

This Week in The Journal

● Cellular/Molecular

Anti-Hebbian LTD Cancels Self-Generated Sensory Signals in Fish

Erik Harvey-Girard, John Lewis, and Leonard Maler

(see pages 6152–6169)

To effectively detect environmental features, an animal must differentiate stimuli that result from its own actions from those produced externally. Electric fish emit electric pulses, creating an electric field that is detected by electroreceptors along the fish's body. Environmental objects create spatially localized amplitude modulations in the electric field, and these distortions are used to detect objects. Tail movements, which vary the distance between the electric organ and electroreceptors, produce spatially diffuse distortions that affect receptors along the entire length of the fish. A negative image of these global signals is transmitted via cerebellar parallel fibers to the central target of electrosensory afferents (principal cells of the electrosensory lateral line lobe) to cancel neural activity produced by self-generated field distortions. Harvey-Girard et al. suggest that this cancellation results in part from long-term depression (LTD) of parallel fiber input to principal cells, and that this occurs when both presynaptic and postsynaptic cells fire bursts of spikes within a short temporal window.

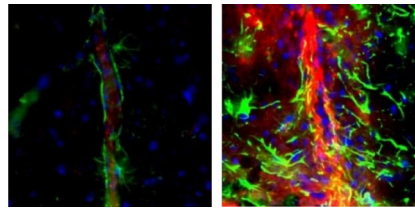
▲ Development/Plasticity/Repair

Blood Fibrinogen Triggers Astrogliosis after Brain Injury

Christian Schachtrup, Jae K. Ryu, Matthew J. Helmrick, Eirini Vagena, Dennis K. Galanakis, et al.

(see pages 5843–5854)

CNS damage triggers reactive astrogliosis. In severe cases, astrocytes proliferate and form a long-lasting glial scar comprised mainly of chondroitin sulfate proteoglycans (CSPGs). Glial scars prevent regrowth of axons and thus are a major impediment to functional recovery. Although the initial molecular triggers of astrogliosis remain



Levels of fibrin (red) and astrogliosis, indicated by glial fibrillary acidic protein labeling (green), are normally low in mouse brain (left), but both increase after cortical injury (right). See the article by Schachtrup et al. for details.

elusive, tumor growth factor β (TGF- β) is thought to trigger CSPG secretion from astrocytes soon after injury. Schachtrup et al. have now identified the blood protein fibrinogen as an inducer of reactive astrogliosis and CSPG deposition. After cortical injury in mice, deposits of fibrin—a clotting protein formed from fibrinogen—colocalized with reactive astrogliosis. Injection of exogenous fibrinogen induced astrogliosis and CSPG deposition. The authors discovered that a latent form of TGF- β was bound to fibrinogen in plasma and was activated upon exposure to cultured astrocytes. In fibrinogen-null mice, injury-induced reactive gliosis, TGF- β levels, and CSPG deposits were greatly reduced, and in wild-type mice, TGF- β receptor inhibitor prevented fibrinogen-induced astrogliosis.

■ Behavioral/Systems/Cognitive

Brain Leptin Regulates Systemic Immune Function

Johannes Tschöp, Ruben Nogueiras, Sarah Haas-Lockie, Kevin R. Kasten, Tamara R. Castañeda, et al.

(see pages 6036–6047)

Leptin, a cytokine produced by adipocytes, plays a major role in food intake, weight control, and reproductive function. Leptin also activates and stimulates proliferation of immune cells, and leptin-resistant people are prone to infections. Having noticed a high susceptibility to sepsis—a systemic inflammatory response to infection that can lead to organ failure—in patients with leptin deficiency, and that low leptin levels are correlated with high mortality in sepsis pa-

tients, Tschöp et al. hypothesized that leptin has an important role in systemic immune defenses. Leptin-deficient mice had a higher incidence of kidney and liver damage and died sooner after experimentally induced sepsis than wild-type mice. Bacteria counts and interleukin 6 levels were higher in blood from mutant mice, and phagocytosis by neutrophils was reduced. Interestingly, injection of leptin directly into the brains of leptin-deficient mice increased survival, normalized interleukin 6 levels, and reduced kidney damage, suggesting that leptin actions in the brain regulate systemic immune function.

◆ Neurobiology of Disease

Autoantibodies against NMDA Receptors Trigger Internalization

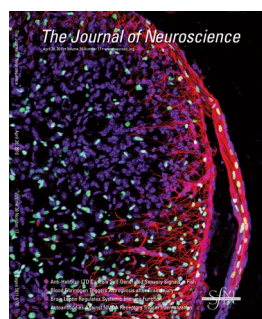
Ethan G. Hughes, Xiaoyu Peng, Amy J. Gleichman, Meizan Lai, Lei Zhou, et al.

(see pages 5866–5875)

In the past few years, autoantibodies against the NMDA receptor NR1 subunit have been identified in many patients with encephalitis. The autoantibodies are thought to be generated when tumors outside the brain, often in the ovaries, express NMDA receptors. The autoantibodies trigger psychiatric disturbances, memory loss, and seizures, which can often be reversed by immunotherapy and tumor removal. Hughes et al. show that antibody-containing CSF from patients caused internalization of NMDA receptors in cultured rat hippocampal neurons without affecting other synaptic proteins, including AMPA receptors, GABA receptors, a glutamate transporter, and the postsynaptic density protein PSD-95. Moreover, the antibodies did not obviously affect synaptic density, dendritic morphology, or cell survival. Surface expression of NMDA receptors returned to normal levels within 4 days after antibodies were removed. In postmortem patients, NMDA receptor density was decreased in the hippocampus, and likewise, infusion of patients' CSF into rat brain caused receptor internalization in the hippocampus.

The Journal of Neuroscience

April 28, 2010 • Volume 30 Number 17 • www.jneurosci.org



Cover legend: Transverse section through the spinal cord and dorsal nerve root of a 14-d-old, Schwann cell-depleted mouse, showing the presence of CNS-type glia in the nerve roots. Oligodendrocytes are labeled with antibodies against Olig2 (green) and astrocytes are labeled with antibodies against GFAP (red). Nuclei were counterstained with Hoechst 33342 (blue). For more information, see the article by Culpier et al. in this issue (pages 5958–5967).

i This Week in The Journal

Brief Communications

- 5825 Emotional Automaticity Is a Matter of Timing**
Qian Luo, Tom Holroyd, Catherine Majestic, Xi Cheng, Julia Schechter, and R. James Blair
- 6116 Cholesterol-Dependent Kinase Activity Regulates Transmitter Release from Cerebellar Synapses**
Alex J. Smith, Shuzo Sugita, and Milton P. Charlton

Articles

CELLULAR/MOLECULAR

- 5811 A Perisynaptic Ménage à Trois between Dlg, DLin-7, and Metro Controls Proper Organization of *Drosophila* Synaptic Junctions**
André Bachmann, Oliver Kobler, Robert J. Kittel, Carolin Wichmann, Jimena Sierralta, Stephan J. Sigrist, Eckart D. Gundelfinger, Elisabeth Knust, and Ulrich Thomas
- 5830 Synaptic Activity Controls Dendritic Spine Morphology by Modulating eEF2-Dependent BDNF Synthesis**
Chiara Verpelli, Giovanni Piccoli, Alice Zanchi, Fabrizio Gardoni, Kun Huang, Dario Brambilla, Monica Di Luca, Elena Battaglioli, and Carlo Sala
- 5855 The Transcription Factor Mef2 Is Required for Normal Circadian Behavior in *Drosophila***
Florence J. Blanchard, Ben Collins, Shawn A. Cyran, Daniel H. Hancock, Michael V. Taylor, and Justin Blau
- 5937 N-Acetylglucosamine 6-O-Sulfotransferase-1-Deficient Mice Show Better Functional Recovery after Spinal Cord Injury**
Zenya Ito, Kazuma Sakamoto, Shiro Imagama, Yukihiro Matsuyama, Haoqian Zhang, Kenichi Hirano, Kei Ando, Toshihide Yamashita, Naoki Ishiguro, and Kenji Kadomatsu
- 6011 M3 Muscarinic Acetylcholine Receptor Expression Confers Differential Cholinergic Modulation to Neurochemically Distinct Hippocampal Basket Cell Subtypes**
Christian A. Cea-del Rio, J. Josh Lawrence, Ludovic Tricoire, Ferenc Erdelyi, Gabor Szabo, and Chris J. McBain
- 6025 Canonical TGF- β Signaling Is Required for the Balance of Excitatory/Inhibitory Transmission within the Hippocampus and Prepulse Inhibition of Acoustic Startle**
Mu Sun, Jonathan C. Gewirtz, Lisa Bofenkamp, Robert J. Wickham, Hong Ge, and Michael B. O'Connor

- 6048 **Dysregulation of Dopamine Transporters via Dopamine D₂ Autoreceptors Triggers Anomalous Dopamine Efflux Associated with Attention-Deficit Hyperactivity Disorder**
Erica Bowton, Christine Saunders, Kevin Erreger, Dhananjay Sakrikar, Heinrich J. Matthies, Namita Sen, Tammy Jessen, Roger J. Colbran, Marc G. Caron, Jonathan A. Javitch, Randy D. Blakely, and Aurelio Galli
- 6122 **Soluble Neuregulin-1 Has Bifunctional, Concentration-Dependent Effects on Schwann Cell Myelination**
Neeraja Syed, Kavya Reddy, David P. Yang, Carla Taveggia, James L. Salzer, Patrice Maurel, and Haesun A. Kim
- 6143 **Type 4 Phosphodiesterase Plays Different Integrating Roles in Different Cellular Domains in Pyramidal Cortical Neurons**
Liliana R. V. Castro, Nicolas Gervasi, Elvire Guiot, Laetitia Cavellini, Viacheslav O. Nikolaev, Danièle Paupardin-Tritsch, and Pierre Vincent
- 6152 **Burst-Induced Anti-Hebbian Depression Acts through Short-Term Synaptic Dynamics to Cancel Redundant Sensory Signals**
Erik Harvey-Girard, John Lewis, and Leonard Maler

