

This Week in The Journal

● Cellular/Molecular

Methylene Blue May Slow Huntington's Disease

Emily Mitchell Sontag, Gregor P. Lotz, Namita Agrawal, Andrew Tran, Rebecca Aron, et al.

(see pages 11109–11119)

Methylene blue (MB) was developed as a textile dye in the late 19th century, but it was found to be better for staining tissues—especially nervous tissue—than for fabrics. The reduced form of MB is lipophilic and easily crosses cell membranes, but inside cells it becomes oxidized and forms a blue precipitate. Because the oxidized and reduced forms are in equilibrium, MB can act as either a peroxidant or antioxidant. Its numerous biological effects include inactivating microbes; inhibiting soluble guanylate cyclase, nitric oxide synthase, and acetylcholinesterase; and modulating membrane transporters and ion channels. Through these and/or other mechanisms, MB can enhance memory and treat psychoses, depression, and possibly Alzheimer's disease. Sontag et al. report that MB might also be useful in treating Huntington's disease (HD). In a mouse HD model, MB slowed the progression of sensorimotor impairment, possibly by reducing aggregation of huntingtin protein or by increasing levels of brain-derived neurotrophic factor.

▲ Development/Plasticity/Repair

Neurons Become Active Early in ENS Development

Marlene M. Hao, Alan E. Lomax, Sonja J. McKeown, Christopher A. Reid, Heather M. Young, et al.

(see pages 10949–10960)

The enteric nervous system (ENS), which lies within the walls of the gut, is essential for normal peristalsis. Although it receives CNS input, the ENS is independently necessary and sufficient to generate gut movements. Malformation of the ENS leads to fatal intestinal blockage. The ENS

derives from the neural crest, and precursors proliferate and differentiate as they migrate caudally and invade the gut. Approximately 20 subtypes of ENS neurons have been identified, as have many genes that regulate precursor proliferation and differentiation. Still, little is known about the functional development of the ENS. Therefore, Hao et al. examined the development of ENS excitability in embryonic mice. Neural markers were expressed before excitable properties were acquired, and the first active property to appear was graded responses to depolarization, which was mediated by an unidentified voltage-gated inward current. Spiking commenced later, after enough voltage-gated Na^+ channels were expressed. Finally, conductances underlying slow afterhyperpolarization emerged perinatally.

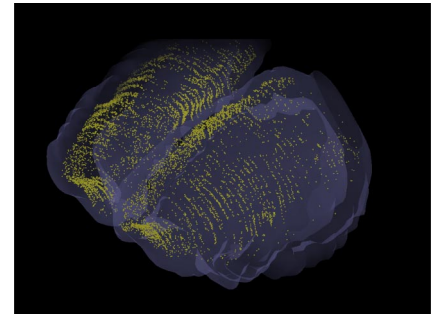
■ Behavioral/Systems/Cognitive

Cerebo-ponto-cerebellar Projections Do Not Adhere to Zones

Lucia Suzuki, Patrice Coulon, Erika H. Sabel-Goedknecht, and Tom J. H. Ruigrok

(see pages 10854–10869)

Cerebellar output is organized as modules comprising longitudinal zones of Purkinje cells that project to different cerebellar nuclei. Each zone receives input from a particular subnucleus of the inferior olive. Relatively little is known about the more complex organization of inputs to the cerebellum from the cerebral cortex via pontine nuclei, however. Suzuki et al. examined this circuitry by coinjecting cholera toxin (CT) and rabies virus (RV) into rat cerebellar cortex. Transsynaptic retrograde transport of RV identified second-order projections from cerebral cortex, while retrograde transport of CT labeled olivary neurons, thus enabling identification of the injected zone(s). Cerebo-ponto-cerebellar projections did not adhere strongly to the zonal organization. Instead, neurons projecting to different zones within a given lobule were largely intermixed in the pons and cerebral cortex. Interestingly, projections to the vermis of lobule VII originated in widespread cortical regions, including retrosplenial and



RV injected into the vermis of cerebellar lobule VII labeled neurons (yellow dots) in wide regions of the cerebral cortex. See the article by Suzuki et al. for details.

orbitofrontal cortices, supporting a role for this lobule in nonmotor functions.

◆ Neurobiology of Disease

Reactive Astrocytes May Promote HIV-Related Pain

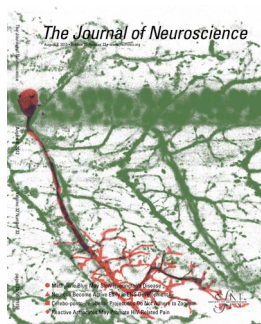
Yuqiang Shi, Benjamin B. Gelman, Joshua G. Lisinicchia, and Shao-Jun Tang

(see pages 10833–10840)

Intense or prolonged activation of nociceptor afferents—which occurs, for example, after peripheral nerve injury—sensitizes central nociception pathways, resulting in chronic pain. In animal models, spinal dorsal horn microglia and astrocytes contribute to the development and maintenance of chronic pain by secreting proinflammatory cytokines. Evidence for glial contributions to chronic pain in humans has been lacking, however. Shi et al. obtained such evidence by examining postmortem dorsal horn tissue from HIV-infected patients. Many, but not all HIV patients develop chronic pain. Markers of reactive astrocytes were expressed at higher levels in patients who had experienced chronic pain than in patients and uninfected controls who had not. The number of reactive astrocytes was also increased in pain-positive patients, as were the levels of proinflammatory cytokines. In contrast, two markers of reactive microglia were not significantly different between pain and pain-free patients, suggesting these cells are not required to maintain HIV-associated pain.

The Journal of Neuroscience

August 8, 2012 • Volume 32 Number 32 • www.jneurosci.org



Cover legend: A confocal z-stack of larval zebrafish axial motoneurons from the *parg^{mn2Et}* enhancer trap line. The image depicts the longitudinal column of green fluorescent protein-labeled cell bodies in the spinal cord and their peripheral axon arborizations in the chevron-shaped trunk muscles (green).

Superimposed on the axial motor pool is a caudal primary motoneuron (CaP) in the same larva, which was electroporated with rhodamine dextran (red). The stack is 80 μm in width; dorsal is up and rostral is to the left. For more information, see the article by Menelaou and McLean (pages 10925–10939).

i This Week in The Journal

Journal Club

- 10795 **Unraveling Acetylcholine Impact on Human Cortical Plasticity**
Antonio Suppa and Odysseas Papazachariadis

Articles

CELLULAR/MOLECULAR

- 10791 **Upregulation of the E3 ligase NEDD4-1 by Oxidative Stress Degrades IGF-1 Receptor Protein in Neurodegeneration**
Young-Don Kwak, