for the inactivation of vasoactive epoxides. In this study, using an in vitro model, we investigated the signaling mechanisms mediating hypoxic responses of the astroglial cells in relation to the cell viability and expression of sEH. In cultures of rat brain astroglial cells, oxygen glucose deprivation (OGD) for 20h followed by reoxygenation (3, 6 and 24h) affected astroglial cell viability as assessed by dual immunocytochemical staining of cells with fluorescent dyes Hoechst/Propidium iodide. The OGD-induced astroglial cell death increased during post-hypoxia oxygen reperfusion: maximum cell death occurred at 6hr returning to that of normoxic controls in 24 hr. Immunocytochemical and quantitative western blot analyses revealed that OGD down-regulated sEH expression and reoxygenation reversed the expression of sEH to their normoxic controls in 24hr in a time-dependent manner suggesting a role for sEH against OGD-induced glial cell death and survival. These results suggest that a differential expression of sEH may be linked to pathophysiology of hypoxia-induced cerebral blood flow and brain damage after cerebral ischemia and stroke.

PROLIFERATIVE BEHAVIOR OF CORTICAL PROGENITORS IN THE INTACT AND INJURED ADULT MOUSE BRAIN

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Dividing progenitor cells in the adult brain may serve as a reservoir for cell replacement. Besides the highly proliferative zones of neurogenesis in the adult mouse brain, a large number of actively cycling progenitor cells resides in the brain parenchyma. The majority of these dividing cells are oligodendrocyte precursor cells (OPCs) that can be identified by the expression of NG2, PDGFRa, Olig2 or Sox10. Recent fate mapping experiments (Olig2::CreER^{TM}, PDGFRa-CreER^{T2}) showed that these cells indeed generate progeny of the oligodendrocyte lineage, with noteable differences between the gray and white matter (Dimou et al. 2008; Rivers et al. 2008). However, the overall proliferation characteristics of this major population of progenitors in the adult brain are still not fully understood. This includes key questions of stem cell biology, such as if these progenitors are able to self-renew and how they react to injury.

To analyze the cell cycle characteristics of cortical progenitors, adult mice received the thymidine analog BrdU for different time periods (4 hours – 8 weeks). BrdU detection was then combined with Ki67-immunoreactivity to label the dividing progenitor pool. These data showed that the majority of OPCs proliferate and that they have a long cell cycle of about a month. When we performed BrdU-label retaining experiments, we could follow the progeny of these cortical progenitors and could show that these proliferating NG2+ generate mature oligodendrocytes. In parallel, these BrdU-retaining experiments revealed for the first time direct evidence of the self-renewal of NG2+ progenitors in the intact cortical gray matter.

OPCs are also well-known to react to brain injury by increasing in number and changing their morphology. Here we examined the entire pool of proliferating cells (including astrocytes, microglia and NG2+ cells) after stab wound injury by cumulative BrdU labeling and Ki67 staining. Our results demonstrate that NG2+ cells are one of the first cells that react to the injury with enhanced proliferation and shortening of the cell cycle. In addition to OPCs, also microglia and later astrocytes start to divide in response to the lesion. Interestingly, the only cells that keep on proliferating within the glial scar at longer time points after injury are the NG2+ cells, being a potential target for approaches in brain repair.

INCREASED EXPRESSION OF GAP JUNCTION PROTEIN CX43 IN LOCALIZED BRAIN INJURY

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Intracranial cerebral laceration is a common traumatic brain injury that results in neuronal death and brain damage. It is therefore conceivable that signaling molecules important in neuroprotection will also be essential for wound repair. The gap junction protein connexin43 (Cx43) is one such protein. By allowing passage of small molecules between two adjacent cells, the up-regulation of Cx43 in reactive astrocytes has been proposed to facilitate removal of harmful substances from the injury site and therefore provide benefits for neuronal survival. However, there are also instances where increased intercellular communication appears to have a detrimental effect on the brain. In addition to reactive astrocytes, activated microglial cells have substantial presence in the injury sites. However, their relative contribution to wound healing is unclear, as is the interaction of microglia with astrocytes. Gap junctions consist of a family of channel proteins with cell type-specific expression in the brain. To examine key players in the wound healing process following brain injury, we determined the up-regulation of gap junction proteins in astrocytes and microglia following a single needle wound to the mouse striatum. We found that Cx43 was up-regulated at the needle wound as early as 2 days post lesion (dpl) and the elevated expression was maintained at 5 dpl. However at 10 dpl, the up-regulation of Cx43 was significantly reduced and the needle wound was no longer obvious. Increased Cx43 expression was detected in both astrocytes and microglia surrounding the needle wound. We are in the process of examining the expression and localization of other connexins in these glial cells to determine the relative importance of gap junction proteins in wound healing. Specific up-regulation of gap junctions in glial cells indicates intercellular communication may be essential for a co-ordinated response to minor brain lesions.

GLIA ARE REQUIRED FOR NEURONAL RECEPTIVE ENDING SHAPE IN *C. ELEGANS*

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Formation and plasticity of neural circuits require precise regulation of dendrite and receptive ending shapes. In vertebrates, dendrite shape development is regulated in part by glial cues. Dendritic spines are also remodeled in a controlled manner in adults during sensory deprivation, information processing (synaptic plasticity) or hormonal surges (e.g. ovulation). Perturbations to dendrite morphologies are correlated with cognitive disorders. Similarly the shapes of sensory receptive endings in *C. elegans* require glial interaction during development and are altered by sensory deprivation and pheromones post-embryonically. While glia are implicated in the control of neuronal receptive ending shape across species, a detailed molecular understanding of relevant glia-neuron interactions is lacking.

We are using the *C. elegans* amphid sheath glia and AFD thermo-sensory neurons to identify genes underlying glial control of neuronal receptive ending architecture. Previous cell ablation studies from our lab suggest that sheath glia are required for development of the microvillar-like receptive endings of the AFD thermo-sensory neuron. We showed that transiently blocking exocytosis in glia by expressing a RAB-1 dominant negative protein promotes loss of AFD fingers, suggesting that an acute signal from glia is required for the maintenance of AFD receptive ending morphology. To identify genes involved in glia-AFD signaling, we conducted a forward genetic screen for mutants with defects in AFD receptive ending morphology. Three independent mutants lacking AFD microvilli have been isolated. Further characterization of these should shed light on the biology of glia, cell shape regulation, and cell-cell communication in the nervous system.

GLIA SPECIFIC REGULATION OF SGK1 ISOFORMS BY DRUGS OF ABUSE.

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One of the important goals in the field of addiction research is to understand alterations in the brain gene expression profile related to rewarding and addictive properties of drugs of abuse. Therefore, we performed whole transcriptome analysis of the mouse striatum after the administration of six addictive substances (Korostynski et al., 2007; Piechota et al. 2010). We have identified serum and glucocorticoid-regulated kinase 1 (Sgk1) as one of the most strongly upregulated transcripts in the striatum after acute drug treatment.

Here we carried out a more detailed study of drug-induced regulation of three transcriptional isoforms resulting from alternative splicing of Sgk1 gene (canonical Sgk1, neuron-specific Sgk1.1 and Sgk1.2). Using qPCR with variant-specific primers, we found robust increase in the abundance of the Sgk1 and Sgk1.2 transcripts after the administration of morphine, heroin, ethanol, amphetamine, cocaine and nicotine. The strongest effect was observed 2h after opioid treatment. In contrast, the expression of Sgk1.1 isoform remained unaltered by the drugs.

Furthermore, to investigate the brain-region specific regulation of individual isoforms we used in situ hybridization. Variant-specific probes revealed striking regional differences in their distribution: 1) Sgk1.1 isoform was mainly detected in the striatum and hippocampus, showing no change in the signal intensity independently of the treatment, 2) Sgk1 isoform was almost undetected in saline-treated animals, whereas upregulation was observed throughout the brain of morphine-treated animals, with the strongest signal in corpus callosum, anterior commisure and internal capsule, 3) the level of Sgk1.2 was below the detection threshold.

To look for cellular specificity we tested expression of the isoforms in cell-type specific primary cultures. We show that Sgk1 and Sgk1.2 isoforms are constitutively expressed in glial cells, but not in neurons, and are strongly upregulated by the GR agonist - dexamethasone. Conversely, Sgk1.1 is expressed in neuronal, but not in glial cultures, and displays no GR-dependent upregulation.

In summary, we describe Sgk1 and Sgk1.2 as glia-specific transcripts regulated by various drugs of addiction. Our data suggest that the overall change in the Sgk1 level reported previously may have originated from alterations in the glial isoforms. The observed alteration in expression of glial Sgk isoforms may represent the unspecific response to a chemical substance, being mediated f.e. via Toll-like receptors or may also be a part of glia-specific pathway involved in drug-induced neuronal plasticity.

NG2+ PROGENITOR RESPONSE AND LINEAGE ANALYSIS IN WHITE MATTER STROKE

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NG2+ cells represent a quiescent oligodendrocyte progenitor (OPC) pool that is found throughout the postnatal brain. In models of immune-mediated demyelinating injuries, NG2+ cells differentiate into myelinating oligodendrocytes in the white matter, although astrocytic fate is also reported. However, the differentiation potential of NG2+ cells in the setting of white matter stroke is still largely unexplored. We investigated the fate of NG2+ cells using an i*n-vivo* model of white matter stroke and an inducible OPC reporter line. Ischemic infarcts are produced by stereotaxic delivery of a potent inhibitor of endothelial cell NO synthase, L-NIO, directly into the white matter below forelimb motor cortex of adult mice. L-NIO injections produce focal ischemic stroke and adjacent demyelination confined to white matter with minimal cortical or striatal damage. Recordings of compound action potentials (CAPs) across the white matter infarcts demonstrated functional axon conduction deficit at 7 days post lesion (dpl). Additionally, mapping of mature oligodendrocytes in ipsilateral white matter identified progressive loss of CC1+ and transferrin+ cells upto 28 dpl. NG2+ cells were found in high numbers in the infarct core and periinfarct white matter within the first week of injury. Ischemic stroke elicited a persistent glial response, as the infarct core remained densely populated with pallisading astrocytes and Iba-1+ microglia/macrophages up to 60 dpl. BrdU pulse given after stroke showed NG2+ cells proliferated upon ischemic insult, and generated BrdU incorporated CC1+ cells as early as 7 dpl. NG2+ cells were mitotically active up to 14 dpl as indicated by Ki 67 immunoreactivity. Preliminary data suggests that some of the newly born oligodendrocytes survive by 28 dpl but the majority of NG2+/BrdU+ cells generated within 7 dpl remain undifferentiated at day 28. Using a tamoxifen inducible NG2-CreERT2/Rosa26-YFP mouse line, YFP gene expression was induced prior to white matter stroke and the phenotype of YFP+ cells was determined at 7, 14 and 28 dpl. YFP+ cells were mostly positive for NG2 and/or PDGFRα during all time points and few YFP/CC1 double positive cells were observed. Despite the low levels of detectable oligodendrocyte differentiation, the YFP+/NG2+ cell population exhibited characteristics of maturing OPCs across the time points evidenced by long elaborate processes. Interestingly, the infarct core and the lesion/striatum border harbored some YFP+ cells with GFAP+ processes starting at 7 dpl. Our findings suggest NG2+ cells remain mostly undifferentiated within the 28 days after white matter stroke with limited differentiation into oligodendrocytes. Astrocytic potential of NG2+ progenitors appear even more limited and possibly influenced by different micro-environments found in white matter stroke.

PROFILING TRANSCRIPTIONAL COREGULATION OF DYNAMICALLY REGULATED GENE LOCI DURING PERIPHERAL MYELINATION

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Myelination of peripheral nerves by Schwann cells depends upon a gene regulatory network controlled by Egr2/Krox20, which is specifically required for myelin formation and maintenance. A number of inherited human peripheral neuropathies including Charcot-Marie-Tooth (CMT) disease have been linked to mutations in major myelin genes such as Connexin-32 and Peripheral Myelin Protein 22 (PMP22), which are normally induced during myelination. Gene dosage appears to be critical as a gene duplication of PMP22 results in CMT1A, the most common inherited peripheral neuropathy. Many myelin genes are regulated by both Egr2 and Sox10, a transcription factor required for Schwann cell specification and other stages of Schwann cell development. To elucidate the mechanism by which Egr2 and Sox10 regulate gene expression during myelination, we have performed chromatin immunoprecipitation analysis on myelinating rat sciatic nerve in vivo. The resulting samples were applied to a tiled microarray consisting of a variety of genes that are deregulated in Egr2-deficient mice. A similar analysis with Sox10 showed substantial colocalization with Egr2 binding at control elements of several myelin genes. Finally, depleting Sox10 in Schwann cells resulted in downregulation of Pmp22 and other target genes suggesting direct regulation of myelin genes by Egr2 and Sox10 during myelin development. The results provide the first locus-wide profile of Egr2 and Sox10 binding patterns within myelin-associated genes, and serves to identify novel targets of Egr2/Sox10 coregulation during peripheral myelination.

GALECTIN-4, A NOVEL REGULATOR OF MYELINATION

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Myelination is essential for proper saltatory nerve conduction, i.e., rapid transmission of nerve impulses, a process which is ultimately irreversibly interrupted in multiple sclerosis (MS). The biogenesis and maintenance of myelin membranes is regulated by extracellular matrix (ECM) molecules, neuronal signaling and axonal adhesion and depends on polarized transport of myelin specific proteins and lipids towards myelin membranes. As galectin-4, which specifically binds to β-galactoside present in glycolipids or glycoproteins, has the ability to regulate (de)adhesion of cells to ECM components and is in addition involved in inter- and intracellular signaling and polarized membrane trafficking, we anticipate that galectin-4 could play a role in (re)myelination. Indeed, here we show that receptor(s) for galectin-4 appeared transiently on processes of immature, pre-myelinating primary oligodendrocytes (OLGs) and disappeared on mature, myelin-sheet forming OLGs. Furthermore, galectin-4 was developmentally downregulated in developing rat brains, i.e., just before the onset of myelination. Interestingly, we identified neurons as a possible source of (secreted) galectin-4, suggesting that neuronal galectin-4 could play a role in OLG myelination. Indeed, when immature OLGs were treated with galectin-4, further maturation and therefore myelin-sheet formation was inhibited, whereas hardly an effect on viability was observed. Moreover, galectin-4 treated OLGs acquired a less complex, i.e., bipolar morphology and were able to proliferate. Intriguingly, neuronal galectin-4 is re-expressed in demyelinating lesions. However, whereas in cuprizone-induced demyelination galectin-4 expression is cleared upon remyelination, in MS lesions axonal galectin-4 expression is persistent. Thus, re-expression of galectin-4 in the adult CNS, as during developmental myelination, might hamper remyelination in MS. Taken together, these results identify galectin-4 as a novel regulator of OLG differentiation and therefore myelination and warrants further research on underlying mechanisms of galectin-4 actions in order to find means to successfully direct remyelination processes under pathological conditions as observed in MS lesions.

DISTINCT ROLES OF SOXD PROTEINS ON OLIGODENDROCYTE DEVELOPMENT

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During nervous system development, Sox proteins as transcription factors are critically involved in neural cell fate decisions and differentiation processes and function in intricate networks where they modulate each other's activity. The SoxD proteins Sox5 and Sox6 are essential components of this network in developing oligodendrocytes. We have previously shown that Sox5 and Sox6 are co-expressed in uncommitted neural precursor cells of the ventricular zone and continue to be present after specification in oligodendrocyte progenitors before being downregulated during terminal differentiation in myelinating oligodendrocytes. In the developing mouse spinal cord, Sox5 and Sox6 jointly repress oligodendroglial specification and terminal differentiation by antagonizing the activating functions of the SoxE proteins Sox9 and Sox10. Sox9 is essential for oligodendrocyte specification, whereas Sox10 is required for terminal differentiation. In the present study, we focus on the role of SoxD proteins in oligodendrocyte lineage progression between these events. By analyzing spinal cord and forebrain of constitutive and conditional SoxD compound mouse mutants we show that Sox5 and Sox6 impair immature oligodendrocyte progenitor dispersal by regulating the expression of PDGF receptor alpha, an important migratory cue for oligodendrocyte progenitors. Our studies thus reveal that SoxD proteins are also crucial in immature oligodendrocyte progenitors for maintenance of PDGF signaling. As PDGF receptor alpha expression in oligodendrocyte progenitors is also dependent on SoxE proteins, SoxD proteins do not always antagonize the SoxE function. Some target genes are apparently activated by both SoxE and SoxD proteins.

MICRORNAS ARE KEY REGULATORS OF THE EARLY EVENTS OF WALLERIAN DEGENERATION

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Post-transcriptional regulation by microRNAs plays an important role in modulating rapid changes in gene expression profile in response to extracellular stimuli such as injury. Using microRNA array (Exiqon, Ver-9.0) analysis on total RNA isolated from uninjured and 24 h post axotomy, sciatic nerve distal segments, we identified differentially expressed microRNAs stimulated by nerve injury. We show that the expression profile of 16 miRNAs changes by more than 50% within 24 h of injury when compared with uninjured nerve. The expression profile of a few microRNAs was validated by Northern analysis and qRT-PCR. In order to examine if the cohort of differentially regulated miRNAs in injury coordinately target a group of genes or processes we used miR-Ontology database. Data mining analysis revealed 20 target genes with potential target sites for all the miRNAs used as constraints. We report genes such as QKI, ADCY1, TSC1 and several transcription factors to be regulated by these microRNAs, which have been shown to play important roles in Schwann cell function. In particular, a correlation between miRNAs expression and putative gene targets involved in signaling pathways leading to cytoskeletal reorganization, repression of myelination and to initiation of de-differentiation of Schwann cells was identified. Comparison of the pattern of miRNAs observed in peripheral nerve injury to that reported in spinal cord injury reveals that the injury response mechanism in PNS and CNS differs in the regulation of certain miRNA population while some show similar expression pattern in both systems.

INCREASE IN ATP RELEASE FROM ASTROCYTES RESULTS IN INCREASE IN THE EXCITABILITY OF THE HIPPOCAMPUS.

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Gliotransmitters released by astrocytes can theoretically regulate neural circuits, however, the consequence of altered gliotransmission, especially in case of the elevated release of gliotransmitters, are largely unknown. In this study, we have examined the astrocyte specific disease model mice, which are known to be vulnerable to convulsant challenge, to explore why altered astrocytic function resulted in altered neural circuit function. Electrophysiological approaches using hippocampal slices revealed increased long-term potentiation due to the inhibition of GABA inhibitory transmission in the model mice. This effect was mimicked by the addition of 100 nM adenosine to wild type slices. We found that cultured astrocytes from model animals exhibited the increased frequency of ATP release in vitro and the level of adenosine in the cerebrospinal fluid was approximately 100 nM higher than that of wild type in vivo. Enhancement of long-term potentiation in the model mice was blocked by the addition of adenosine A1 antagonist, strengthening the idea that the elevated level of adenosine inhibited GABA transmission via adenosine receptors. Consequently, increase in ATP gliotransmitter release resulted in the suppression of inhibitory tone and increase in the excitability of hippocampal circuit.

DEPLETION OF *DICER* IN ASTROCYTES CAUSES CELL NON-AUTONOMOUS NEURONAL DEGENERATION

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Astrocytes play pivotal roles in maintaining normal brain functions. MicroRNAs (miRNAs) are posttranscriptional regulators of gene expression and have been implicated in cell differentiation and neuronal survival. However, the role of miRNAs in regulation of astroglial physiology remains unknown. Here we demonstrate that *Dicer* depletion in cerebellar astrocytes results in ataxia gait, massive cerebellar granule neuron apoptosis and Purkinje cell degeneration. Transcriptional profiling of pre-symptomatic stage cerebella indicates that loss of *Dicer* may lock the astroglial cells in a false reactive astrogliosis status while shutting down important house-keeping functions, combination of which may lead to neuronal degeneration. Therefore our study provides in vivo evidence that miRNAs are indispensible regulators of astroglial homeostasis, malfunction of which might be involved in neurological disorders such as cerebellar ataxia.

MICROGLIAL INTERACTIONS WITH SYNAPSES ARE REGULATED BY VISUAL EXPERIENCE

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Microglia are the immune cells of the brain. In the absence of pathological insult, their highly motile processes continually survey the brain parenchyma and transiently contact synaptic elements. Aside from monitoring, their physiological roles at synapses are not known. To gain insight into possible roles of microglia in the modification of synaptic structures, we used immunocytochemical electron microscopy (EM), serial section EM with three-dimensional reconstructions, and two-photon in vivo imaging to characterize microglial interactions with synapses during normal sensory experience and sensory deprivation, in the visual cortex of juvenile mice. During normal visual experience, most microglial processes displayed direct apposition with multiple synapse-associated elements, including synaptic clefts. Microglial processes were also distinctively surrounded by pockets of extracellular space. In terms of dynamics, microglial processes specifically targeted small and transiently growing dendritic spines, which were typically lost over two days. Following brief visual deprivation, microglial processes changed their morphology, displayed phagocytic structures, apposed synaptic clefts more frequently and enveloped synapse-associated element more extensively. Their associated extracellular spaces also became more extended. While microglia became less motile, their preference of contact changed to a subset of larger dendritic spines that persistently shrank. Taken together, these findings reveal different modalities of microglial interactions with synapses that are subtly altered by sensory experience. These suggest that microglia may actively contribute to the experience-dependent modification or elimination of a specific subset of synapses in the healthy brain.

CYTOKINE RELEASE BY MICROGLIA AND ASTROGLIA IN PRION DISEASE PATHOGENESIS

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Activation of glia is seen in many neurodegenerative diseases including prion diseases. Prion diseases are fatal infectious neurodegenerative disorders that affect both humans and animals. A key event in the pathogenesis is the conformational change of a normal host protein (PrPsen, also known as PrP^{C}) into an abnormal, partially protease-resistant, disease-associated form (PrPres or PrP^{Sc}). The specific roles of glia in prion disease pathogenesis are still not clear. Activated glial cells produce cytokines as a protective response against certain pathogens, and as part of the host inflammatory response to brain damage. In addition, cytokines might also exacerbate tissue damage initiated by other processes. In the present work using multiplex assays to analyze protein levels of 24 cytokines in prion-infected C57BL/10 mouse brains, we observed elevation of CCL2, CCL5, CXCL1, CXCL10, GM-CSF, IFN-γ, IL-1α, IL-1β, IL-6, and IL-12p40. To study the role of astroglia and microglia in these cytokine responses, primary glial cultures were exposed to prion-infected brain homogenates. Microglia produced only IL-12p40 and CXCL10, whereas astroglia produced these cytokines plus CCL2, CCL3, CCL5, CXCL1, G-CSF, IL-1β, IL-6, IL-12p70 and IL-13. PrPres is believed to cause neurodegeneration in prion diseases, and may also be involved in stimulation of cytokine release by glia. To identify the main stimulant for glial cell cytokine release, we fractionated prion-infected brain homogenates by decreasing molecular size based on differential centrifugation. Surprisingly, the fraction giving the highest glial stimulation was devoid of PrPres. Furthermore, purified PrPres was found not to stimulate cytokine release by either astroglia or microglia. Future work is in progress to identify other stimulators of glial cytokines present in prion disease brain.

CYP450S EXPRESSION IN HUMAN PRIMARY GLIAL CELLS DURING BRAIN DEVELOPMENT: REGULATION OF GENE EXPRESSION UNDER INFLAMMATORY CONDITIONS.

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The brain cytochromes P450 (CYP450s) are involved in the metabolism of drugs with or without CNS tropism. The toxicity impact of xenobiotics varies significantly depending on patients' age due to gene expression variability in children and adults. Glial cells dysfunction as well as some inflammatory mediators such as endothelin-1 (ET-1) are involved in neurodegenerative disorders. Glial cells are potential sources of ET-1 release in response to cerebral injury. This could be expected in the very rare neuroinflammatory Rasmussen's disease (RD). CYP450s gene expression was compared in human primary glial cells isolated from brain parenchyma samples of 6 pediatric and 20 adults. Then, the *in vitro* modulation of CYP450s gene expression by the effect of ET-1 on primary glial cells was compared to CYP450s gene expression in RD patients.
The mRNA expression of 16 CYP450 genes (CYP1A1, 1A2, 1B1, 2B6, 2C8, 2C9, 2D6, 2E1, 2J2, 2U1, 3A4, 3A5, 4A11, 4F2, 11B2, 46A1) was studied by RT-PCR. These CYP450s are involved in exogenous (*i.e.* drugs) and endogenous substrates (*i.e.* arachidonic acid) metabolism. The mRNA expression profile of 7 transcription factors involved in the regulation of the CYP450s pathways (GR, LXRβ, PPARα, PPARγ, CAR, AhR and PXR) was also established. Wilcoxon and Spearman tests assessed statistical significance.
Our findings indicate that 8 out of the 16 CYP450s are expressed in human primary glial cells of paediatric and adult patients and differences in the mRNA expression profiles were found for CYP450s [CYP1B1 ($p<0.005$), 3A5 ($p<0.005$), 2E1 ($p<0.001$) and 2U1 ($p<0.01$)] as well as for transcription factors (LXRβ, PPARα, AhR) between both groups. In adult patients, CYP3A5 and 2E1 mRNA expression was correlated with AhR expression ($p<0.01$), while CYP2U1 mRNA expression was associated with GR, LXRβ, PPARγ and CAR ($p<0.05$), PPARα ($p<0.001$) expression. We demonstrated that exposure of human primary glial cells to ET-1 downregulates CYP1B1, 3A5 and 2E1 mRNA. Findings on mRNA CYP2U1 expression tend to show an up or down regulation depending on ET-1 high or low concentrations, consistent with the CYP2U1 mRNA expression observed in glial cells isolated from RD patients.
This is the first study highlighting CYP450s mRNA expression differences in human glial cells during brain development and pointing to the putative physiological impact of CYP2U1 in neuroinflammatory diseases.

THE ROLE OF POLYPLOIDY IN SUBPERINEURIAL GLIA IN DROSOPHILA

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Polyploidy is a widely used strategy in plants and animals to increase cell size, and the resulting increase in gene templates may facilitate robust gene expression. Through the examination of nuclear DNA content in situ, we found that the subperineurial glial cells of Drosophila melanogaster are polyploid. This demonstration of polyploid glial cells in the adult brain parallels previous observations that the surface glia in the larval ventral nerve cord (VNC) undergo DNA replication without cell division (1). However, the role of polyploidy in these glia is not known. Given that glial morphogenesis and its function in the mature nervous system are similar in Drosophila and vertebrates, elucidating the function of endoreplication of the Drosophila glia could provide a valuable comparative study towards the understanding of glia in both Drosophila and vertebrates.

To define the function of glial polyploidy in Drosophila, we constructed a strain in which DNA replication can be blocked specifically in the subperineurial glia when they are undergoing endoreplication. We find that when DNA replication and polyploidy are inhibited in the subperineurial glia, larval lethality occurs. Current experiments are directed towards defining the impact of reduced ploidy on the function of these glial cells.

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PROTEOMIC ANALYSIS OF SIGNALING PROCESSES IN GLIOBLASTOMA CELLS SUBJECTED TO SUB-LETHAL PHOTODYNAMIC TREATMENT

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Numerous signalling processes in control cell responses to oxidative stress induced by photodynamic treatment (PDT). The Panorama Ab Cell Signaling Microarray (Sigma-Aldrich) contains antibodies against 224 proteins involved in cell signalling, cell cycle, apoptosis, cytoskeleton, etc. We used it for the study of sub-lethal effect of 5-aminolevulinic acid-mediated PDT (1 mM, 1 hour incubation, 1 min 410 nm irradiation; 95% cell survival) on cultured glioblastoma D54Mg cells in order to explore the processes occurring at the initial cell response stage. Such treatment induced diverse biochemical changes. The phosphorylation of adhesion-related signaling proteins FAK and Pyk2 was especially high. The phosphorylation of Raf, a central component of the Ras/Raf/MEK/ERK pathway, and the expression of protein kinase C were also observed. Increase in the expression of microtubule protein MAP-1beta, the phosphorylation of microtubule-associated protein tau, and simultaneous down-regulation of dystrophin, vinculin, MAP2, and cytokeratins reflect cytoskeleton reorganization probably associated with changes in cell adhesion. The level of proteins regulating cell cycle was also changed. At 30 min after PDT, the levels of cyclin D1, c-Myc, checkpoint proteins chk1 and chk2 were reduced that could arrest G0/G1, G1/S and G2/M transitions. At 1 hour after PDT cyclins A and D3, protein kinases cdk6 and cdc27 were down-regulated. This could also delay G0/G1, G1/S and G2/M transitions. However, simultaneous increase in cyclin D1, transcription factors E2F1 and Smad4 could facilitate G0/G1 and G1/S transitions. We also observed up-regulation of anti-apoptotic protein Bcl-xL and simultaneous decrease in the level of caspase 9 promoting apoptosis that have demonstrated the protective cell response. Neuro-specific proteins S100-beta, CNPase and increase in the expression of beta-synuclein and proapoptotic NGF receptor p75 were down-regulated. Thus, the complex response of glioblastoma cells to sub-lethal PDT treatment included dynamic changes in the expression of signaling proteins associated with cell adhesion and cytoskeleton reorganization, cell cycle control and apoptosis. Supported by RFBR grant No 08-04-01322 and Minobrnauki RF grant No 2.1.1/6185.

NEUROGLIAL INTERACTIONS IN THE CRAYFISH STRETCH RECEPTOR AT THE ULTRASTRUCTURAL LEVEL

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In order to explore neuroglial relationships in a simple nervous system, the ultrastructure of crayfish stretch receptor (CSR) that consists of only two mechanoreceptor neurons (MRN) enveloped by glial cells (GC) was studied. After isolation and control registration of neuron firing the preparations were fixed by glutaraldehide, stained with OsO4 and uranyl acetate, embedded into an epon, and studied with the electron microscope Tecnai Spirit 12 (Phillips, Netherlands). MRN envelope consists of 10-30 glial layers separated by collagen. Intercellular space is usually no more than 10-15 nm width that facilitates diffusion between these cells but limits MRN communication with the external medium. Microtubule bundles passing from dendrites to axon form the fibrillar envelope around the neuron perikaryon, which reduces vesicular transport to the plasma membrane and glial cells. Numerous glial protrusions into the neuron cytoplasm up to 1 μm deep shorten the diffusion pathway between GC and MRN perikaryon through the fibrillar layer. The neuron cytoplasm also contains double-wall vesicles, which may represent the captured parts of these protrusions. They contain fragments of glial but not neuronal cytoplasm. Specific structural triads consisting of flattened submembrane cisterns, vesicles and mitochondria are present in the peripheral neuron regions contacting to glial cells. These triads are presumably involved in formation of invaginations in the neuronal membrane and double-wall vesicles. They are typically associated with these structures and presumably participate in the neuroglial exchange. The tubular lattice in glial cells can participate in the transfer of ions and metabolites between glial layers. In some places the integrity of neuroglial membranes is impaired but free neuroglial passage is prevented by the dense diffuse material accumulated in these regions. Thus, the neuroglial exchange with some cell components may be mediated by diffusion through glial protrusions and submembrane cisterns, by formation of double-walled vesicles, in which big glial masses are captured, and by transfer through tubular lattices.

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ON THE MECHANISM OF FIBRONECTIN AGGREGATE FORMATION IN MULTIPLE SCLEROSIS. IMPLICATIONS FOR REMYELINATION

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Demyelinating diseases, such as multiple sclerosis (MS), are characterized by trauma to the myelin membrane and oligodendrocytes. Whereas demyelination impedes nerve conduction, ultimate failure of remyelination by oligodendrocytes leads to neurodegeneration. An altered microenvironment, as a result of chronic inflammation, likely contributes to remyelination failure. For example, the extracellular matrix molecule fibronectin (Fn), which is virtually absent in healthy adult CNS, accumulates in MS lesions, and inhibits myelin-like formation in vitro. Our recent findings indicate that also in a healthy environment plasma and astrocyte-derived Fn accumulate in demyelinated lesions, however, and in contrast to MS lesions, Fn is subsequently cleared upon remyelination. The underlying mechanism of persistent Fn expression in MS lesions appears to be astrocyte-mediated Fn aggregation, which might render Fn more resistant to proteolytic degradation.
Our current research focuses on the underlying mechanism(s) of Fn aggregation. As a model for micro-environmental changes induced by inflammation, the role of various cytokines and TLR agonists on astrocyte-mediated Fn aggregation are examined. Furthermore, the contribution of plasma Fn , as well as the role of astrocyte-derived Fn splice variants is determined. In parallel, the effect of the different Fn matrixes on myelin like membrane formation is analyzed. Evidently, knowledge on the underlying mechanism of Fn aggregation, will add to our understanding of remyelination failure in MS lesions.