DEVELOPMENT/PLASTICITY/REPAIR

- 5843 **Fibrinogen Triggers Astrocyte Scar Formation by Promoting the Availability of Active TGF- β after Vascular Damage**
Christian Schachtrup, Jae K. Ryu, Matthew J. Helmrick, Eirini Vagena, Dennis K. Galanakis, Jay L. Degen, Richard U. Margolis, and Katerina Akassoglou
- 5927 ***In Vivo* Proliferation of Postmitotic Cochlear Supporting Cells by Acute Ablation of the Retinoblastoma Protein in Neonatal Mice**
Yiling Yu, Thomas Weber, Tetsuji Yamashita, Zhiyong Liu, Marcus B. Valentine, Brandon C. Cox, and Jian Zuo
- 5958 **CNS/PNS Boundary Transgression by Central Glia in the Absence of Schwann Cells or Krox20/Egr2 Function**
Fanny Couplier, Laurence Decker, Benoît Funalot, Jean-Michel Vallat, Federico Garcia-Bragado, Patrick Charnay, and Piotr Topilko
- 6080 **Pharmacological and Genetic Accumulation of Hypoxia-Inducible Factor-1 α Enhances Excitatory Synaptic Transmission in Hippocampal Neurons through the Production of Vascular Endothelial Growth Factor**
Yu-Fei Huang, Chih-Hao Yang, Chiung-Chun Huang, Ming-Hong Tai, and Kuei-Sen Hsu
- 6094 **The Death Receptor Antagonist FLIP-L Interacts with Trk and Is Necessary for Neurite Outgrowth Induced by Neurotrophins**
Rana S. Moubarak, Carme Solé, Marta Pascual, Humberto Gutierrez, Marta Llovera, M. José Pérez-García, Raffaella Gozzelino, Miguel F. Segura, Victoria Iglesias-Guimaraes, Stéphanie Reix, Rosa M. Soler, Alun M. Davies, Eduardo Soriano, Victor J. Yuste, and Joan X. Comella

BEHAVIORAL/SYSTEMS/COGNITIVE

- 5876 **Differential Neuromodulation of Acquisition and Retrieval of Avoidance Learning by the Lateral Habenula and Ventral Tegmental Area**
Jason Shumake, Anton Ilango, Henning Scheich, Wolfram Wetzel, and Frank W. Ohl
- 5894 **Sequentially Switching Cell Assemblies in Random Inhibitory Networks of Spiking Neurons in the Striatum**
Adam Ponzi and Jeff Wickens

- 5912 **Parallel Input Channels to Mouse Primary Visual Cortex**
Enquan Gao, Gregory C. DeAngelis, and Andreas Burkhalter
- 5968 **Integration of Goal- and Stimulus-Related Visual Signals Revealed by Damage to Human Parietal Cortex**
Paul M. Bays, Victoria Singh-Curry, Nikos Gorgoraptis, Jon Driver, and Masud Husain
- 5979 **Priming of Hippocampal Population Bursts by Individual Perisomatic-Targeting Interneurons**
Tommas J. Ellender, Wiebke Nissen, Laura L. Colgin, Edward O. Mann, and Ole Paulsen
- 5992 **Genetic Variation in *FGF20* Modulates Hippocampal Biology**
Herve Lemaitre, Venkata S. Mattay, Fabio Sambataro, Beth Verchinski, Richard E. Straub, Joseph H. Callicott, Raja Kittappa, Thomas M. Hyde, Barbara K. Lipska, Joel E. Kleinman, Ronald McKay, and Daniel R. Weinberger
- 6036 **CNS Leptin Action Modulates Immune Response and Survival in Sepsis**
Johannes Tschöp, Ruben Nogueiras, Sarah Haas-Lockie, Kevin R. Kasten, Tamara R. Castañeda, Nadine Huber, Kelsey Guanciale, Diego Perez-Tilve, Kirk Habegger, Nickki Ottaway, Stephen C. Woods, Brian Oldfield, Iain Clarke, Streamson Chua Jr, I. Sadaf Farooqi, Stephen O'Rahilly, Charles C. Caldwell, and Matthias H. Tschöp
- 6058 **Aging-Related Gene Expression in Hippocampus Proper Compared with Dentate Gyrus Is Selectively Associated with Metabolic Syndrome Variables in Rhesus Monkeys**
Eric M. Blalock, Richard Grondin, Kuey-chu Chen, Olivier Thibault, Veronique Thibault, Jignesh D. Pandya, Amy Dowling, Zhiming Zhang, Patrick Sullivan, Nada M. Porter, and Philip W. Landfield
- 6072 **Ignoring the Elephant in the Room: A Neural Circuit to Downregulate Salience**
Carmel Mevorach, John Hodsoll, Harriet Allen, Lilach Shalev, and Glyn Humphreys
- 6106 **Rapid Glucocorticoid Receptor-Mediated Inhibition of Hypothalamic–Pituitary–Adrenal Ultradian Activity in Healthy Males**
Georgina M. Russell, David E. Henley, Jack Leendertz, Jennie A. Douthwaite, Susan A. Wood, Adam Stevens, Wolfram W. Woltersdorf, Bernard W. M. M. Peeters, Ge S. F. Ruigt, Anne White, Johannes D. Veldhuis, and Stafford L. Lightman

NEUROBIOLOGY OF DISEASE

- 5866 **Cellular and Synaptic Mechanisms of Anti-NMDA Receptor Encephalitis**
Ethan G. Hughes, Xiaoyu Peng, Amy J. Gleichman, Meizan Lai, Lei Zhou, Ryan Tsou, Thomas D. Parsons, David R. Lynch, Josep Dalmau, and Rita J. Balice-Gordon
- 5884 **Dynamic Time Course of Typical Childhood Absence Seizures: EEG, Behavior, and Functional Magnetic Resonance Imaging**
Xiaoxiao Bai, Matthew Vestal, Rachel Berman, Michiro Negishi, Marisa Spann, Clemente Vega, Matthew Desalvo, Edward J. Novotny, Robert T. Constable, and Hal Blumenfeld
- 5948 **β -Mediated NMDA Receptor Endocytosis in Alzheimer's Disease Involves Ubiquitination of the Tyrosine Phosphatase STEP₆₁**
Pradeep Kurup, Yongfang Zhang, Jian Xu, Deepa V. Venkitaramani, Vahram Haroutunian, Paul Greengard, Angus C. Nairn, and Paul J. Lombroso

5998 **Norrin Mediates Neuroprotective Effects on Retinal Ganglion Cells via Activation of the Wnt/ β -Catenin Signaling Pathway and the Induction of Neuroprotective Growth Factors in Müller Cells**
Roswitha Seitz, Simon Hackl, Thomas Seibuchner, Ernst R. Tamm,
and Andreas Ohlmann

6132 **Identification of Caspase-6-Mediated Processing of the Valosin Containing Protein (p97) in Alzheimer's Disease: A Novel Link to Dysfunction in Ubiquitin Proteasome System-Mediated Protein Degradation**
Dalia Halawani, Sylvain Tessier, Dominique Anzellotti, David A. Bennett,
Martin Latterich, and Andréa C. LeBlanc

Erratum: For the article “‘Slow Activity Transients’ in Infant Rat Visual Cortex: A Spreading Synchronous Oscillation Patterned by Retinal Waves” by Matthew T. Colonnese and Rustem Khazipov, which appeared on pages 4325–4337 of the March 24, 2010, issue, the authors would like to issue the following correction: Our paper incorrectly stated that infra-slow activity transients have not been described in animal models and implied that no animal models of human SATs had been proposed. In fact, using wide-band recording methods, Seelke and Blumberg (2008) [Seelke AMH, Blumberg MS (2008) The microstructure of active and quiet sleep as cortical delta activity emerges in infant rats. *Sleep* 31:691–699] described SATs in the parietal cortex of P9–P13 rats that they defined as “discrete, high-amplitude, cortical events with a duration of approximately 5 seconds,” though absent consistent rapid oscillations. We apologize for this oversight. Published online after our paper’s acceptance, Seelke and Blumberg (2010) [Seelke AMH, Blumberg MS (2010) Developmental appearance and disappearance of cortical events and oscillations in infant rats. *Brain Res* 1324:34–42], recording from the frontal, parietal, and occipital lobes of P5–P13 rats, also reported SATs, some of which contained high-frequency embedded activity and some of which did not.

Persons interested in becoming members of the Society for Neuroscience should contact the Membership Department, Society for Neuroscience, 1121 14th St., NW, Suite 1010, Washington, DC 20005, phone 202-962-4000.

Instructions for Authors are available at <http://www.jneurosci.org/misc/itoa.shtml>. Authors should refer to these Instructions online for recent changes that are made periodically.

Brief Communications Instructions for Authors are available via Internet (http://www.jneurosci.org/misc/ifa_bc.shtml).

Submissions should be submitted online using the following url: <http://jneurosci.msubmit.net>. Please contact the Central Office, via phone, fax, or e-mail with any questions. Our contact information is as follows: phone, 202-962-4000; fax, 202-962-4945; e-mail, jn@sfn.org.

Emotional Automaticity Is a Matter of Timing

Qian Luo,¹ Tom Holroyd,² Catherine Majestic,¹ Xi Cheng,³ Julia Schechter,¹ and R. James Blair¹

¹Mood and Anxiety Disorders Program, ²Magnetoencephalography Core Facility, and ³Genes, Cognition, and Psychosis Program, National Institute of Mental Health, National Institutes of Health, Bethesda, Maryland 20892

There has been a long controversy concerning whether the amygdala's response to emotional stimuli is automatic or dependent on attentional load. Using magnetoencephalography and an advanced beamformer source localization technique, we found that amygdala automaticity was a function of time: while early amygdala responding to emotional stimuli (40–140 ms) was unaffected by attentional load, later amygdala response (280–410 ms), subsequent to frontoparietal cortex activity, was modulated by attentional load.

The Journal of Neuroscience, April 28, 2010 • 30(17):5825–5829

Cholesterol-Dependent Kinase Activity Regulates Transmitter Release from Cerebellar Synapses

Alex J. Smith,¹ Shuzo Sugita,^{1,2} and Milton P. Charlton¹

¹Department of Physiology, University of Toronto, Toronto, Ontario M5S 1A8, Canada, and ²Toronto Western Research Institute, University Health Network, Toronto, Ontario M5T 2S8, Canada

Changes in membrane cholesterol content can alter protein kinase activity, however, it is not known whether kinases regulating transmitter release are sensitive to membrane cholesterol content. Here we have used the cholesterol extracting agent methyl- β -cyclodextrin to measure the effects of acute cholesterol reduction on transmitter release from cultured cerebellar neurons. Cholesterol depletion increased the frequency of spontaneous transmitter release without altering the amplitude and time course of mEPSCs. Evoked transmitter release was decreased by cholesterol extraction and the paired pulse ratio was also decreased. Alterations in synaptic transmission were not associated with failure of action potential generation or changes in presynaptic Ca^{2+} signaling. Both the increase in mEPSC frequency and the change in paired pulse ratio were blocked by the broad spectrum protein kinase inhibitor staurosporine. The increase in mEPSC frequency was also sensitive to selective inhibitors of PKC and PKA. Our results therefore demonstrate that the activity of presynaptic protein kinases that regulate spontaneous and evoked neurotransmitter release is sensitive to changes of membrane cholesterol content.

The Journal of Neuroscience, April 28, 2010 • 30(17):6116–6121

Articles

CELLULAR/MOLECULAR

A Perisynaptic Ménage à Trois between Dlg, DLin-7, and Metro Controls Proper Organization of *Drosophila* Synaptic Junctions

André Bachmann,^{1*} Oliver Kobler,^{2*} Robert J. Kittel,^{3,4} Carolin Wichmann,^{5,6} Jimena Sierralta,⁷ Stephan J. Sigrist,⁶ Eckart D. Gundelfinger,² Elisabeth Knust,^{1,8} and Ulrich Thomas²

¹Institut für Genetik, Heinrich-Heine-Universität Düsseldorf, D-40225 Düsseldorf, Germany, ²Leibniz Institute for Neurobiology, Department of Neurochemistry, D-39118 Magdeburg, Germany, ³Institut für Physiologie, Universität Würzburg, D-97070 Würzburg, Germany, ⁴Rudolf Virchow Deutsche Forschungsgemeinschaft Research Center for Experimental Medicine, D-97078 Würzburg, Germany, ⁵European Neuroscience Institute, D-37077 Göttingen, Germany, ⁶Institut für Biologie, Freie Universität Berlin, 14195 Berlin, Germany, ⁷Instituto de Ciencias Biomédicas, Facultad de Medicina, Universidad de Chile, Santiago 6530499, Chile, and ⁸Max Planck Institute of Cell Biology and Genetics, D-01307 Dresden, Germany

Structural plasticity of synaptic junctions is a prerequisite to achieve and modulate connectivity within nervous systems, e.g., during learning and memory formation. It demands adequate backup systems that allow remodeling while retaining sufficient stability to prevent unwanted synaptic disintegration. The strength of submembranous scaffold complexes, which are fundamental to the architecture of synaptic junctions, likely constitutes a crucial determinant of synaptic stability. Postsynaptic density protein-95 (PSD-95)/ Discs-large (Dlg)-like membrane-associated guanylate kinases (DLG-MAGUKs) are principal scaffold proteins at both vertebrate and invertebrate synapses. At *Drosophila* larval glutamatergic neuromuscular junctions (NMJs) DlgA and DlgS97 exert pleiotropic functions, probably reflecting a few known and a number of yet-unknown binding partners. In this study we have identified Metro, a novel p55/MPP-like *Drosophila* MAGUK as a major binding partner of perisynaptic DlgS97 at larval NMJs. Based on homotypic LIN-2,-7 (L27) domain interactions, Metro stabilizes junctional DlgS97 in a complex with the highly conserved adaptor protein DLin-7. In a remarkably interdependent manner, Metro and DLin-7 act downstream of DlgS97 to control NMJ expansion and proper establishment of synaptic boutons. Using quantitative 3D-imaging we further demonstrate that the complex controls the size of postsynaptic glutamate receptor fields. Our findings accentuate the importance of perisynaptic scaffold complexes for synaptic stabilization and organization.

The Journal of Neuroscience, April 28, 2010 • 30(17):5811–5824

Synaptic Activity Controls Dendritic Spine Morphology by Modulating eEF2-Dependent BDNF Synthesis

Chiara Verpelli,^{1,2*} Giovanni Piccoli,^{1*} Alice Zanchi,¹ Fabrizio Gardoni,⁴ Kun Huang,⁶ Dario Brambilla,⁵ Monica Di Luca,⁴ Elena Battaglioli,³ and Carlo Sala^{1,7}

¹Consiglio Nazionale delle Ricerche Neuroscience Institute and Department of Pharmacology, ²Neurosurgery, Department of Neurological Sciences, ³Department of Biology and Genetics for Medical Sciences, ⁴Department of Pharmacological Sciences and Centre of Excellence for Neurodegenerative Diseases, and ⁵Department of Human Physiology, University of Milan, 20122 Milan, Italy, ⁶Department of Psychiatry, University of British Columbia, Vancouver, British Columbia V6T 2A1, Canada, and ⁷Neuromuscular Diseases and Neuroimmunology, Neurological Institute Foundation "Carlo Besta," 20133 Milan, Italy

Activity-dependent changes in synaptic structure and spine morphology are required for learning and memory, and depend on protein translation. We show that the kinase for eukaryotic elongation factor 2 (eEF2K) regulates dendritic spine stability and synaptic structure by modulating activity-dependent dendritic BDNF synthesis. Specifically RNAi knockdown of eEF2K reduces dendritic spine stability and inhibits dendritic BDNF protein expression; whereas overexpression of a constitutively activated eEF2K induces spine maturation and increases expression of dendritic BDNF. Furthermore, BDNF overexpression rescues the spine stability reduced by RNAi knockdown of eEF2K. We also show that synaptic activity-dependent spine maturation and dendritic BDNF protein expression depend on mGluR/EF2K-induced eEF2 phosphorylation. We propose that the eEF2K/eEF2 pathway is a key biochemical sensor that couple neuronal activity to spine plasticity, by controlling the dendritic translation of BDNF. The Journal of Neuroscience, April 28, 2010 • 30(17):5830–5842

The Transcription Factor Mef2 Is Required for Normal Circadian Behavior in *Drosophila*

Florence J. Blanchard,¹ Ben Collins,¹ Shawn A. Cyran,¹ Daniel H. Hancock,² Michael V. Taylor,² and Justin Blau¹

¹Department of Biology, New York University, New York, New York 10003, and ²School of Biosciences, Cardiff University, Cardiff CF10 3AX, United Kingdom

The transcription factor Mef2 has well established roles in muscle development in *Drosophila* and in the differentiation of many cell types in mammals, including neurons. Here, we describe a role for Mef2 in the *Drosophila* pacemaker neurons that regulate circadian behavioral rhythms. We found that Mef2 is normally produced in all adult clock neurons and that Mef2 overexpression in clock neurons leads to long period and complex rhythms of adult locomotor behavior. Knocking down Mef2 expression via RNAi or expressing a repressor form of Mef2 caused flies to lose circadian behavioral rhythms. These behavioral changes are correlated with altered molecular clocks in pacemaker neurons: Mef2 overexpression causes the oscillations in individual pacemaker neurons to become desynchronized, while Mef2 knockdown strongly dampens molecular rhythms. Thus, a normal level of Mef2 activity is required in clock neurons to maintain robust and accurate circadian behavioral rhythms. The Journal of Neuroscience, April 28, 2010 • 30(17):5855–5865

N-Acetylglucosamine 6-O-Sulfotransferase-1-Deficient Mice Show Better Functional Recovery after Spinal Cord Injury

Zenya Ito,^{1,2} Kazuma Sakamoto,^{1*} Shiro Imagama,^{1,2*} Yukihiro Matsuyama,² Haoqian Zhang,¹ Kenichi Hirano,² Kei Ando,² Toshihide Yamashita,³ Naoki Ishiguro,² and Kenji Kadomatsu^{1,4}

Departments of ¹Biochemistry and ²Orthopedics, Nagoya University Graduate School of Medicine, Nagoya 466-8550, Japan, ³Department of Molecular Neuroscience, Graduate School of Medicine, Osaka University, Osaka 565-0871, Japan, and ⁴Institute for Advanced Research, Nagoya University, Nagoya 464-8601, Japan

Neurons in the adult CNS do not spontaneously regenerate after injuries. The glycosaminoglycan keratan sulfate is induced after spinal cord injury, but its biological significance is not well understood. Here we investigated the role of keratan sulfate in functional recovery after spinal cord injury, using mice deficient in N-acetylglucosamine 6-O-sulfotransferase-1 that lack 5D4-reactive keratan sulfate in the CNS. We made contusion injuries at the 10th thoracic level. Expressions of N-acetylglucosamine 6-O-sulfotransferase-1 and keratan sulfate were induced after injury in wild-type mice, but not in the deficient mice. The wild-type and deficient mice showed similar degrees of chondroitin sulfate induction and of CD11b-positive inflammatory cell recruitment. However, motor function recovery, as assessed by the footprint test, footprint test, and Basso mouse scale locomotor scoring, was significantly better in the deficient mice. Moreover, the deficient mice showed a restoration of neuromuscular system function below the lesion after electrical stimulation at the occipito-cervical area. In addition, axonal regrowth of both the corticospinal and raphespinal tracts was promoted in the deficient mice. *In vitro* assays using primary cerebellar granule neurons demonstrated that keratan sulfate proteoglycans were required for the proteoglycan-mediated inhibition of neurite outgrowth. These data collectively indicate that keratan sulfate expression is closely associated with functional disturbance after spinal cord injury. N-acetylglucosamine 6-O-sulfotransferase-1-deficient mice are a good model to investigate the roles of keratan sulfate in the CNS. The Journal of Neuroscience, April 28, 2010 • 30(17):5937–5947

M3 Muscarinic Acetylcholine Receptor Expression Confers Differential Cholinergic Modulation to Neurochemically Distinct Hippocampal Basket Cell Subtypes

Christian A. Cea-del Rio,^{1,*} J. Josh Lawrence,^{1,2,*} Ludovic Tricoire,¹ Ferenc Erdelyi,³ Gabor Szabo,³ and Chris J. McBain¹

¹Program in Developmental Neurobiology, Eunice Kennedy Shriver National Institute of Child Health and Human Development, Bethesda, Maryland 20892, ²Center for Structural and Functional Neuroscience, Department of Biomedical and Pharmaceutical Sciences, The University of Montana, Missoula, Montana 59812, and ³Laboratory of Molecular Biology and Genetics, Institute of Experimental Medicine, Hungarian Academy of Sciences, H-1450 Budapest, Hungary

Cholinergic neuromodulation of hippocampal circuitry promotes network oscillations and facilitates learning and memory through cellular actions on both excitatory and inhibitory circuits. Despite widespread recognition that neurochemical content discriminates between functionally distinct interneuron populations, there has been no systematic examination of whether neurochemically distinct interneuron classes undergo differential cholinergic neuromodulation in the hippocampus. Using GFP transgenic mice that enable the visualization of perisomatically targeting parvalbumin-positive (PV+) or cholecystokinin-positive (CCK+) basket cells (BCs), we tested the hypothesis that neurochemically distinct interneuron populations are differentially engaged by muscarinic acetylcholine receptor (mAChR) activation. Cholinergic fiber activation revealed that CCK BCs were more sensitive to synaptic release of ACh than PV BCs. In response to depolarizing current steps, mAChR activation of PV BCs and CCK BCs also elicited distinct cholinergic response profiles, differing in mAChR-induced changes in action potential (AP) waveform, firing frequency, and intrinsic excitability. In contrast to PV BCs, CCK BCs exhibited a mAChR-induced afterdepolarization (mADP) that was frequency and activity-dependent. Pharmacological, molecular, and loss-of-function data converged on the presence of M3 mAChRs in distinguishing CCK BCs from PV BCs. Firing frequency of CCK BCs was controlled through M3 mAChRs but PV BC excitability was altered solely through M1 mAChRs. Finally, upon mAChR activation, glutamatergic transmission enhanced cellular excitability preferentially in CCK BCs but not in PV BCs. Our findings demonstrate that cell type-specific cholinergic specializations are present on neurochemically distinct interneuron subtypes in the hippocampus, revealing an organizing principle that cholinergic neuromodulation depends critically on neurochemical identity.