A LINK BETWEEN NEURONOPHAGIC ACTIVITY AND INCREASED EXPRESSION OF CILIARY NEUROTROPHIC FACTOR BY PERIVASCULAR CELLS IN THE RAT NEUROHYPOPHYSIS

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Unilateral axotomy of the magnocellular hypothalamo-neurophypophysial tract (HNT) results in an initial period of axonal degeneration followed by collateral sprouting of uninjured axons resulting in the restoration of the neurosecretory axonal density within the rat neurohypophysis (NH) to normal values by 30 days post-lesion. However, the specific factors that initiate and maintain axonal sprouting of magnocellular neurons remain ill-defined. In this regard, we have reported previously that partial denervation of the rat NH results in a significant increase in the number of perivascular cells immunoreactive for ciliary neurotrophic factor (CNTF). Furthermore, this increase was sustained throughout the period during which axonal sprouting of magnocellular neurosecretory axons occurs. Magnocellular neurons express CNTF receptor alpha and this expression is upregulated during the sprouting event, concurrent with the increase in CNTF-immunoreactive perivascular cells. Therefore, we hypothesized that CNTF derived from phagocytically-active perivascular cells acts as a target derived sprouting factor. However, a causal link between phagocytic activity and increased CNTF expression has not yet been established. Therefore, in the present study, we sought to; 1) establish a link between phagocytic activity and the up-regulation of CNTF by perivascular cells; 2) identify whether or not perivascular cells are neuronophagic in the injured MNS and, 3) further characterize the immunophenotype of perivascular cells in the NL following endogenously and exogenously induced phagocytic activity. We used unilateral lesion of the HNT and LPS injections to induce neuronophagic and phagocytic activity, respectively. Subsequent dual label immunoperoxidase experiments revealed an increased co-localization of CNTF and ED1 immunoreactive profiles within phagocytically active perivascular cells. In contrast, phagocytically active OX-42-immunoreactive microglial cells were not immunoreactive for CNTF, but were immunoreactive for CD4, ED1, interleukin 1 beta, and MHC I and II. The participation of perivascular cells in the process of neuronophagia was determined by iontophoretic injection of Mini Ruby (MR) resulted in anterograde labeling of neurosecretory axons. Following unilateral lesion of the HNT, MR labeled neurosecretory axons degenerated into small fluorescent granules. Subsequent dual immunofluorescence labeling revealed incorporation of these small MR fluorescent granules within CNTF-immunoreactive perivascular cells. Taken together, our results provide novel evidence for a link between neuronophagia and CNTF upregulation by perivascular cells indicating their role in providing trophic support of neuronal sprouting in the magnocellular neurosecretory system.

A WHOLE CELL ASSAY FOR EVALUATING KAT II INHIBITION

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Kynurenic acid (KYNA) has been implicated in the pathophysiology of schizophrenia. Elevated levels of KYNA that have been reported in the prefrontal cortex and CSF of schizophrenia patients could produce altered glutamatergic and/or cholinergic transmission and negatively affect cognition. Kynurenic acid (KYNA) is produced by the irreversible transamination of L-kynurenine, by the action of multiple kynurenine aminotransferases (KATs). KAT II, present in astrocytes, is responsible for the majority of KYNA synthesis in the brain. Therefore, an inhibitor of KATII may provide may provide a therapeutic benefit to the cognitive deficits in schizophrenia. Screening strategy for our KATII program includes a high throughput whole cell assay in 96 well format to confirm potencies from our primary purified enzyme assay. The whole cell assay utilizes the human hepatocellular carcinoma cell line HEPG2 which expresses high levels of KAT II. The assay demonstrates KYNA accumulation over time and is responsive to L-kynurenine concentration in agreement with the literature. We have also optimized a whole cell assay in primary human hepatocytes and primary human astrocytes, the target cells in the brain. In addition, we have developed a robust, rapid, and sensitive LC/MS/MS analytical method for KYNA to support this assay. Our KAT II inhibitor has an IC50 of 28 nM in HEPG2 cells, 49nM in primary human hepatocytes, and 50 nM in primary human astrocytes. This assay allows us to compare potencies across multiple cells types and in several different formats. The whole cell assay for KAT II inhibitors has proven a valuable second tier screening platform to help drive compound selection for the KAT II program

PROTEOME ANALYSIS OF PERIPHERAL NERVE MYELIN AND BIOINFORMATICAL INTEGRATION WITH MRNA ABUNDANCE PROFILES

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Peripheral nerve myelin allows rapid impulse conduction and normal nerve homeostasis, and mutations affecting a variety of its constituents cause neuropathies. However, knowledge of myelin protein composition has remained sketchy. Combining gel-based and gel-free proteomic approaches we have identified 545 proteins in purified sciatic nerve myelin, including 38 known myelin proteins. By mass spectrometry-based quantification, P0, periaxin, and myelin basic protein constitute 21%, 16%, and 8% of total myelin protein, respectively. We suggest that their relative abundance was previously misestimated due to limitations regarding protein separation and visualization, and that the variety of myelin proteins is much larger than assumed. For validation that novel myelin constituents have been identified using this strategy, we have selected several of them for further characterization by immuno-based methods. Proteins that may stem from fraction contaminations such as blood cells or mitochondria were determined by bioinformatical comparison with mRNA abundance profiles and predicted subcellular localizations. Neuropathy candidate genes were suggested based on a systematic comparison of the positions of the corresponding genes in the human genome with neuropathy disease loci. Our compendium of integrated proteome, transcriptome, and genome data constitutes a versatile resource for further understanding of myelin biogenesis and metabolism, glia-axonal interactions, and hereditary neuropathies.

THE EQUILIBRIUM BETWEEN HISTONE DEACETYLASE 1 (HDAC1) AND HISTONE ACETYTRANSFERASE PCAF MODULATES CHROMATIN COMPACTION AND NUCLEAR SIZE REDUCTION DURING OLIGODENDROCYTE DIFFERENTIATION

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Epigenetic mechanisms regulate mature oligodendrocyte differentiation from oligodendrocyte precursor cells (OPCs). Histone deacetylation plays a critical role in this process and is regulated by the activity of histone deacetylases and histone acetytransferases. In this study we show that during oligodendrocyte progenitor differentiation, there is a progressive reduction of nucleus size, which is coupled with progressive chromatin compaction, as defined by electron microscopy. The profiles of histone deacetylation (AcH3K9 and AcH3K18) correlate with the nuclear size changes. Using lentiviruses to silence HDAC1, we define the critical role of this enzyme in chromatin compaction. Silencing HDAC1 prevents the nuclear size changes, and the deacetylation of AcH3K9 and AcH3K18. Conversely, silencing histone acetytransferases (i.e. PCAF) promotes oligodendrocyte differentiation and up-regulation of myelin basic protein (MBP) gene expression. These findings indicate that the equilibrium between histone deacetylases and histone acetytransferases initiates chromatin changes, leading to nuclear compaction and oligodendrocyte differentiation.

CORTICAL RADIAL GLIA MEDIATES A HOMOCELLULAR NETWORK OF COUPLING IN THE EARLY POSTNATAL SUBVENTRICULAR ZONE

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We developed a novel technique, coined pial loading, to reveal a cellular coupling network mediated by gap junctions (GJ) involving RG cells in the subventricular zone (SVZ), an important postnatal neurogenic niche. During the first postnatal week (P0, P4 and P6), widespread dye coupling is observed within the SVZ, as revealed by direct loading of ruptured pial processes of RG with a mixture of GJ permeant (0,5% lucifer yellow 443Da, LY) and non permeant (0,5% rhodamine dextran 3KDa, RD) fluorochromes. Coupled cells (LY+RD-) were distributed in two strata, one lining the lateral ventricle and the other at the SVZ/WM (white matter) border. RG mediated cell coupling diminishes when animals were intraventricular injected with the pharmacological gap junction blocker flufenamic acid (FFA; 100μM) (control: 1.767± 0.0456 mean ± SEM; FFA: 1.187± 0,01331 mean ± SEM; p value<0.0001; Mann Whitney test; n=9 animals, 3 independent experiments). Exclusively LY labeled cells express a glial immunophenotypic profile (BLBP+; GFAP+; Nestin+; TuJ1-), suggesting restricted homocellular coupling networks. We also analyzed the expression of connexins (Cxs) 43 and 45 in the SVZ/RMS (rostral migratory stream), as well as by its major cell types. RG and neuroblasts/immature neurons were revealed by Cell Tracker Red™ (CMTPX; 10mM) injections at the pial surface or directly into SVZ. Although presented in both neuronal and glial cell types, Cx43 and Cx45 levels seemed inversely related, with Cx45 prevailing in neuroblasts. To further discriminate single cell expression we dissociated the SVZ, separating the cortical (SVZc) and olfactory bulb (SVZb) regions, acute plated on Matrigel (15μM) and performed immunohistochemical assays. Double labeling with major SVZ cell phenotypic markers (TuJ1, PSA-NCAM, GFAP and BLBP) and Cxs showed that all cell types express Cxs similarly. The percentage of SVZb cells expressing Cx45 (91.90 ± 1.33 mean ± SEM) is significantly greater than in SVZc (87.48 ± 1.56; p value=0.03; Unpaired t test; 48 fields, n=9 animals, 3 independent experiments). In contrast, no significative difference is observed for Cx43 expression (94.23 ± 0.79 and 92.52 ± 1.07; p value=0.20). Together, these results demonstrate the expression of connexins and GJ cell coupling in the neonatal SVZ/RMS, suggesting an important role of these proteins on cell migration and to the proliferative capacity in this notable neurogenic region.

INTEGRIN FUNCTION IN PERIPHERAL GLIA OF *DROSOPHILA*

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In Drosophila and vertebrates, peripheral nerves share similar structures. Axons are centrally located and are wrapped by membrane processes from wrapping glial in fruitfly and Schwann cells (SCs) in vertebrates. The axons and inner glial cells are subsequently surrounded by superficial cellular layers, the Drosophila subperineurial and perineurial glia and vertebrate perineurial cells. Non-cellular layers composed of ECM components are formed on the surface of nerves, which are the Drosophila neural lamella and vertebrate epineurium respectively. A tubular sheath is established around axons by these glial cells and surrounding extracellular matrix and protects axons from physical damage and pathogens. Adhesion and interactions with the ECM is mediated in part by different integrin complexes. ECM-integrins interactions have been shown to play a role in the development of vertebrate glial layers and have diverse functions including: migration of neural crest cells, radial sorting and myelination of Schwann cells. However the identify and role of integrin complexes in the other classes of vertebrate glia and in the equivalent glial layers of the Drosophila peripheral nerve has not been determined. Here we show that Drosophila αPS2βPS1 and αPS3βPS1 integrins are expressed in different glial layers in the larval peripheral nerves. These integrins are located to cytoplasm membrane and form adhesion complexes with ILK and Talin. Inducible RNA interference technique was employed to knock down integrin expression specifically in glial cells and more importantly in specific glial subtypes. Expression of βPS1 RNAi in all glial cells results in animal lethality and glial defects. Specifically, induction of βPS1 RNAi in perineurial glia gives wrapping failure, which is phenocopied by degradation of neural lamella. Knocking down of βPS1 integrin in wrapping glia causes loss of membrane processes around axons. Our data suggest that integrin complexes are employed in different glial layers to mediate formation of glial ensheathment in larval peripheral nerves.

SCULPTING CIRCUITS AND CLEANING UP MESSES: GLIAL CRK-II/DOCK-180/ELMO REGULATE AXON PATHFINDING DURING DEVELOPMENT, AND CLEARANCE OF DEGENERATING AXONS FROM THE ADULT BRAIN.