The Journal of Neuroscience, April 28, 2010 • 30(17):6011–6024

Canonical TGF- β Signaling Is Required for the Balance of Excitatory/Inhibitory Transmission within the Hippocampus and Prepulse Inhibition of Acoustic Startle

Mu Sun,^{1,2} Jonathan C. Gewirtz,³ Lisa Bofenkamp,^{1,2} Robert J. Wickham,³ Hong Ge,¹ and Michael B. O'Connor^{1,2}

¹Department of Genetics, Cell Biology and Development, ²Howard Hughes Medical Institute, and ³Departments of Psychology and Neuroscience, University of Minnesota, Minnesota 55455

Smad4 is a unique nuclear transducer for all TGF- β signaling pathways and regulates gene transcription during development and tissue homeostasis. To elucidate the postnatal role of TGF- β signaling in the mammalian brain, we generated forebrain-specific Smad4 knock-out mice. Surprisingly, the mutants showed no alteration in long-term potentiation and water maze, suggesting that Smad4 is not required for spatial learning and memory. However, these mutant mice did show enhancement of paired-pulse facilitation in excitatory synaptic transmission and stronger paired-pulse depression of GABA_A currents in the hippocampus. The alteration of hippocampal electrophysiology correlated with mouse hyperactivity in homecage and open field tests. Mutant mice also showed overgrooming as well as deficits of prepulse inhibition, a widely used endophenotype of schizophrenia. With a specific real-time PCR array focused on TGF- β signaling pathway, we identified a novel regulation mechanism of the pathway in the hippocampal neurons, in which Smad4-mediated signaling suppresses the level of extracellular antagonism of TGF- β ligands through transcriptional regulation of follistatin, a selective inhibitor to activin/TGF- β signaling in the hippocampus. In summary, we suggest that the canonical TGF- β signaling pathway is critical for use-dependent modulation of GABA_A synaptic transmission and dendritic homeostasis; furthermore, a disruption in the balance of the excitatory and inhibitory hippocampal network can result in psychiatric-like behavior.

The Journal of Neuroscience, April 28, 2010 • 30(17):6025–6035

Dysregulation of Dopamine Transporters via Dopamine D₂ Autoreceptors Triggers Anomalous Dopamine Efflux Associated with Attention-Deficit Hyperactivity Disorder

Erica Bowton,^{1,4} Christine Saunders,² Kevin Erreger,¹ Dhananjay Sakrikar,⁴ Heinrich J. Matthies,¹ Namita Sen,^{5,6,7} Tammy Jessen,² Roger J. Colbran,¹ Marc G. Caron,⁸ Jonathan A. Javitch,^{5,6,7} Randy D. Blakely,^{2,3,4} and Aurelio Galli^{1,2,4}

Departments of ¹Molecular Physiology & Biophysics, ²Pharmacology, and ³Psychiatry and ⁴Center for Molecular Neuroscience, Vanderbilt University School of Medicine, Nashville, Tennessee 37232–8548, Departments of ⁵Psychiatry and ⁶Pharmacology and ⁷Center for Molecular Recognition, College of Physicians and Surgeons, Columbia University, New York, New York 10032, and ⁸Department of Cell Biology, Duke University Medical Center, Durham, North Carolina 27710

The neurotransmitter dopamine (DA) modulates brain circuits involved in attention, reward, and motor activity. Synaptic DA homeostasis is primarily controlled via two presynaptic regulatory mechanisms, DA D₂ receptor (D₂R)-mediated inhibition of DA synthesis and release, and DA transporter (DAT)-mediated DA clearance. D₂Rs can physically associate with DAT and regulate DAT function, linking DA release and reuptake to a common mechanism. We have established that the attention-deficit hyperactivity disorder-associated human DAT coding variant Ala559Val (hDAT A559V) results in anomalous DA efflux (ADE) similar to that caused by amphetamine-like psychostimulants. Here, we show that tonic activation of D₂R provides support for hDAT A559V-mediated ADE. We determine in hDAT A559V a pertussis toxin-sensitive, CaMKII-dependent phosphorylation mechanism that supports D₂R-driven DA efflux. These studies identify a signaling network downstream of D₂R activation, normally constraining DA action at synapses, that may be altered by DAT mutation to impact risk for DA-related disorders.

The Journal of Neuroscience, April 28, 2010 • 30(17):6048–6057

Soluble Neuregulin-1 Has Bifunctional, Concentration-Dependent Effects on Schwann Cell Myelination

Neeraja Syed,^{1*} Kavya Reddy,^{1*} David P. Yang,¹ Carla Taveggia,² James L. Salzer,³ Patrice Maurel,¹ and Haesun A. Kim¹

¹Department of Biological Sciences, Rutgers University, Newark, New Jersey 07102, ²Instituto di Neurologia Sperimentale, San Raffaele Scientific Institute, 20132 Milan, Italy, and ³Smilow Neuroscience Program, Department of Cell Biology and Neurology, New York University School of Medicine, New York, New York 10016

Members of the neuregulin-1 (Nrg1) growth factor family play important roles during Schwann cell development. Recently, it has been shown that the membrane-bound type III isoform is required for Schwann cell myelination. Interestingly, however, Nrg1 type II, a soluble isoform, inhibits the process. The mechanisms underlying these isoform-specific effects are unknown. It is possible that myelination requires juxtacrine Nrg1 signaling provided by the membrane-bound isoform, whereas paracrine stimulation by soluble Nrg1 inhibits the process. To investigate this, we asked whether Nrg1 type III provided in a paracrine manner would promote or inhibit myelination. We found that soluble Nrg1 type III enhanced myelination in Schwann cell-neuron cocultures. It improved myelination of Nrg1 type III^{+/-} neurons and induced myelination on normally nonmyelinated sympathetic neurons. However, soluble Nrg1 type III failed to induce myelination on Nrg1 type III^{-/-} neurons. To our surprise, low concentrations of Nrg1 type II also elicited a similar promyelinating effect. At high doses, however, both type II and III isoforms inhibited myelination and increased c-Jun expression in a manner dependent on Mek/Erk (mitogen-activated protein kinase/extracellular signal-regulated kinase) activation. These results indicate that paracrine Nrg1 signaling provides concentration-dependent bifunctional effects on Schwann cell myelination. Furthermore, our studies suggest that there may be two distinct steps in Schwann cell myelination: an initial phase dependent on juxtacrine Nrg1 signaling and a later phase that can be promoted by paracrine stimulation.

The Journal of Neuroscience, April 28, 2010 • 30(17):6122–6131

Type 4 Phosphodiesterase Plays Different Integrating Roles in Different Cellular Domains in Pyramidal Cortical Neurons

Liliana R. V. Castro,^{1,2} Nicolas Gervasi,³ Elvire Guiot,^{1,2} Laetitia Cavellini,^{1,2} Viacheslav O. Nikolaev,⁴ Danièle Paupardin-Tritsch,^{1,2} and Pierre Vincent^{1,2}

¹Centre National de la Recherche Scientifique, Unité Mixte de Recherche (UMR) 7102, Paris, France, ²Université Pierre et Marie Curie, UMR 7102, Paris, France, ³ESPCI (École Supérieure de Physique et de Chimie Industrielles de la Ville de Paris), UMR 7637, Paris, France, and ⁴Institute of Pharmacology and Toxicology, University of Würzburg, D-97078 Würzburg, Germany

We investigated the role of phosphodiesterases (PDEs) in the integration of cAMP signals and protein kinase A (PKA) activity following β -adrenergic stimulation, by carrying out real-time imaging of male mouse pyramidal cortical neurons expressing biosensors to monitor cAMP levels (Epac1-camps and Epac2-camps300) or PKA activity (AKAR2). In the soma, isoproterenol (ISO) increased the PKA signal to approximately half the maximal response obtained with forskolin, with a characteristic β_1 pharmacology and an EC₅₀ of 4.5 nM. This response was related to free cAMP levels in the submicromolar range. The specific type 4 PDE (PDE4) inhibitor rolipram had a very small effect alone, but strongly potentiated the PKA response to ISO. Blockers of other PDEs had no effect. PDE4 thus acts as a brake in the propagation of the β_1 -adrenergic signal from the membrane to the bulk somatic cytosol. The results for a submembrane domain were markedly different, whether recorded with a PKA-sensitive potassium current related to the slow AHP or by two-photon imaging of small distal dendrites. The responses to ISO were stronger than in the bulk cytosol. This is consistent with the cAMP/PKA signal being strong at the membrane, as shown by electrophysiology, and favored in cellular domains with a high surface area to volume ratio, in which this signal was detected by imaging. Rolipram alone also produced a strong cAMP/PKA signal, revealing tonic cAMP production. PDE4 thus appears as a crucial integrator with different physiological implications in different subcellular domains.

The Journal of Neuroscience, April 28, 2010 • 30(17):6143–6151

Burst-Induced Anti-Hebbian Depression Acts through Short-Term Synaptic Dynamics to Cancel Redundant Sensory Signals

Erik Harvey-Girard,¹ John Lewis,² and Leonard Maler¹

¹Department of Cellular and Molecular Medicine, Faculty of Medicine, University of Ottawa, Ottawa, Ontario K1H 8M5, Canada, and ²Department of Biology, Faculty of Science, University of Ottawa, Ottawa, Ontario K1N 6N5, Canada

Weakly electric fish can enhance the detection and localization of important signals such as those of prey in part by cancellation of redundant spatially diffuse electric signals due to, e.g., their tail bending. The cancellation mechanism is based on descending input, conveyed by parallel fibers emanating from cerebellar granule cells, that produces a negative image of the global low-frequency signals in pyramidal cells within the first-order electrosensory region, the electrosensory lateral line lobe (ELL). Here we demonstrate that the parallel fiber synaptic input to ELL pyramidal cell undergoes long-term depression (LTD) whenever both parallel fiber afferents and their target cells are stimulated to produce paired burst discharges. Paired large bursts (4–4) induce robust LTD over pre–post delays of up to ± 50 ms, whereas smaller bursts (2–2) induce weaker LTD. Single spikes (either presynaptic or postsynaptic) paired with bursts did not induce LTD. Tetanic presynaptic stimulation was also ineffective in inducing LTD. Thus, we have demonstrated a form of anti-Hebbian LTD that depends on the temporal correlation of burst discharge. We then demonstrated that the burst-induced LTD is postsynaptic and requires the NR2B subunit of the NMDA receptor, elevation of postsynaptic Ca²⁺, and activation of CaMKII β . A model incorporating local inhibitory circuitry and previously identified short-term presynaptic potentiation of the parallel fiber synapses further suggests that the combination of burst-induced LTD, presynaptic potentiation, and local inhibition may be sufficient to explain the generation of the negative image and cancellation of redundant sensory input by ELL pyramidal cells.