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We are exploring molecular mechanisms how glia sense and respond to acute brain injury in *Drosophila*. Fly glia rapidly respond to axotomy by extending membranes to degenerating axons and phagocytosing axon debris. In *draper* (MEGF-10/Jedi) null mutants, glia fail to respond to axon injury: glial membranes are not recruited to degenerating axons, and axon debris persist in the CNS. During cell corpse engulfment in *C. elegans*, the CED-1/Draper pathway functions partially redundantly with a second pathway, which includes the Rac guanine nucleotide exchange factor (GEF) CED-2/CED5/CED-12 (CrkII/Dock-180/Elmo). Loss of either pathway partially suppresses cell corpse engulfment, but loss of both completely blocks clearance. Here we show that the Draper pathway and CrkII/Dock-180/Elmo complex are essential, non-redundant signaling pathways that promote clearance of degenerating axons.

We find that severed axons linger in the CNS for weeks after axotomy in *draper* mutants, indicating that Draper is absolutely essential for all axon clearance, and arguing against the presence of a redundant second pathway. In addition, we find that glial-specific knockdown of any component in the CrkII/Dock-180/Elmo complex suppresses clearance of degenerating axons, with axons lingering in the CNS for weeks after injury. Interestingly, each of these signaling pathways plays a unique functional role during clearance. While Draper is essential for the recruitment of glial membranes to severed axons (above), loss of CrkII/Dock-180/Elmo function, in contrast, does not affect the recruitment of glia to degenerating axons: glial membranes still accumulate on severed axons, remain on axons for ~5 days, and appear to leave the injury site. Thus, recruitment of glial membranes to sites of injury in the brain and phagocytosis of axon debris are genetically separable events, with Draper signaling modulating the former and CrkII/Dock-180/Elmo regulating the latter.

Surprisingly, we also found that glial-specific loss of CrkII/Dock-180/Elmo during development resulted in striking defects in axon pathfinding. Cell type and temporally-controlled RNAi, as well as conditional blockade of endocytosis, demonstrated a pivotal role for these genes in glia at the precise time when axons are projecting to their target sites. We are currently exploring whether axon wiring defects arise from defective phagocytic glial signaling during this stage, or whether this represents a novel (i.e. non-engulfment) role for CrkII/Dock-180/Elmo in glia during axon pathfinding.

CELLULAR AND MOLECULAR REGULATION OF THE BLOOD-BRAIN BARRIER

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Vascular endothelial cells in the central nervous system (CNS) form a barrier that restricts the movement of molecules and ions between the blood and the brain. This blood-brain barrier (BBB) is crucial to ensure proper neuronal function and protect the CNS from injury and disease. Although the properties of the BBB are manifested in the endothelial cells, transplantation studies have demonstrated that the BBB is not intrinsic to the endothelial cells, but is induced by interactions with the neural cells. Here we use a genomic, genetic and molecular approach to elucidate the cellular and molecular mechanisms that regulate the formation of the BBB. We have developed methods to highly purify and gene profile endothelial cells from different tissues, and by comparing the transcriptional profile of brain endothelial cells with those purified from the liver and lung, we have generated a comprehensive resource of transcripts that are specific to the BBB forming endothelial cells of the brain. Through this comparison we have identified novel tight junction proteins, molecular transporters, metabolic enzymes, signaling components, and unknown transcripts that are expressed specifically by CNS endothelial cells. This analysis has identified that Wnt/beta-catenin signaling is critical for BBB formation.

THE CAMP EFFECTOR PKA MEDIATES GPCR SIGNALING IN GLIAL BLOOD-BRAIN BARRIER DEVELOPMENT

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The blood-brain barrier (BBB) plays a crucial role in insulating the nervous system against the surrounding hemolymph and is established by surface glia during embryonic development in *Drosophila*. We have previously identified Moody, a novel G protein-coupled receptor (GPCR), as a key factor in the development and maintenance of the BBB. However, the precise mechanism underlying GPCR signaling during BBB development has remained unclear. Here we show that cAMP-dependent protein kinase (PKA), a major effector of cAMP, is required for the insulation of the nervous system and plays a critical role in the Moody signaling pathway. At the cellular level, PKA not only regulates cell shape by reorganization of the actin and microtubule cytoskeleton, but also affects the expression and proper localization of SJ components to the interface with neighboring surface glia, which are required for effective insulation. Strikingly, the PKA catalytic subunit DC0 shows highly polarized localization in the surface glia, and both over- and underactivity of PKA result in severe insulation defects, indicating that the cAMP/PKA pathway produces a local signal within the cell. Our study demonstrates a novel role of cAMP/PKA signaling in the regulation of the intercellular SJ organization that generates BBB integrity, and provides new insights into the molecular and cellular mechanism of Moody-regulated BBB development and maintenance.

VEGFR3 SIGNALING IN SUBVENTRICULAR ZONE ASTROCYTES IS REQUIRED FOR POSTNATAL NEUROGENESIS

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Neural stem cells are intimately associated with capillary endothelial cells in the lateral ventricle walls of the CNS. Functionally, members of the vascular endothelial growth factor (VEGF) family can stimulate neurogenesis as well as angiogenesis, but it remains unclear if they act directly via VEGF receptors expressed by neurons, or indirectly via release of growth factors from angiogenic capillaries. Here, we show that the lymphangiogenic receptor VEGFR-3 is expressed by slowly dividing type B neural stem cells in the adult subventricular zone (SVZ) and is required for postnatal neurogenesis. Neurosphere assays and adeno-associated virus (AAV)-mediated VEGF-C overexpression in the adult SVZ promote SVZ cell survival. Finally, neural-specific Vegfr3 deletion using a Cre/lox approach impairs SVZ and olfactory bulb neurogenesis, without affecting angiogenesis. Thus, neural stem cells in the postnatal forebrain require VEGF-C/VEGFR-3 signaling, demonstrating that this lymphangiogenic growth factor-receptor system is essential for murine CNS neurogenesis.

TWENTY YEARS OF GLIOTRANSMISSION - EXPERIMENTAL ARTIFACT OR REALITY?

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The neuronal doctrine, developed a century ago, regards the neuronal networks as a sole substrate for the higher brain function. Recent advances in glial physiology promoted an alternative hypothesis, which places information processing in the brain into the integrated neuronal-glial circuitries that utilize both binary (neuronal action potentials) and analogue (glial Ca2+ waves) signal encoding. The feed-forward and feed-back communications between two cellular circuits are accomplished by the release of neuro- and gliotransmitters that are believed to control information transfer and processing. There remain, however, important questions and controversies that need further probing.

CX32 AND CX47 HAVE DISTINCT ROLES IN OLIGODENDROCYTE:ASTROCYTE GAP JUNCTION COUPLING

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We have investigated the roles of connexin32 (Cx32) and Cx47 in oligodendrocyte:astrocyte (O:A) coupling by whole-cell patch clamp studies in acute brain slices from P15-P32 *Gjb1/Cx32*-null, *Gjc2/Cx47*-null mice, *Gjb1- // Gjc2*-double-null mice, or their littermates. Because the *Egfp* gene had been "knocked into" the *Gjc2* locus, oligodendrocytes could be directly visualized owing to their expression of EGFP. EGFP-positive cells were patched with an electrode containing 0.1% Sulforhodamine-B (SR-B), and the dye was allowed to diffuse for 20 minutes, then the section was analyzed optically to determine whether nearby EGFP-positive and/or –negative cells were labeled by SR-B.

• In the corpus callosum of *Gjb1+/+ // Gjc2+/-* mice, SR-B labeled EGFP-positive cells (but not EGFP-negative cells); in the neocortex, SR-B labeled EGFP-positive and -negative (presumed to be astrocytes) cells.
• In the corpus callosum of *Gjb1-/- // Gjc2+/-* mice, SR-B did not label EGFP-positive cells (or EGFP-negative cells); in the neocortex, SR-B labeled EGFP-positive and -negative cells.
• In the corpus callosum of *Gjb1+/+// Gjc2-/-* mice, SR-B labeled EGFP-positive cells (but not EGFP-negative cells); in the neocortex, SR-B did not label EGFP-positive or -negative cells.
• In the corpus callosum and the neocortex of *Gjb1-/- // Gjc2-/-* mice, SR-B did not label EGFP-positive or -negative cells.

These data demonstrate that O:A coupling is mediated by Cx47 in the neocortex, and suggests that Cx32 may mediate O:O coupling in the corpus callosum. The loss of O:A and possibly O:O coupling could be the basis of the severe myelinopathy in *Gjb1- // Gjc2*-double-null mice, which are an animal model of Pelizaeus-Merzbacher-like disease of humans.

ANTI-DEPRESSIVE EFFECTS OF SLEEP DEPRIVATION REQUIRE THE ASTROCYTE-DEPENDENT SLEEP-HOMEOSTAT

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Approximately one in 20 Americans will suffer from depression in a given year, and one in 10 Americans will suffer from major depression at least once in their lifetime. Current pharmacological treatments take weeks for efficacy limiting the ability to bring instant relief to suicidal patients. One non-pharmacological intervention that rapidly alleviates symptoms of depression is sleep deprivation (SD) for one or more nights. This treatment is effective in approximately 60% of all depressed patients, and in about 70% of those patients diagnosed with “endogenous” depression. In stark contrast to pharmacological agents, SD produces a rapid improvement in mood. The mechanism by which SD can produce these rapid and dramatic improvements in depressive symptoms remains unknown.

Recent work in our laboratory has discovered that astrocytes regulate behavioral responses to SD including changes in NREM slow wave activity as well as increased sleep time and cognitive impairments that follow SD. The conditional expression of the SNARE domain of the vesicle protein VAMP2 within astrocytes reduces the extracellular adenosine level as assessed by changes in the tonic activation of neuronal A_1 receptors. This reduced activation of A_1 receptors leads to a reduction in the pressure to sleep as well as reduced responses to SD. We have asked whether this astrocytic modulation of the sleep homeostat contributes to anti-depressive effects of a total night of SD.

C57Bl/6J mice, the genetic background of dnSNARE mice, are considered to exhibit depressive-like behaviors as measured in two models of behavioral despair. A total night of SD produces a robust reduction of murine depressive-like behaviors as measured by immobility time in both the tail suspension test (TST) and forced swim task (FST) models of depression. Inhibition of the glial sleep homeostat by astrocytic dn-SNARE expression prevents SD changing immobility times suggesting that the SNARE-sensitive accumulation of adenosine mediates these anti-depressive-like actions. This hypothesis is supported by the observation that the beneficial effects of SD are prevented in $A1R^{-/-}$ mice, and in mice treated with the A1 receptor antagonist cyclopentyltheophylline (CPT; 4mM in osmotic mini-pump). These data suggest that the beneficial effects of SD are mediated by a glial-dependent adenosine A1R pathway and provides a unique opportunity to develop novel, fast acting therapeutic strategies to treat depression.

ASTROCYTE STIMULATION CAN TRIGGER CORTICAL UP STATES

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Although the effect of neuronal activity on astrocytic function is well documented, it remains unclear whether or how astrocytes influence neuronal circuit function. We have explored potential circuit-wide effects of astrocytes and investigate how astrocytic activity affects coherent neuronal activity in a cortical mouse slice, using UP states as a measure of neuronal synchronization. We use simultaneous whole-cell patch clamping of neurons and astrocytes, and find that cortical astrocytes become depolarized during UP states, with an amplitude that is correlated with the duration of the UP states in the neurons. Further, we depolarize individual astrocytes and observe that this depolarization increases the probability that neurons in the circuit will undergo an UP state transition. We also use two-photon microscopy to visualize calcium dynamics in astrocytic networks, and find a significant increase in calcium transients in surrounding astrocytes when a single astroycte in the syncytium is depolarized. In addition, spontaneous and evoked astrocytic calcium transients tend to precede UP states, and blocking these transients significantly reduces the number of UP states we observe, demonstrating that calcium increases throughout the astrocytic network are causally linked to the subsequent UP state. These experiments indicate that astrocytic stimulation can trigger synchronized neuronal activity in cortical circuits.

DEVELOPMENT OF A NOVEL METHOD TO PURIFY AND CULTURE MATURE RAT BRAIN ASTROCYTES

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The active role that astrocytes play in brain development and function have been brought to the forefront by several seminal studies in recent years. Despite these advances, however, many fundamental properties of astrocytes still remain a mystery. In order to answer these questions, we have developed a novel immunopanning method to purify relatively mature postnatal astrocytes from P7 to P14 rodent forebrains. We found that these highly purified astrocytes rapidly undergo apoptosis in culture. We analyzed their gene profiles to determine which signalling receptors were highly expressed, and then screened their cognate ligands to identify one trophic factor that is sufficient to strongly promote their survival in a defined, serum-free media. We have validated that the gene profile of these astrocytes in culture is close to that of acutely purified astrocytes, and thus more representative of the *in vivo* state than the previous McCarthy-de Vellis method of preparing and culturing neonatal astrocytes. By studying co-cultures of neurons and astrocytes, we show that these astrocytes strongly promote neuronal survival and synapse formation. Together, the data suggests that we have developed a culture system that is more representative of the *in vivo* state and will be helpful for future *in vitro* studies of neuron-glial interactions and astrocyte function.

MOLECULAR DISSECTION OF REACTIVE ASTROGLIOSIS AND GLIAL SCAR FORMATION

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Although reactive astrogliosis is a ubiquitous feature of the CNS response to all forms of injury and disease, until recently this response has been poorly defined and its mechanisms poorly understood. Genetic tools are now enabling the molecular dissection of the functions and mechanisms of reactive astrogliosis *in vivo*. This presentation will examine findings from our and other laboratories indicating that reactive astrogliosis is a complex, multifaceted process comprising numerous potential cellular and molecular changes with a wide range of potential effects regulated by different signaling molecules. It is now clear that reactive astrogliosis is not a simple all-or-none phenomenon but is a finely gradated continuum of changes that occur in context dependent manners regulated by specific signaling events. These changes range from reversible alterations in gene expression and cell hypertrophy with preservation of cellular domains and tissue structure, to long lasting scar formation with rearrangement of tissue structure. Although reactive astrocytes have often been regarded as uniformly detrimental to clinical outcome, it is now clear that reactive astrogliosis, including scar formation, can exert both beneficial and detrimental effects in a context-dependent manner determined by specific molecular signaling cascades. In addition, increasing evidence points towards the potential that reactive astrogliosis may play either primary or contributing roles in a wide variety of CNS disorders via loss of normal astrocyte functions or gain of abnormal effects. A better understanding of astrocyte signaling mechanisms and the mechanisms of reactive astrogliosis has the potential to open the door to identifying molecules that might serve as novel therapeutic targets for a wide range of neurological disorders.

For recent reviews see:
Sofroniew MV (2009) Molecular dissection of reactive astrogliosis and glial scar formation. Trends Neurosci 32:638-647.
Sofroniew MV, Vinters HV (2010) Astrocytes: biology and pathology. Acta Neuropathol 119:7-35.

DECIPHERING THE DAMAGING AND PROTECTIVE ROLES OF GLIA IN GLAUCOMA

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Glaucoma is a neurodegenerative disease affecting 70 million worldwide. Vision loss in glaucoma is caused by the death of retinal ganglion cells (RGCs). Studies in humans and primates established that early damage occurs to RGC axons in the lamina cribrosa, located at the optic nerve head (ONH). The lamina cribrosa is comprised of extracellular matrix (ECM) plates that are lined with astrocytes. Mice do not have an ECM-based lamina, but do have a similar robust network of astrocytes. We provided the first functional evidence that an early insult occurs to RGC axons at the ONH in DBA/2J mice, a widely used mouse model of glaucoma. Although RGCs are the most susceptible to damage, changes also occur to glial cells including astrocytes, microglia and Muller glia. Astrocytes and Muller glia become gliotic and responding microglia accumulate at the junction of myelination in the optic nerve. Currently, it is not clear what the molecular changes are that underlie these morphological changes in glial cells, or whether targeting these changes will be beneficial.

To identify molecular events in glaucoma, we used microarray-generated gene expression data and a series of clustering methods to identify early stages of glaucoma in both the ONH and retina in DBA/2J mice. Our methods successfully subdivided eyes with no detectable glaucoma by conventional assays into molecularly defined stages of disease. Events identified as early include (i) upregulation of ECM-receptor interactions in the ONH, (ii) upregulation of pro-inflammatory cytokines in the ONH and retina, (iii) complement activation in ONH and retina and (iv) Endothelin-2 upregulation in retina. Immunohistochemistry and RNA *in situ* hybridization shows these events are occurring in different populations of glial cells. Since targeting early changes is likely to provide effective new treatments, we are testing the efficacy of manipulating pathways that we have found to change early in the disease. Targeting pro-inflammatory cytokines by administering anti-inflammatory drugs does not significantly protect DBA/2J eyes from glaucoma. In fact, they appear to make glaucoma worse under certain conditions. To assess the complement cascade, we generated DBA/2J mice with a mutation in the complement component 1a gene (*C1qa*) and importantly, these mice develop substantially less glaucoma than wild–type littermates.

Our successful implementation of clustering methodologies to discover early events in an inherited, later-onset, complex glaucoma suggests that similar approaches will prove valuable for uncovering early processes in other diseases and for identifying novel therapeutic targets.

USING EMBRYONIC STEM CELLS TO STUDY MOTOR NEURON/GLIA INTERACTIONS IN ALS

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Amyotrophic Lateral Sclerosis (ALS) is a result of the selective dysfunction and progressive degeneration of motor neurons. Although the underlying disease mechanisms remain unknown, recent in vivo and cell culture experiments have implicated glial cells in motor neuron degeneration in ALS.

We have made use of the SOD1 mouse model of ALS to study the effect of glial cells bearing the mutant SOD1 transgene on motor neuron viability in cell culture. Specifically, we have studied the gene expression profiles of co-cultured mouse embryonic stem (ES) cell derived motor neurons and primary glia using the Illumina deep sequencing platform (RNAseq). In this study, we vary both the genotype of the motor neurons and glia, as well as time in culture as a means of examining both cell autonomous and non-cell autonomous effects of the mutant transgene. In addition, we carry out parallel studies with spinal cord samples from mutant and wildtype SOD1 mice, and compare both the in vivo and in vitro derived data sets with laser capture microdissection studies of both the ALS mouse model and human ALS patient samples.

We have detected significant cell autonomous and non-autonomous changes in gene expression in both motor neurons and glia, indicating that the two cell types profoundly affect each other's gene expression. In addition, we find a remarkable concordance between the different data sets mentioned above, thus validating the in vitro approach. We are currently analyzing these data sets to identify changes in the expression of specific genes and signaling pathways that may contribute to motor neuron degeneration in ALS.

THE APPLICATION OF MADM, A MOUSE GENETIC MOSAIC SYSTEM, FOR GLIOMA MODELING

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Cancer is a disease of genetic mosaicism. “Mosaicism” as a genetic term describes the phenomenon that cells within a multi-cellular organism harbor distinct genomic content. In the case of cancer, it refers to the distinct genotype in cancerous cells from normal ones in the same individual. Cancerous mosaicism in human is often initiated by the loss of heterozygosity (LOH) of tumor suppressor genes (TSGs) in as few as a single cell. The TSG null mutant cell then evolves through acquiring further gene mutations and via interactions with its normal stromal niche to eventually develop into malignant tumors.

To study the cancerous process in vivo, a genetic mosaic animal model should be ideal. Previously, Drosophila geneticists have developed a powerful mosaic system to address these problems. Many elegant and otherwise unachievable studies have been carried out with the mosaic system, such as mutant-WT cell competition behavior, cell and organ size control mechanisms, recessive genetic screening for tumor suppressor genes or metastasis-promoting genes. Based on a similar design principle to the Drosophila system, we established a mouse genetic mosaic system termed MADM (Mosaic Analysis with Double Markers) [Zong et al 2005 Cell]. Through Cre-loxP mediated inter-chromosomal mitotic recombination, the MADM system can generate homozygous mutant cells that are unequivocally labeled by green fluorescent protein (GFP) and their sibling WT cells labeled by red fluorescent protein (RFP) within a heterozygous colorless mouse. Due to the low probability of the recombination between chromosomes, mutant cells generated by MADM are very sparse (0.1-1% or much lower). The sparseness of mutant cells, although not as few as the monoclonal origin of human cancers, allow them to be dispersed among normal surrounding cells to mimic the heterotypic interactions during tumorigenesis. Furthermore, the dependence of cell type-specific Cre for mutant cell generation provides us a precise control to study the cell of origin problem. With the permanent GFP-labeling, progeny of mutant cells can be studied along each and every lineages for their tumorigenic potentials. Last but not least, the RFP-labeled WT sibling cells provide an excellent internal control for in vivo phenotypic analysis. Here I will present our recent work in glioma modeling with the MADM system, which revealed the involvement of a specific glial cell lineage in gliomagenesis. Finally, it is important to point out that MADM can be applied to study other diseases and normal development for in-depth mechanistic studies.

THE LINEAGE-RESTRICTED TRANSCRIPTION FACTOR OLIG2 OPPOSES P53 RESPONSES TO GENOTOXIC DAMAGE IN NEURAL PROGENITORS

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The *p53* tumor suppressor and some of its downstream target genes have been shown to suppress self-renewal of adult neural stem cells. In murine neural progenitors, the lineage-restricted bHLH transcription factor Olig2 promotes cell survival and functionally opposes p53-mediated responses to genotoxic damage. This cross-antagonistic relationship between Olig2 and p53 is also observed in malignant neural progenitors and in human glioma-derived progenitor lines. The data sheds light on the pervasive expression of Olig2 in human diffuse gliomas and may, in part, explain why these tumors are notoriously resistant to radiation and chemotherapy. In the absence of Olig2, even attenuated levels of p53 function (seen for example in *p53* null heterozygotes or $p19^{Arf}$ null cells) are sufficient to initiate biological responses to genotoxic damage. Olig2-mediated vulnerability to malignant transformation might be the price paid by the adult central nervous system to maintain replication competence in these cells and sustain a reserve of neural progenitors for response to injury and for normal turnover of certain neural populations.