The Journal of Neuroscience, April 28, 2010 • 30(17):6152–6169

Fibrinogen Triggers Astrocyte Scar Formation by Promoting the Availability of Active TGF- β after Vascular Damage

Christian Schachtrup,¹ Jae K. Ryu,¹ Matthew J. Helmrick,¹ Eirini Vagena,¹ Dennis K. Galanakis,³ Jay L. Degen,⁴ Richard U. Margolis,⁵ and Katerina Akassoglou^{1,2}

¹Gladstone Institute of Neurological Disease and ²Department of Neurology, University of California, San Francisco, San Francisco, California 94158, ³Department of Pathology, State University of New York, Stony Brook, New York 11794, ⁴Children's Hospital Research Foundation and the University of Cincinnati College of Medicine, Cincinnati, Ohio 45229, and ⁵Department of Pharmacology, New York University Medical Center, New York, New York 10016

Scar formation in the nervous system begins within hours after traumatic injury and is characterized primarily by reactive astrocytes depositing proteoglycans that inhibit regeneration. A fundamental question in CNS repair has been the identity of the initial molecular mediator that triggers glial scar formation. Here we show that the blood protein fibrinogen, which leaks into the CNS immediately after blood–brain barrier (BBB) disruption or vascular damage, serves as an early signal for the induction of glial scar formation via the TGF- β /Smad signaling pathway. Our studies revealed that fibrinogen is a carrier of latent TGF- β and induces phosphorylation of Smad2 in astrocytes that leads to inhibition of neurite outgrowth. Consistent with these findings, genetic or pharmacologic depletion of fibrinogen in mice reduces active TGF- β , Smad2 phosphorylation, glial cell activation, and neurocan deposition after cortical injury. Furthermore, stereotactic injection of fibrinogen into the mouse cortex is sufficient to induce astrogliosis. Inhibition of the TGF- β receptor pathway abolishes the fibrinogen-induced effects on glial scar formation *in vivo* and *in vitro*. These results identify fibrinogen as a primary astrocyte activation signal, provide evidence that deposition of inhibitory proteoglycans is induced by a blood protein that leaks in the CNS after vasculature rupture, and point to TGF- β as a molecular link between vascular permeability and scar formation.

The Journal of Neuroscience, April 28, 2010 • 30(17):5843–5854

In Vivo Proliferation of Postmitotic Cochlear Supporting Cells by Acute Ablation of the Retinoblastoma Protein in Neonatal Mice

Yiling Yu,^{1,3} Thomas Weber,¹ Tetsuji Yamashita,¹ Zhiyong Liu,^{1,4} Marcus B. Valentine,² Brandon C. Cox,¹ and Jian Zuo¹

Departments of ¹Developmental Neurobiology, and ²Genetics and Tumor Cell Biology, St. Jude Children's Research Hospital, Memphis, Tennessee 38105, ³Shanghai Medical School, Fudan University, Shanghai 200032, People's Republic of China, and ⁴Integrated Program in Biomedical Sciences, University of Tennessee Health Science Center, Memphis, Tennessee 38163

Cochlear hair cells (HCs) are mechanosensory receptors that transduce sound into electrical signals. HC damage in nonmammalian vertebrates induces surrounding supporting cells (SCs) to divide, transdifferentiate and replace lost HCs; however, such spontaneous HC regeneration does not occur in the mammalian cochlea. Here, we acutely ablate the retinoblastoma protein (Rb), a crucial cell cycle regulator, in two subtypes of postmitotic SCs (pillar and Deiters' cells) using an inducible Cre line, *Prox1-CreER*^{T2}. Inactivation of Rb in these SCs results in cell cycle reentry of both pillar and Deiters' cells, and completion of cell division with an increase in cell number of pillar cells. Interestingly, nuclei of *Rb*^{−/−} mitotic pillar and Deiters' cells migrate toward the HC layer and divide near the epithelial surface in a manner similar to the SCs in the regenerating avian auditory epithelium. In contrast to postmitotic *Rb*^{−/−} HCs which abort cell division, postmitotic *Rb*^{−/−} pillar cells can proliferate, maintain their SC fate and survive for more than a week. However, no newly formed HCs are detected and SC death followed by HC loss occurs. Our studies accomplish a crucial step toward functional HC regeneration in the mammalian cochlea *in vivo*, demonstrating the critical role of Rb in maintaining quiescence of postmitotic pillar and Deiters' cells and highlighting the heterogeneity between these two cell types. Therefore, the combination of transient Rb inactivation and further manipulation of transcription factors (i.e., Atoh1 activation) in SCs may represent an effective therapeutic avenue for HC regeneration in the mammalian cochlea.

The Journal of Neuroscience, April 28, 2010 • 30(17):5927–5936

CNS/PNS Boundary Transgression by Central Glia in the Absence of Schwann Cells or Krox20/Egr2 Function

Fanny Culpier,^{1,2} Laurence Decker,² Benoît Funalot,^{3,4} Jean-Michel Vallat,⁴ Federico Garcia-Bragado,⁵ Patrick Charnay,² and Piotr Topilko²

¹Plate-forme Transcriptome and ²Developmental Biology Section, Inserm, Unité 1024, Centre National de la Recherche Scientifique, Unité Mixte de Recherche 8197, Institut de Biologie de l'Ecole Normale Supérieure, 75230 Paris, France, ³Laboratoire de Biochimie et Génétique Moléculaire and ⁴National Reference Center "Rare Peripheral Neuropathies," Department of Neurology, Centre Hospitalier Universitaire de Limoges, 87042 Limoges, France, and ⁵Servicio de Anatomía Patológica, Hospital Virgen del Camino, 31008 Pamplona, Spain

CNS/PNS interfaces constitute cell boundaries, because they delimit territories with different neuronal and glial contents. Despite their potential interest in regenerative medicine, the mechanisms restricting oligodendrocytes and astrocytes to the CNS and Schwann cells to the PNS in mammals are not known. To investigate the involvement of peripheral glia and myelin in the maintenance of the CNS/PNS boundary, we have first made use of different mouse mutants. We show that depletion of Schwann cells and boundary cap cells or inactivation of *Krox20/Egr2*, a master regulatory gene for myelination in Schwann cells, results in transgression of the CNS/PNS boundary by astrocytes and oligodendrocytes and in myelination of nerve root axons by oligodendrocytes. In contrast, such migration does not occur with the *Trembler*¹ mutation, which

prevents PNS myelination without affecting *Krox20* expression. Altogether, these data suggest that maintenance of the CNS/PNS boundary requires a *Krox20* function separable from myelination control. Finally, we have analyzed a human patient affected by a congenital amyelinating neuropathy, associated with the absence of the KROX20 protein in Schwann cells. In this case, the nerve roots were also invaded by oligodendrocytes and astrocytes. This indicates that transgression of the CNS/PNS boundary by central glia can occur in pathological situations in humans and suggests that the underlying mechanisms are common with the mouse.

The Journal of Neuroscience, April 28, 2010 • 30(17):5958–5967

Pharmacological and Genetic Accumulation of Hypoxia-Inducible Factor-1 α Enhances Excitatory Synaptic Transmission in Hippocampal Neurons through the Production of Vascular Endothelial Growth Factor

Yu-Fei Huang,^{1,2} Chih-Hao Yang,^{1,2} Chiung-Chun Huang,^{1,3} Ming-Hong Tai,⁴ and Kuei-Sen Hsu^{1,2,3}

¹Department of Pharmacology and ²Institute of Basic Medical Sciences, College of Medicine, and ³Center for Gene Regulation and Signal Transduction Research, National Cheng Kung University, Tainan 701, Taiwan, and ⁴Institute of Biomedical Sciences, National Sun Yat-Sen University, Kaohsiung 804, Taiwan

Hypoxia-inducible factor-1 (HIF-1) is an important transcriptional factor in mammalian cells for coordination of adaptive responses to hypoxia. It consists of a regulatory subunit HIF-1 α , which accumulates under hypoxic conditions, and a constitutively expressed subunit HIF-1 β . In addition to the well characterized oxygen-dependent mode of action of HIF-1, recent work has shown that various growth factors and cytokines stimulate HIF-1 α expression, thereby triggering transcription of numerous hypoxia-inducible genes by oxygen-independent mechanisms. In this study, we examined whether accumulation of HIF-1 α induced by insulin-like growth factor-1 (IGF-1) has a regulatory role in excitatory synaptic transmission in hippocampal neuron cultures. Our results show that IGF-1 induced a time- and dose-dependent increase in HIF-1 α expression that was blocked by pretreatment with selective IGF-1 receptor antagonist, transcriptional inhibitor, and translational inhibitors. In addition, pharmacological blockade of the phosphatidylinositol 3-kinase/Akt/mammalian target of the rapamycin signaling pathway, but not extracellular signal-regulated kinase, inhibited IGF-1-induced HIF-1 α expression. More importantly, the increase in HIF-1 α expression induced by IGF-1 was accompanied by increasing levels of vascular endothelial growth factor (VEGF) mRNA and protein, which enhanced excitatory synaptic transmission. In parallel, blockade of HIF-1 α activity by echinomycin or lentiviral infection with dominant-negative mutant HIF-1 α or short hairpin RNA targeting HIF-1 α inhibited the increase in expression of VEGF and the enhancement of synaptic transmission induced by IGF-1. Conversely, transfection of constitutively active HIF-1 α into neurons mimicked the effects of IGF-1 treatment. Together, these results suggest that HIF-1 α accumulation can enhance excitatory synaptic transmission in hippocampal neurons by regulating production of VEGF.

The Journal of Neuroscience, April 28, 2010 • 30(17):6080–6093

The Death Receptor Antagonist FLIP-L Interacts with Trk and Is Necessary for Neurite Outgrowth Induced by Neurotrophins

Rana S. Moubarak,^{1,3,4*} Carme Solé,^{2*} Marta Pascual,^{4,5} Humberto Gutierrez,⁶ Marta Llovera,² M. José Pérez-García,² Raffaella Gozzelino,² Miguel F. Segura,² Victoria Iglesias-Guimaraes,^{1,4} Stéphanie Reix,^{1,3,4} Rosa M. Soler,² Alun M. Davies,⁶ Eduardo Soriano,^{4,5†} Victor J. Yuste,^{1,4†} and Joan X. Comella^{1,2,3,4†}

¹Institut de Neurociències, Departament de Bioquímica i Biologia Molecular, Facultat de Medicina, Universitat Autònoma de Barcelona, Campus de Bellaterra (Edifici M), 08193 Bellaterra, Spain, ²Cell Signaling and Apoptosis Group, Departament de Ciències Mèdiques Bàsiques, Institut de Recerca Biomèdica de Lleida/Universitat de Lleida, 25198 Lleida, Spain, ³Institut de Recerca de l'Hospital Universitari de la Vall d'Hebron, 08035 Barcelona, Spain, ⁴Centro de Investigación Biomédica en Red sobre Enfermedades Neurodegenerativas (CIBERNED), Spain, ⁵Developmental Neurobiology and Regeneration Unit, Institute for Research in Biomedicine, Parc Científic de Barcelona and Department of Cell Biology, University of Barcelona, Barcelona 08028, Spain, and ⁶School of Biosciences, Cardiff CF10 3US, United Kingdom

FLICE-inhibitory protein (FLIP) is an endogenous inhibitor of the signaling pathway triggered by the activation of death receptors. Here, we reveal a novel biological function for the long form of FLIP (FLIP-L) in neuronal differentiation, which can be dissociated from its antiapoptotic role. We show that FLIP-L is expressed in different regions of the mouse embryonic nervous system. Immunohistochemistry of mouse brain sections at different stages reveals that, in neurons, FLIP is expressed early during the embryonic neuronal development (embryonic day 16) and decreases at later stages (postnatal days 5–15), when its expression is essentially detected in glial cells. FLIP-L overexpression significantly enhances neurotrophin-induced neurite outgrowth in motoneurons, superior cervical ganglion neurons, and PC12 cells. Conversely, the downregulation of FLIP-L protein levels by specific RNA interference significantly reduces neurite outgrowth, even in the presence of the appropriate neurotrophin stimulus. Moreover, NGF-dependent activation of two main intracellular pathways involved in the regulation of neurite outgrowth, extracellular signal-regulated kinases (ERKs) and nuclear factor κ B (NF- κ B), is impaired when endogenous FLIP-L is downregulated, although TrkA remains activated. Finally, we demonstrate that FLIP-L interacts with TrkA, and not with p75^{NTR}, in an NGF-dependent manner, and endogenous FLIP-L interacts with TrkB in whole-brain lysates from embryonic day 15 mice embryos. Altogether, we uncover a new role for FLIP-L as an unexpected critical player in neurotrophin-induced mitogen-activated protein kinase/ERK- and NF- κ B-mediated control of neurite growth in developing neurons.