GLIAL PROGENITOR CELLS AS THE ORIGIN AND ESSENCE OF GLIOBLASTOMA

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Identifying the cells that give rise to glioblastomas (GBMs) and defining the molecular/genetic alterations that are needed to transform these cells have important implications for understanding the genesis and progression of brain tumors. In this study we examine the tumorigenic potential of progenitors that reside in subcortical white matter. To this end, we performed stereotactic injection of PDGF–IRES-CRE (PIC) retrovirus into the brains of adult mice that harbor floxed tumor suppressors (PTEN, p53 or both), and stop-flox reporter systems (YFP or luciferase). Fate mapping showed that injecting control retrovirus into the lateral white matter selectively infects local glial progenitors that give rise to a mixture of astrocytes, oligodendrocytes and NG2 glia. In contrast, injection with PIC retrovirus gives rise to brain tumors that harbored genetic deletion in PTEN and/or p53. Tumors showing the histological features of glioblastoma form with 100% penetrance, although the rate of tumor formation depends on the initial genetic alterations, with combined deletion of PTEN and p53 inducing the most rapid tumor formation. Expression profiling showed that all tumors had a similar phenotype defined by a set of “proneural” genes that are known to regulate oligodendrocyte progenitor proliferation and differentiation. Array CGH and sequencing analysis revealed that the mouse tumors consistently accumulate additional genomic alterations. A comprehensive comparison with the TCGA data on human GBMs showed that a subset of genetic alterations seen in the mouse models is also found in the “proneural” subtype of human GBM. Thus, the PDGF driven mouse tumors recapitulate both the phenotype and genomic alteration seen in a specific subset of human GBMs and provide a powerful tool to study the molecular/genetic alterations that occur during the evolution from glial PROGENITOR TO GLIOBLASTOMA.

THE NG2 PROTEIN EXPRESSED BY OLIGODENDROCYTE PROGENITOR CELLS BINDS TO THE MITOCHONDRIAL SERINE PROTEASE OMI/HTRA2: A ROLE FOR NG2 IN STRESS-PROTECTION?

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The NG2 proteoglycan is expressed by oligodendrocyte progenitor cells (OPC). In order to understand the function of the protein we are defining its binding partners. NG2 has a PSD95/Discs Large/ZhO-1 (PDZ) binding domain at the intracellular C terminus: in a yeast two-hybrid screen, we identified the PDZ mitochondrial serine protease Omi/HtrA2 as a potential NG2 binding partner. Biochemical analysis confirmed this interaction in OPC in vitro as well as in cells transfected with constructs for Omi/HtrA2 and NG2. Omi/HtrA2 is imported into mitochondria where it is processed and then retained in the intermembrane space. It is thought to function in mitochondrial homeostasis and may act as a chaperone. Omi possesses a serine protease domain and loss of function mutations of this leads to neuromuscular disease in mice (1). Upon cellular stress, Omi/HtrA2 is released from the mitochondria and enters the cytosol. Here it binds to the inhibitor of apoptosis proteins (IAPs), leading the cell into caspase-dependent apoptosis. The Omi/HtrA2 serine protease domain can also degrade the IAPs. In the presence of hydrogen peroxiode, which is a model of cellular stress, increased amounts of released Omi/HtrA2 bind to NG2 in OPC. Binding of NG2 to Omi/HtrA2 may thus be a way to sequester Omi released from mitochondria to protect the cells from apoptosis. In the absence of NG2, OPC would be expected to be more sensitive to cellular stress.
We have generated a knock-out mouse for NG2 by inserting the EYFP chromophore into the first exon of the endogeneous NG2 gene (2). The most striking feature of the homozygous knock-out mouse is an increased synthesis of GFAP indicating gliosis. This increases with age and may represent an astrocytic reaction to a altered turnover of OPC, or an altered differentiation of astrocytes. OPC are known to be particularly sensitive to cellular stress and white matter disease of the newborn is a major pathology in situations where premature infants are exposed to hypoxia. The ability of the NG2 protein to bind Omi/HtrA2 may help protect OPC from cellular stress.
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THE GLIAL REGENERATIVE RESPONSE TO CENTRAL NERVOUS SYSTEM INJURY IS ENABLED BY PROS-NOTCH AND PROS-NFκB FEEDBACK

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Injury to the central nervous system (CNS) induces proliferation of ensheathing glia, leading to axonal re-enwrapment and functional recovery. The glial regenerative response is found across species, from insects to humans, invoking an underlying genetic mechanism. However the gene network controlling the glial regenerative proliferation is unknown. It is a key to unravel how glial proliferation might be controlled to promote repair while avoiding tumorigenesis. Using stabbing injury and in vivo gene manipulation in glial cells, here we show that the proliferative glial response to injury critically depends on the gene prospero (pros/Prox1) in the *Drosophila* larval CNS. While Pros-positive ensheathing glia rarely proliferate in the unstabbed CNS, injury signals conveyed by Eiger/TNF through Dorsal/NFκB trigger their proliferation. We demonstrate that two positive feedback loops, one involving Pros and Notch, and another one Pros and Dorsal/NFκB, enable this response. Pros negatively regulates the cell cycle and promotes glial differentiation, while Notch and Dorsal/NFκB positively promote proliferation, and they maintaining each other through positive feedback. The balance of opposing functions enables the glial cells (1) to maintains them on the brink of dividing, (2) to respond to injury, and subsequently, (3) to re-establish cell cycle arrest. Breakdown of these feedback loops resulted in loss of the injury response and tumourigenesis. In *pros egr* double mutants, there was no injury response and wounds were larger. Gain of function of Notch increased glial number, whilst concomitantly reducing wound size, the extent of injury-induced apoptosis and vacuolisation. Time-lapse analysis in wild-type CNS showed that vacuoles emerged in axonal bundles after stabbing, some of which were filled with glial projections and subsequently repaired. These results show that the proliferative glial response is regenerative and that manipulating the gene network in glial cells can facilitate neuropile repair. We have thus identified a gene network underlying the glial regenerative response, which is favourable for repair. Our findings are likely to provide valuable insights for mammalian models of CNS regeneration.

REGION-RESTRICTED ORIGINS AND DISTRIBUTION OF ASTROCYTES IN THE MAMMALIAN SPINAL CORD

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Recent studies indicate that region-restricted expression of transcription factors regulate astrogenesis in the ventral neural tube according to a segmental template, providing a putative model for generation of astroglial diversity. Here we investigate the long-term consequences of this "segmental model" for glial cell organization and diversity throughout the postnatal spinal cord in vivo. Using a genetic lineage tracing approach, we delineate multiple embryonic origins for astrocytes along the dorsal-ventral (DV) axis. We show that developing astrocytes migrate in strictly radial fashion and permanently settle in stereotypic "wedge-shaped" territories, reflecting their derivation from radial glia and cell-intrinsic restrictions on migration patterns. Although some ventrally-derived oligodendrocytes exhibit multi-modal migration, and tangential astrocyte migration has been reported in brain, our findings show that mature astrocytes in all spinal cord domains migrate radially but not tangentially. We find that DV domains differ dramatically in their allocation of oligodendrocyte versus astrocyte precursors, but that region-restricted fibrous and protoplasmic astrocytes arise from similar domains from precursors that are largely Olig2-negative. These findings indicate that the spatial arrangement of astrocytes in the mature spinal cord, which delimits their range of influence and cell-cell interactions, is determined by patterning of the embryonic neural tube.

IDENTIFICATION AND CHARACTERIZATION OF A FUNCTIONAL SCHWANN CELL ENHANCER REGULATING OCT6 GENE EXPRESSION

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The intimate and continued interactions between Schwann cells and their associated axons drive the development, maintenance and regeneration of the peripheral nervous system. One major function of Schwann cells is the elaboration and maintenance of the myelin sheath that surrounds the axons which in turn allows for rapid saltatory propagation of electric impulses.

Previous studies have shown that Oct-6, a POU – homeodomain transcription factor is a major regulator of Schwann cells, driving the timely progression of cell differentiation from promyelinating to the myelinating stage. Oct-6 is one of the first transcription factors in the genetic hierarchy of myelination. In Oct-6 deficient mice, Schwann cells are transiently arrested at the promyelinating stage of cell-differentiation. Understanding the regulation of oct-6 gene expression would help uncover the identity of yet unknown axon mediated signals that drive its expression.

Previously we have identified a cis-acting regulatory element downstream of the Oct-6 gene promoter, the Oct-6 Schwann cell enhancer (SCE), which is sufficient to drive the expression of Oct-6 in Schwann cell lineage both spatially and temporally. Phylogenetic analysis of the SCE revealed the presence of two highly conserved regions named homology region 1 (HR1) and 2 (HR2). Deletion analysis showed that both elements contribute to full enhancer activity in vivo and in primary Rat Schwann cells.

We further narrowed down the 4.5kb SCE to a 1kb mini SCE element, which recapitulates Oct6 expression in time and space. This expression is independent of the promoter used, to drive the LacZ reporter, as the Oct6 promoter and hsp68 promoter worked equally well. Within the 1kb mini SCE, a highly evolutionarily conserved 38bp element of HR1 was found to be absolutely essential for the function of the SCE. Mutating the 38bp element (block1) abolishes enhancer activity. Extending our phylogenetic analysis to Opossum, we further narrowed down the 1kb mini SCE to a 300bp micro SCE containing 4 core elements, which are essential and sufficient for the enhancer function. Mass spectrometry of proteins binding to core element block1 identified Sox10. Taken together our characterization of the Schwann cell enhancer reveals an evolutionarily conserved, Sox10 binding, 38bp core element within a 300bp functional Schwann cell enhancer that is capable of regulating the expression of Oct6 in a tissue and time specific manner.

NEURAL CREST ORIGIN OF OLFACTORY ENSHEATHING GLIA

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Olfactory ensheathing cells (OECs) are a unique class of glial cells with exceptional translational potential because of their ability to support axon regeneration in the central nervous system. Current dogma holds that OECs arise from the olfactory epithelium. OECs for use in cell transplantation therapy for spinal cord repair are generally cultured from explants of the olfactory mucosa, but this tissue also includes antigenically-similar Schwann cells, making it difficult to identify OECs reliably in such cultures. Here, using fate-mapping techniques in chick embryos and genetic lineage-tracing in mice, we show that OECs originate from the neural crest, hence share a common developmental heritage (including expression of the transcription factor Sox10) with Schwann cells. This explains the similarities between OECs and Schwann cells, overturns the existing dogma on the developmental origin of OECs, and opens up a new area of research in neural crest and glial cell biology, i.e., the control of neural crest cell differentiation into OECs. Since neural crest stem cells persist in adult tissue, including skin and hair follicles, our results also raise the possibility that patient-derived neural crest stem cells could in future provide an abundant and accessible source of autologous OECs for cell transplantation therapy for the injured central nervous system.

HELPING TO START AND STOP MYELINATION: CSK HAS DUAL REGULATORY FUNCTIONS DURING OLIGODENDROCYTE DEVELOPMENT

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The ability of oligodendrocyte progenitors (OPCs) to generate sufficient numbers of myelinating oligodendrocytes is critical both for developmental myelination and for repair following demyelination. The timing and location of gliogenesis are tightly controlled but the molecular mechanisms that underlie this control are poorly understood. Here we report that the C-terminal Src Kinase (Csk) acts as a molecular switch for Src Family Kinase activity in oligodendrocytes, with distinct and opposing roles for Csk in early versus late stages of development. Early in development Csk is critical for the appropriate onset of oligodendrocyte progenitor differentiation. Csk suppresses OPC proliferation such that Csk depletion in OPCs in vitro and in vivo led to proliferation under conditions that normally promoted cell cycle exit. Hyperproliferation of Csk-deficient OPCs resulted in delayed oligodendrocyte maturation accompanied by delayed myelination onset, while survival of newly-formed oligodendrocytes was increased. These data suggest that, during myelination onset, Csk is a pro-differentiation factor that promotes timely OPC cell cycle exit. Adult Csk null mice, however, developed hypermyelination, suggesting that Csk also contributes to myelination arrest. Thus, Csk deletion caused increased levels of myelin basic protein as well as increased myelination in the cortex, cerebellum and spinal cord, although there was no change in the number of mature oligodendrocytes. Further analysis of myelin ultrastructure using transmission electron microscopy revealed increased numbers of myelin wraps such that the g-ratio was significantly decreased, particularly in small caliber axons. To address a putative role for Csk during myelin repair we have recently begun to evaluate the response of Csk null mice to cuprizone-induced demyelination. Preliminary analyses reveal that during remyelination Csk mutant animals exhibit increased numbers of OPCs and mature oligodendrocytes, increased myelin content, and lower g-ratio as well as decreased axonal degeneration. We propose that Csk is a novel regulator of oligodendrocyte development with two distinct roles: generating appropriate numbers of oligodendrocytes at the onset of myelination, and terminating wrapping during myelination.

HISTONE DEACETYLASES BETWEEN DAMAGE AND REPAIR

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Oligodendrocytes are the myelin-forming cells of the CNS and are essential for trophic neuronal support. Previous studies have supported the existence of an obligatory relationship between cell cycle exit and the initiatiorenders the chromatin transcriptionally inactive, decreasing the levels of the inhibitors, and favoring myelin gene expression. Similar mechanisms are implemented during repair of cuprizone-induced demyelination in young adults, but not in old mice. Histones, however, are not the only substrates of HDACs, and distinct effects can be detected in diverse cell types in response to HDAC activity. We propose that the combined use of HDAC inhibitors and small molecules may promote repair in demyelinating disorders and overcome the age-related changes. Supported by R01NS-42925-08 and ARRA R01NS-42925-07-S1 n of the transcriptional program of differentiation. In this presentation we shall discuss experimental evidence supporting the hypothesis that epigenetic mechanisms of repression involving post-translational changes of nucleosomal histones, histone variants and microRNAs are necessary for differentiation. Progenitors are characterized by high levels of inhibitors and histone acetylation favors a transcriptionally competent chromatin at their promoters. As progenitors begin differentiating, histone deacetylation (MEDIATED BY HDACS)

SCHWANN CELL SURVIVAL AND MYELINATION ARE CRITICALLY DEPENDENT ON HDAC1 AND HDAC2 FUNCTION

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Histone deacetylases (HDACs) are major epigenetic regulators. We show that HDAC1 and HDAC2 functions are critical for myelination and integrity of the peripheral nervous system. Using mouse genetics, we have ablated Hdac1 and Hdac2 specifically in Schwann cells resulting in virtually no myelin in mutant sciatic nerves and massive Schwann cell loss. Expression of Krox20, the main transcriptional regulator of Schwann cell myelination, was strongly reduced. We demonstrate that HDAC1/2 control the myelination program at the transcriptional level. In a highly redundant and compensation-competent milieu, HDAC2 regulates myelination while HDAC1 controls Schwann cell survival.

CHD4 IS REQUIRED FOR EFFICIENT MYELINATION OF THE PERIPHERAL NERVOUS SYSTEM

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Peripheral nerve myelination requires the interaction of the Early growth response 2 (Egr2/Krox20) transcription factor with the Nab1/ Nab2 corepressors. We have recently shown that these corepressors interact with the chromodomain helicase DNA-binding protein 4 (Chd4) subunit of the nucleosome remodeling and deacetylase (NuRD) complex. To understand the developmental importance of Chd4 in peripheral nerve myelination, we generated mice with a conditional gene ablation in cells of the Schwann cell lineage ($Chd4^{f/f}$ P0-CRE). Chd4 conditional null mice were found to have a general delay in myelination, hypomyelination, and the persistence of promyelinating Schwann cells. Furthermore, as development progresses Chd4-deficient peripheral nerves display evidence of axon/myelin degeneration. Expression analysis indicates that loss of Chd4 leads to reduced expression of myelin genes and elevated expression of the promyelinating Schwann cell gene, Oct6/Pou3f1. These data provide the first demonstration that a chromatin remodeling complex is required for efficient peripheral nerve myelination by Schwann cells.

GLIAL CELL DIFFERENTIATION AND NEURAL STEM REPROGRAMMING IN DROSOPHILA MELANOGASTER

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The molecular and epigenetic pathways controlling neural stem cells plasticity and differentiation towards a specific fate are still poorly understood.
In Drosophila, the Gcm/Glide (Glial cell missing/Glial Cell Deficient) transcription factor constitutes the glial determinant. In its absence, glia transform into neurons and its overexpression triggers ectopic glial markers. We here show that Gcm produces ectopic glia by reprogramming neural stem cells, even in the absence of cell division. Strikingly, a pulse of Gcm expression is sufficient to induce a complete and irreversible cell fate transformation.
Reprogramming involves an initial metastable state and subsequent consolidation, which relies on threshold Gcm levels and produces a stable glial fate. Gcm thus competes for the general transcription machinery in order to activate its own targets and trigger the glial pathway.
Gcm-dependent reprogramming is regulated by the CBP co-activator, a histone acetyl transferase (HAT) that targets lysine 9 and 56 of H3, and is associated with overall changes in such epigenetic landmarks.
These in vivo data demonstrate that a single transcription factor is sufficient to direct a multipotent cell towards a specific fate via epigenetic mechanisms that stably modify the endogenous developmental program.
Data from microarrays and from recent molecular and genetics screens performed in the laboratory support this hypothesis and have consequence onto the evolutionary conservation of Gcm function.
The different aspects of the Gcm cascade will be discussed as a model to understand the molecular and epigenetic pathways driving cell differentiation in the nervous system during evolution.

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VISITOR INFORMATION

EMERGENCY	CSHL	BANBURY
Fire	**(9) 742-3300**	**(9) 692-4747**
Ambulance	**(9) 742-3300**	**(9) 692-4747**
Poison	**(9) 542-2323**	**(9) 542-2323**
Police	**(9) 911**	**(9) 549-8800**
Safety-Security	**Extension 8870**	

Emergency Room **Huntington Hospital** 270 Park Avenue, Huntington	**631-351-2300** **(1037)**
Dentists Dr. William Berg Dr. Robert Zeman	 **631-271-2310** **631-271-8090**
Doctor MediCenter 234 W. Jericho Tpke., Huntington Station	**631-423-5400** (**1034**)
Drugs - 24 hours, 7 days Rite-Aid 391 W. Main Street, Huntington	**631-549-9400** (**1039**)

Free Speed Dial
Dial the four numbers (********) from any **tan house phone** to place a free call.

GENERAL INFORMATION

Books, Gifts, Snacks, Clothing, Newspapers
BOOKSTORE 367-8837 (hours posted on door)
Located in Grace Auditorium, lower level.

Photocopiers, Journals, Periodicals, Books, Newspapers
Photocopying – Main Library
Hours: 8:00 a.m. – 9:00 p.m. Mon-Fri
10:00 a.m. – 6:00 p.m. Saturday
Helpful tips - Obtain PIN from Meetings & Courses Office to enter Library after hours. See Library staff for photocopier code.

Computers, E-mail, Internet access
Grace Auditorium
Upper level: E-mail only
Lower level: Word processing and printing.
STMP server address: mail.optonline.net
To access your E-mail, you must know the name of your home server.

Dining, Bar
Blackford Hall
Breakfast 7:30–9:00, Lunch 11:30–1:30, Dinner 5:30–7:00
Bar 5:00 p.m. until late
Helpful tip - If there is a line at the upper dining area, try the lower dining room

Messages, Mail, Faxes

Message Board, Grace, lower level

Swimming, Tennis, Jogging, Hiking

June–Sept. Lifeguard on duty at the beach. 12:00 noon–6:00 p.m. Two tennis courts open daily.

Russell Fitness Center

Dolan Hall, west wing, lower level
PIN#: **Press 64435 (then enter #)**

Concierge

On duty daily at Meetings & Courses Office.
After hours – From tan house phones, dial x8870 for assistance

Pay Phones, House Phones

Grace, lower level; Cabin Complex; Blackford Hall; Dolan Hall, foyer

CSHL's Green Campus

Cold Spring Harbor Laboratory is pledged to operate in an environmentally responsible fashion wherever possible. In the past, we have removed underground oil tanks, remediated asbestos in historic buildings, and taken substantial measures to ensure the pristine quality of the waters of the harbor. Water used for irrigation comes from natural springs and wells on the property itself. Lawns, trees, and planting beds are managed organically whenever possible. And trees are planted to replace those felled for construction projects.

Two areas in which the Laboratory has focused recent efforts have been those of waste management and energy conservation. The Laboratory currently recycles most waste. Scrap metal, electronics, construction debris, batteries, fluorescent light bulbs, toner cartridges, and waste oil are all recycled. For general waste, the Laboratory uses a "single stream waste management" system, removing recyclable materials and sending the remaining combustible trash to a cogeneration plant where it is burned to provide electricity, an approach considered among the most energy efficient, while providing a high yield of recyclable materials.

Equal attention has been paid to energy conservation. Most lighting fixtures have been replaced with high efficiency fluorescent fixtures, and thousands of incandescent bulbs throughout campus have been replaced with compact fluorescents. The Laboratory has also embarked on a project that will replace all building management systems on campus, reducing heating and cooling costs by as much as twenty-five per cent.

Cold Spring Harbor Laboratory continues to explore new ways in which we can reduce our environmental footprint, including encouraging our visitors and employees to use reusable containers, conserve energy, and suggest areas in which the Laboratory's efforts can be improved. This book, for example, is printed on recycled paper.

1-800 Access Numbers

AT&T	**9-1-800-321-0288**
MCI	**9-1-800-674-7000**

Local Interest

Fish Hatchery	631-692-6768
Sagamore Hill	516-922-4447
Whaling Museum	631-367-3418
Heckscher Museum	631-351-3250
CSHL DNA Learning Center	x 5170

New York City

Helpful tip -

Take Syosset Taxi to Syosset Train Station ($8.00 per person, 15 minute ride), then catch Long Island Railroad to Penn Station (33rd Street & 7th Avenue). Train ride about one hour.

TRANSPORTATION

Limo, Taxi

Syosset Limousine	516-364-9681 (**1031**)
Super Shuttle	800-957-4533 (**1033**)
To head west of CSHL - Syosset train station	
Syosset Taxi	516-921-2141 (**1030**)
To head east of CSHL - Huntington Village	
Orange & White Taxi	631-271-3600 (**1032**)
Executive Limo	631-696-8000 (**1047**)

Trains

Long Island Rail Road	822-LIRR
Schedules available from the Meetings & Courses Office.	
Amtrak	800-872-7245
MetroNorth	800-638-7646
New Jersey Transit	201-762-5100

Ferries

Bridgeport / Port Jefferson	631-473-0286 **(1036)**
Orient Point/ New London	631-323-2525 **(1038)**

Car Rentals

Avis	631-271-9300
Enterprise	631-424-8300
Hertz	631-427-6106

Airlines

American	800-433-7300
America West	800-237-9292
British Airways	800-247-9297
Continental	800-525-0280
Delta	800-221-1212
Japan Airlines	800-525-3663
Jet Blue	800-538-2583
KLM	800-374-7747
Lufthansa	800-645-3880
Northwest	800-225-2525
United	800-241-6522
US Airways	800-428-4322