The Journal of Neuroscience, April 28, 2010 • 30(17):6094–6105

Differential Neuromodulation of Acquisition and Retrieval of Avoidance Learning by the Lateral Habenula and Ventral Tegmental Area

Jason Shumake, Anton Ilango, Henning Scheich, Wolfram Wetzell, and Frank W. Oehl

Leibniz Institute for Neurobiology, D-39118 Magdeburg, Germany

Several studies suggest an opponent functional relationship between the lateral habenula (LHb) and the ventral tegmental area (VTA). Previous work has linked LHb activation to the inhibition of dopaminergic neurons during loss of reward, as well as to deficits in escape and avoidance learning. We hypothesized that a dopamine signal might underlie the negative reinforcement of avoidance responses and that LHb activation could block this signal and thereby cause avoidance deficits. To test this idea, we implanted stimulating electrodes in either the VTA or LHb of gerbils engaged in two-way active avoidance learning, a task that shows learning-associated dopamine changes and that is acquired faster following LHb lesions. We delivered brief electrical brain stimulation whenever the animal performed a correct response, i.e., when the successful avoidance of foot shock was hypothesized to trigger an intrinsic reward signal. During the acquisition phase, VTA stimulation improved avoidance performance, while LHb stimulation impaired it. VTA stimulation appeared to improve both acquisition and asymptotic performance of the avoidance response, as VTA-stimulated animals reached above-normal performance but reverted to normal responding when stimulation was discontinued. The effects of LHb stimulation during avoidance acquisition were long lasting and persisted even after stimulation was discontinued. However, when given after successful acquisition of avoidance behavior, LHb stimulation had no effect, indicating that LHb stimulation specifically impaired avoidance acquisition without affecting memory retrieval or motivation or ability to perform the avoidance response. These results demonstrate opponent roles of LHb and VTA during acquisition but not during retrieval of avoidance learning.

The Journal of Neuroscience, April 28, 2010 • 30(17):5876–5883

Sequentially Switching Cell Assemblies in Random Inhibitory Networks of Spiking Neurons in the Striatum

Adam Ponzi and Jeff Wickens

Neurobiology Research Unit, Okinawa Institute of Science and Technology, Onna-Son, Okinawa 904-0412, Japan

The striatum is composed of GABAergic medium spiny neurons with inhibitory collaterals forming a sparse random asymmetric network and receiving an excitatory glutamatergic cortical projection. Because the inhibitory collaterals are sparse and weak, their role in striatal network dynamics is puzzling. However, here we show by simulation of a striatal inhibitory network model composed of spiking neurons that cells form assemblies that fire in sequential coherent episodes and display complex identity–temporal spiking patterns even when cortical excitation is simply constant or fluctuating noisily. Strongly correlated large-scale firing rate fluctuations on slow behaviorally relevant timescales of hundreds of milliseconds are shown by members of the same assembly whereas members of different assemblies show strong negative correlation, and we show how randomly connected spiking networks can generate this activity. Cells display highly irregular spiking with high coefficients of variation, broadly distributed low firing rates, and interspike interval distributions that are consistent with exponentially tailed power laws. Although firing rates vary coherently on slow timescales, precise spiking synchronization is absent in general. Our model only requires the minimal but striatally realistic assumptions of sparse to intermediate random connectivity, weak inhibitory synapses, and sufficient cortical excitation so that some cells are depolarized above the firing threshold during up states. Our results are in good qualitative agreement with experimental studies, consistent with recently determined striatal anatomy and physiology, and support a new view of endogenously generated metastable state switching dynamics of the striatal network underlying its information processing operations.

The Journal of Neuroscience, April 28, 2010 • 30(17):5894–5911

Parallel Input Channels to Mouse Primary Visual Cortex

Enquan Gao,¹ Gregory C. DeAngelis,² and Andreas Burkhalter¹

¹Department of Anatomy and Neurobiology, Washington University School of Medicine, St. Louis, Missouri 63110, and ²Department of Brain and Cognitive Sciences, Center for Visual Sciences, University of Rochester, Rochester, New York 14627

It is generally accepted that in mammals visual information is sent to the brain along functionally specialized parallel pathways, but whether the mouse visual system uses similar processing strategies is not known. It is important to resolve this issue because the mouse brain provides a tractable system for developing a cellular and molecular understanding of disorders affecting spatiotemporal visual processing. We have used single-unit recordings in mouse primary visual cortex to study whether individual neurons are more sensitive to one set of sensory cues than another. Our quantitative analyses show that neurons with short response latencies have low spatial acuity and high sensitivity to contrast, temporal frequency, and speed, whereas neurons with long latencies have high spatial acuity, low sensitivities to contrast, temporal frequency, and speed. These correlations suggest that neurons in mouse V1 receive inputs from a weighted combination of parallel afferent pathways with distinct spatiotemporal sensitivities.

The Journal of Neuroscience, April 28, 2010 • 30(17):5912–5926

Integration of Goal- and Stimulus-Related Visual Signals Revealed by Damage to Human Parietal Cortex

Paul M. Bays,^{1,2} Victoria Singh-Curry,^{1,2} Nikos Gorgoraptis,^{1,2} Jon Driver,¹ and Masud Husain^{1,2}

¹UCL Institute of Cognitive Neuroscience, London WC1N 3AR, United Kingdom, and ²UCL Institute of Neurology, London WC1N 3BG, United Kingdom

Where we look is determined both by our current intentions and by the tendency of visually salient items to “catch our eye.” After damage to parietal cortex, the normal process of directing attention is often profoundly impaired. Here, we tracked parietal patients’ eye movements during visual search to separately map impairments in goal-directed orienting to targets versus stimulus-driven gaze shifts to salient but task-irrelevant probes. Deficits in these two distinct types of attentional selection are shown to be identical in both magnitude and spatial distribution, consistent with damage to a “priority map” that integrates goal- and stimulus-related signals to select visual targets. When goal-relevant and visually salient items compete for attention, the outcome depends on a biased competition in which the priority of contralesional targets is undervalued. On the basis of these findings, we further demonstrate that parietal patients’ spatial bias (neglect) in goal-directed visual exploration can be corrected and even reversed by systematically manipulating the spatial distribution of stimulus salience in the visual array.

The Journal of Neuroscience, April 28, 2010 • 30(17):5968–5978

Priming of Hippocampal Population Bursts by Individual Perisomatic-Targeting Interneurons

Tommas J. Ellender,¹ Wiebke Nissen,^{1,2} Laura L. Colgin,³ Edward O. Mann,^{1,4} and Ole Paulsen^{1,5}

¹Department of Physiology, Anatomy and Genetics, University of Oxford, Oxford OX1 3PT, United Kingdom, ²Department of Pharmacology, University of Oxford, Oxford OX1 3QT, United Kingdom, ³Centre for the Biology of Memory, Norwegian University of Science and Technology, NO-7489 Trondheim, Norway, ⁴Department of Neurology, David Geffen School of Medicine at the University of California, Los Angeles, California 90095, and ⁵Department of Physiology, Development and Neuroscience, University of Cambridge, Cambridge CB2 3EG, United Kingdom

Hippocampal population bursts (“sharp wave–ripples”) occur during rest and slow-wave sleep and are thought to be important for memory consolidation. The cellular mechanisms involved are incompletely understood. Here we investigated the cellular mechanisms underlying the initiation of sharp waves using a hippocampal slice model. To this end, we used a combination of field recordings with planar multielectrode arrays and whole-cell patch-clamp recordings of individual anatomically identified pyramidal neurons and interneurons. We found that GABA_A receptor-mediated inhibition is necessary for sharp wave generation. Moreover, the activity of individual perisomatic-targeting interneurons can both suppress, and subsequently enhance, the local generation of sharp waves. Finally, we show that this is achieved by the tight control of local excitation and inhibition by perisomatic-targeting interneurons. These results suggest that perisomatic-targeting interneurons assist in selecting the subset of pyramidal neurons that initiate each hippocampal sharp wave–ripple.

The Journal of Neuroscience, April 28, 2010 • 30(17):5979–5991

Genetic Variation in *FGF20* Modulates Hippocampal Biology

Herve Lemaitre,¹ Venkata S. Mattay,¹ Fabio Sambataro,¹ Beth Verchinski,¹ Richard E. Straub,¹ Joseph H. Callicott,¹ Raja Kittappa,² Thomas M. Hyde,¹ Barbara K. Lipska,¹ Joel E. Kleinman,¹ Ronald McKay,² and Daniel R. Weinberger¹

¹Clinical Brain Disorder Branch, Genes, Cognition, and Psychosis Program, National Institutes of Health–National Institute of Mental Health, and

²Laboratory of Molecular Biology, National Institutes of Health–National Institute of Neurological Disorders and Stroke, Bethesda, Maryland 20892

We explored the effect of single-nucleotide polymorphisms (SNPs) in the fibroblast growth factor 20 gene (*FGF20*) associated with risk for Parkinson’s disease on brain structure and function in a large sample of healthy young-adult human subjects and also in elderly subjects to look at the interaction between genetic variations and age ($N = 237$; 116 men; 18–87 years). We analyzed high-resolution anatomical magnetic resonance images using voxel-based morphometry, a quantitative neuroanatomical technique. We also measured *FGF20* mRNA expression in postmortem human brain tissue to determine the molecular correlates of these SNPs ($N = 108$; 72 men; 18–74 years). We found that the T allele carriers of rs12720208 in the 3′-untranslated region had relatively larger hippocampal volume ($p = 0.0059$) and diminished verbal episodic memory ($p = 0.048$) and showed steeper decreases of hippocampal volume with normal aging ($p = 0.026$). In postmortem brain, T allele carriers had greater expression of hippocampal *FGF20* mRNA ($p = 0.037$), consistent with a previously characterized microRNA mechanism. The C allele matches a predicted miR-433 microRNA binding domain, whereas the T allele disrupts it, resulting in higher *FGF20* protein translation. The strong *FGF20* genetic effects in hippocampus are presumably mediated by activation of the FGFR1 (FGF receptor 1), which is expressed in mammalian brain most abundantly in the hippocampus. These associations, from mRNA expression to brain morphology to cognition and an interaction with aging, confirm a role of *FGF20* in human brain structure and function during development and aging.

The Journal of Neuroscience, April 28, 2010 • 30(17):5992–5997

CNS Leptin Action Modulates Immune Response and Survival in Sepsis

Johannes Tschöp,^{1,2} Ruben Nogueiras,³ Sarah Haas-Lockie,^{3,4} Kevin R. Kasten,² Tamara R. Castañeda,³ Nadine Huber,² Kelsey Guanciale,² Diego Perez-Tilve,³ Kirk Habegger,³ Nickki Ottaway,³ Stephen C. Woods,³ Brian Oldfield,⁴ Iain Clarke,⁴ Streamson Chua Jr,⁵ I. Sadaf Farooqi,⁶ Stephen O'Rahilly,⁶ Charles C. Caldwell,² and Matthias H. Tschöp^{3,7}

¹Clinic for Anaesthesiology, Klinikum Großhadern, Ludwig Maximilians University, D-81375 Munich, Germany, ²Department of Surgery, ³Metabolic Disease Institute, Division of Endocrinology, Department of Internal Medicine, University of Cincinnati College of Medicine, Cincinnati, Ohio 45267, ⁴Department of Physiology, Monash University, Melbourne, Victoria 3800, Australia, ⁵Departments of Medicine and Neuroscience, Albert Einstein College of Medicine, Bronx, New York 10461, ⁶University of Cambridge Metabolic Research Laboratories, Institute of Metabolic Science, Addenbrooke's Hospital, Cambridge CB2 0QQ, United Kingdom, and ⁷Department of Pharmacology, German Institute of Human Nutrition, D-14558 Potsdam-Nuthetal, Germany

Sepsis describes a complex clinical syndrome that results from an infection, setting off a cascade of systemic inflammatory responses that can lead to multiple organ failure and death. Leptin is a 16 kDa adipokine that, among its multiple known effects, is involved in regulating immune function. Here we demonstrate that leptin deficiency in *ob/ob* mice leads to higher mortality and more severe organ damage in a standard model of sepsis in mice [cecal ligation and puncture (CLP)]. Moreover, systemic leptin replacement improved the immune response to CLP. Based on the molecular mechanisms of leptin regulation of energy metabolism and reproductive function, we hypothesized that leptin acts in the CNS to efficiently coordinate peripheral immune defense in sepsis. We now report that leptin signaling in the brain increases survival during sepsis in leptin-deficient as well as in wild-type mice and that endogenous CNS leptin action is required for an adequate systemic immune response. These findings reveal the existence of a relevant neuroendocrine control of systemic immune defense and suggest a possible therapeutic potential for leptin analogs in infectious disease. *The Journal of Neuroscience*, April 28, 2010 • 30(17):6036–6047

Aging-Related Gene Expression in Hippocampus Proper Compared with Dentate Gyrus Is Selectively Associated with Metabolic Syndrome Variables in Rhesus Monkeys

Eric M. Blalock,^{1*} Richard Grondin,^{2*} Kuey-chu Chen,¹ Olivier Thibault,¹ Veronique Thibault,¹ Jignesh D. Pandya,^{2,3} Amy Dowling,¹ Zhiming Zhang,² Patrick Sullivan,^{2,3} Nada M. Porter,¹ and Philip W. Landfield¹

Departments of ¹Molecular and Biomedical Pharmacology and ²Anatomy and Neurobiology and ³Spinal Cord and Brain Injury Research Center, University of Kentucky, Lexington, Kentucky 40536

Age-dependent metabolic syndrome (MetS) is a well established risk factor for cardiovascular disease, but it also confers major risk for impaired cognition in normal aging or Alzheimer's disease (AD). However, little is known about the specific pathways mediating MetS–brain interactions. Here, we performed the first studies quantitatively linking MetS variables to aging changes in brain genome-wide expression and mitochondrial function. In six young adult and six aging female rhesus monkeys, we analyzed gene expression in two major hippocampal subdivisions critical for memory/cognitive function [hippocampus proper, or cornu ammonis (CA), and dentate gyrus (DG)]. Genes that changed with aging [aging-related genes (ARGs)] were identified in each region. Serum variables reflecting insulin resistance and dyslipidemia were used to construct a quantitative MetS index (MSI). This MSI increased with age and correlated negatively with hippocampal mitochondrial function (state III oxidation). More than 2000 ARGs were identified in CA and/or DG, in approximately equal numbers, but substantially more ARGs in CA than in DG were correlated selectively with the MSI. Pathways represented by MSI-correlated ARGs were determined from the Gene Ontology Database and literature. In particular, upregulated CA ARGs representing glucocorticoid receptor (GR), chromatin assembly/histone acetyltransferase, and inflammatory/immune pathways were closely associated with the MSI. These results suggest a novel model in which MetS is associated with upregulation of hippocampal GR-dependent transcription and epigenetic coactivators, contributing to decreased mitochondrial function and brain energetic dysregulation. In turn, these MSI-associated neuroenergetic changes may promote inflammation, neuronal vulnerability, and risk of cognitive impairment/AD.

The Journal of Neuroscience, April 28, 2010 • 30(17):6058–6071

Ignoring the Elephant in the Room: A Neural Circuit to Downregulate Salience

Carmel Mevorach,¹ John Hodsoll,¹ Harriet Allen,¹ Lilach Shalev,² and Glyn Humphreys¹

¹Behavioural Brain Sciences Centre, School of Psychology, University of Birmingham, Birmingham B15 2TT, United Kingdom, and ²Division of Learning Disability, School of Education, Hebrew University of Jerusalem, Jerusalem 91905, Israel

How do we ignore stimuli that are salient but irrelevant when our task is to select a lower salient stimulus? Since bottom–up processes favor high saliency, detection of a low-salient target in the presence of highly salient distractors requires top–down attentional guidance. Previous studies have demonstrated that top–down attention can modulate perceptual processing and also that the control of attention is driven by frontoparietal regions. However, to date, there is no direct evidence on the cause and effect relationship between control regions and perceptual processing. Here, we report the first evidence demonstrating a neural circuit for the downregulation of salient distractors when a low-salient target is selected, combining brain imaging using functional magnetic resonance imaging with brain stimulation by transcranial magnetic stimulation. Using these combined techniques, we were able to identify a cause and effect relationship in the suppression of saliency, based on an interaction between the left intraparietal sulcus (IPS) and a region implicated in visual processing in our task (the left occipital pole). In particular, low-salient stimuli were selected by the left IPS suppressing early visual areas that would otherwise respond to a high-saliency distractor in the task. Apart from providing a first documentation of the neural circuit supporting selection by saliency, these data can be critical for understanding the underlying causes of problems in ignoring irrelevant salience that are found in both acquired and neurodevelopmental disorders (e.g., attention deficit/hyperactivity disorder or autism).

The Journal of Neuroscience, April 28, 2010 • 30(17):6072–6079

Rapid Glucocorticoid Receptor-Mediated Inhibition of Hypothalamic–Pituitary–Adrenal Ultradian Activity in Healthy Males

Georgina M. Russell,¹ David E. Henley,^{1,2} Jack Leendertz,¹ Jennie A. Douthwaite,¹ Susan A. Wood,¹ Adam Stevens,³ Wolfram W. Woltersdorf,⁴ Bernard W. M. M. Peeters,⁵ Ge S. F. Ruigt,⁵ Anne White,³ Johannes D. Veldhuis,⁶ and Stafford L. Lightman¹

¹Henry Wellcome Laboratories for Integrative Neurosciences and Endocrinology, University of Bristol, Bristol BS1 3NY, United Kingdom, ²Faculty of Medicine, Dentistry and Health Sciences, University of Western Australia, Crawley 6009, Western Australia, Australia, ³Endocrine Sciences Research Group, Manchester Academic Health Sciences Center, University of Manchester, Manchester M13 9PT, United Kingdom, ⁴University Hospitals Bristol National Health Science Foundation Trust, Department of Laboratory Medicine, Bristol BS2 8HW, United Kingdom, ⁵Schering-Plough, 5340BH Oss, The Netherlands, and ⁶Endocrine Research Unit, Mayo Clinic College of Medicine, Rochester, Minnesota 55905

A complex dynamic ultradian rhythm underlies the hypothalamic–pituitary–adrenal (HPA) circadian rhythm. We have investigated in normal human male subjects the importance, site of action, and receptor-mediated processes involved in rapid basal corticosteroid feedback and its interaction with corticotrophin releasing hormone (CRH) drive. Pro-opiomelanocortin (POMC), ACTH, and cortisol were measured every 10 min from healthy males during the awakening period or late afternoon using an automated blood sampling system. Mathematical modeling into discrete pulses of activity revealed that intravenous infusion of the synthetic mixed glucocorticoid/mineralocorticoid agonist prednisolone produced rapid inhibition of ACTH and cortisol pulsatility within 30 min in the morning and afternoon. Any pulse that had commenced at the time of injection was unaffected, and subsequent pulsatility was inhibited. Prednisolone also inhibited ACTH and cortisol secretion in response to exogenous CRH stimulation, inferring rapid feedback inhibition at the anterior pituitary. Circulating POMC peptide concentrations were unaffected, suggesting that the rapid corticosteroid inhibitory effect specifically targeted ACTH secretion from pituitary corticotrophs. Prednisolone fast feedback was only reduced by glucocorticoid receptor antagonist pretreatment and not by mineralocorticoid receptor antagonism, suggesting a glucocorticoid receptor-mediated pathway. The intravenous prednisolone suppression test provides a powerful new tool to investigate HPA abnormalities underlying metabolic and psychiatric disease states.

The Journal of Neuroscience, April 28, 2010 • 30(17):6106–6115

NEUROBIOLOGY OF DISEASE

Cellular and Synaptic Mechanisms of Anti-NMDA Receptor Encephalitis

Ethan G. Hughes,^{1*} Xiaoyu Peng,^{1*} Amy J. Gleichman,¹ Meizhan Lai,² Lei Zhou,² Ryan Tsou,¹ Thomas D. Parsons,⁴ David R. Lynch,^{2,3} Josep Dalmau,² and Rita J. Balice-Gordon¹

Departments of ¹Neuroscience and ²Neurology, University of Pennsylvania, ³Department of Pediatrics, Children's Hospital of Philadelphia, and

⁴Department of Clinical Studies, New Bolton Center, University of Pennsylvania School of Veterinary Medicine, Philadelphia, Pennsylvania 19104

We recently described a severe, potentially lethal, but treatment-responsive encephalitis that associates with autoantibodies to the NMDA receptor (NMDAR) and results in behavioral symptoms similar to those obtained with models of genetic or pharmacologic attenuation of NMDAR function. Here, we demonstrate that patients' NMDAR antibodies cause a selective and reversible decrease in NMDAR surface density and synaptic localization that correlates with patients' antibody titers. The mechanism of this decrease is selective antibody-mediated capping and internalization of surface NMDARs, as Fab fragments prepared from patients' antibodies did not decrease surface receptor density, but subsequent cross-linking with anti-Fab antibodies recapitulated the decrease caused by intact patient NMDAR antibodies. Moreover, whole-cell patch-clamp recordings of miniature EPSCs in cultured rat hippocampal neurons showed that patients' antibodies specifically decreased synaptic NMDAR-mediated currents, without affecting AMPA receptor-mediated currents. In contrast to these profound effects on NMDARs, patients' antibodies did not alter the localization or expression of other glutamate receptors or synaptic proteins, number of synapses, dendritic spines, dendritic complexity, or cell survival. In addition, NMDAR density was dramatically reduced in the hippocampus of female Lewis rats infused with patients' antibodies, similar to the decrease observed in the hippocampus of autopsied patients. These studies establish the cellular mechanisms through which antibodies of patients with anti-NMDAR encephalitis cause a specific, titer-dependent, and reversible loss of NMDARs. The loss of this subtype of glutamate receptors eliminates NMDAR-mediated synaptic function, resulting in the learning, memory, and other behavioral deficits observed in patients with anti-NMDAR encephalitis.

The Journal of Neuroscience, April 28, 2010 • 30(17):5866–5875

Dynamic Time Course of Typical Childhood Absence Seizures: EEG, Behavior, and Functional Magnetic Resonance Imaging

Xiaoxiao Bai,¹ Matthew Vestal,¹ Rachel Berman,¹ Michiro Negishi,² Marisa Spann,⁵ Clemente Vega,⁵ Matthew Desalvo,¹ Edward J. Novotny,^{1,3,5} Robert T. Constable,² and Hal Blumenfeld^{1,4,5}

Departments of ¹Neurology, ²Diagnostic Radiology, ³Pediatrics, ⁴Neurobiology, and ⁵Neurosurgery, Yale University School of Medicine, New Haven, Connecticut 06520

Absence seizures are 5–10 s episodes of impaired consciousness accompanied by 3–4 Hz generalized spike-and-wave discharge on electroencephalography (EEG). The time course of functional magnetic resonance imaging (fMRI) changes in absence seizures in relation to EEG and behavior is not known. We acquired simultaneous EEG–fMRI in 88 typical childhood absence seizures from nine pediatric patients. We investigated behavior concurrently using a continuous performance task or simpler repetitive tapping task. EEG time–frequency analysis revealed abrupt onset and end of 3–4 Hz spike-wave discharges with a mean duration of 6.6 s. Behavioral analysis also showed

rapid onset and end of deficits associated with electrographic seizure start and end. In contrast, we observed small early fMRI increases in the orbital/medial frontal and medial/lateral parietal cortex >5 s before seizure onset, followed by profound fMRI decreases continuing >20 s after seizure end. This time course differed markedly from the hemodynamic response function (HRF) model used in conventional fMRI analysis, consisting of large increases beginning after electrical event onset, followed by small fMRI decreases. Other regions, such as the lateral frontal cortex, showed more balanced fMRI increases followed by approximately equal decreases. The thalamus showed delayed increases after seizure onset followed by small decreases, most closely resembling the HRF model. These findings reveal a complex and long-lasting sequence of fMRI changes in absence seizures, which are not detectable by conventional HRF modeling in many regions. These results may be important mechanistically for seizure initiation and termination and may also contribute to changes in EEG and behavior.

The Journal of Neuroscience, April 28, 2010 • 30(17):5884–5893

A β -Mediated NMDA Receptor Endocytosis in Alzheimer's Disease Involves Ubiquitination of the Tyrosine Phosphatase STEP₆₁

Pradeep Kurup,^{1*} Yongfang Zhang,^{1*} Jian Xu,¹ Deepa V. Venkitaramani,¹ Vahram Haroutunian,³ Paul Greengard,⁴ Angus C. Nairn,² and Paul J. Lombroso¹

¹Child Study Center and ²Department of Psychiatry, Yale University School of Medicine, New Haven, Connecticut 06520, ³Department of Psychiatry, The Mount Sinai School of Medicine, New York, New York 10029, and ⁴Laboratory of Molecular and Cellular Neuroscience, The Rockefeller University, New York, New York, 10021

Amyloid β (A β) is involved in the etiology of Alzheimer's disease (AD) and may contribute to cognitive deficits by increasing internalization of ionotropic glutamate receptors. Striatal-enriched protein tyrosine phosphatase 61 (STEP₆₁), which is targeted in part to the postsynaptic terminal, has been implicated in this process. Here we show that STEP₆₁ levels are progressively increased in the cortex of Tg2576 mice over the first year, as well as in prefrontal cortex of human AD brains. The increased STEP₆₁ was associated with greater STEP activity, dephosphorylation of phospho-tyr¹⁴⁷² of the NR2B subunit, and decreased NR1 and NR2B subunits on neuronal membranes. Treatment with A β -enriched medium also increased STEP₆₁ levels and decreased NR1/NR2B abundance in mouse cortical cultures as determined by biotinylation experiments. In STEP knock-out cultures, A β treatment failed to induce NMDA receptor internalization. The mechanism for the increase in STEP₆₁ levels appears to involve the ubiquitin proteasome system. Blocking the proteasome resulted in elevated levels of STEP₆₁. Moreover, STEP₆₁-ubiquitin conjugates were increased in wild-type cortical slices upon A β treatment as well as in 12 month Tg2576 cortex. These findings reveal a novel mechanism by which A β -mediated accumulation of STEP₆₁ results in increased internalization of NR1/NR2B receptor that may contribute to the cognitive deficits in AD.

The Journal of Neuroscience, April 28, 2010 • 30(17):5948–5957

Norrin Mediates Neuroprotective Effects on Retinal Ganglion Cells via Activation of the Wnt/ β -Catenin Signaling Pathway and the Induction of Neuroprotective Growth Factors in Müller Cells

Roswitha Seitz, Simon Hackl, Thomas Seibuchner, Ernst R. Tamm, and Andreas Ohlmann

Institute of Human Anatomy and Embryology, University of Regensburg, D-93053 Regensburg, Germany

Norrin is a secreted protein that binds to frizzled 4 and controls development of capillaries in retina and inner ear. We provide evidence that Norrin has distinct neuroprotective properties that are independent from its effects on vascular development. The function of Norrin was investigated in a mouse model of excitotoxic retinal ganglion cell (RGC) damage after intravitreal injection of NMDA, and in cultured Müller glia or immortalized RGC-5 cells. Intravitreal injection of Norrin significantly increased the number of surviving RGC axons in the optic nerve and decreased apoptotic death of retinal neurons following NMDA-mediated damage. This effect could be blocked by adding dickkopf (DKK)-1, an inhibitor of the Wnt/ β -catenin signaling pathway. Treatment of eyes with combined Norrin/NMDA activated Wnt/ β -catenin signaling and increased the retinal expression of leukemia inhibitory factor and endothelin-2, as well as that of neurotrophic growth factors such as fibroblast growth factor-2, brain-derived neurotrophic factor, lens epithelium-derived growth factor, and ciliary neurotrophic factor. A similar activation of Wnt/ β -catenin signaling and an increased expression of neurotrophic factors was observed in cultured Müller cells after treatment with Norrin, effects that again could be blocked by adding DKK-1. In addition, conditioned cell culture medium of Norrin-treated Müller cells increased survival of differentiated RGC-5 cells. We conclude that Norrin has pronounced neuroprotective properties on retinal neurons with the distinct potential to decrease the damaging effects of NMDA-induced RGC loss. The effects of Norrin involve activation of Wnt/ β -catenin signaling and subsequent induction of neurotrophic growth factors in Müller cells.

The Journal of Neuroscience, April 28, 2010 • 30(17):5998–6010

Identification of Caspase-6-Mediated Processing of the Valosin Containing Protein (p97) in Alzheimer's Disease: A Novel Link to Dysfunction in Ubiquitin Proteasome System-Mediated Protein Degradation

Dalia Halawani,¹ Sylvain Tessier,² Dominique Anzellotti,¹ David A. Bennett,³ Martin Latterich,^{1,4} and Andréa C. LeBlanc^{5,6}

¹Department of Anatomy and Cell Biology, McGill University, Montréal, Québec H3T 1E2, Canada, ²Institut de Recherches Cliniques de Montréal, Université de Montréal, Montréal, Québec H2W 1R7, Canada, ³Rush University Medical Center, Chicago, Illinois 60612, ⁴Proteogenomics Research Institute for Systems Medicine, San Diego, California, 92121-1206, ⁵Lady Davis Institute for Medical Research, Sir Mortimer B. Davis Jewish General Hospital, and ⁶Department of Neurology and Neurosurgery, McGill University, Montréal, Québec H3A 2B4, Canada

The valosin-containing protein (p97) is a ubiquitin-dependent ATPase that plays central roles in ubiquitin proteasome system (UPS)-mediated protein degradation pathways. p97 has been recently identified as a putative substrate of active Caspase-6 (Casp6) in primary human neurons. Since Casp6 is activated in mild cognitive impairment (MCI) and Alzheimer's disease (AD) patients' brains, the targeting of p97 by Casp6 may represent an important step that leads to UPS impairment in AD. Here, we show that p97 is a Casp6 substrate *in vitro* and *in vivo*. Casp6 cleavage of recombinant p97 generated two N-terminal fragments of 28 and 20 kDa, which were not generated by the other two effector caspases, Caspase-3 and Caspase-7. ATP binding to the D1 ATPase ring of p97 reduced the susceptibility of the N-domain to caspase-mediated proteolysis. Mass spectrometric analysis identified VAPD¹⁷⁹ as a Casp6 cleavage site within p97's N-domain. An anti-neoepitope serum immunohistochemically detected p97 cleaved at VAPD¹⁷⁹ in the cytoplasm of the cell soma and neurites of hippocampal neurons in MCI and AD. Overexpression of p97 (1-179) fragment, representing p97 cleaved at D179, impaired the degradation of model substrates in the ubiquitin-fusion degradation and the N-end rule pathways, and destabilized endogenous p97. Collectively, these results show that p97 is cleaved by Casp6 in AD and suggest p97 cleavage as an important mechanism for UPS impairment.

The Journal of Neuroscience, April 28, 2010 • 30(17):6132–6142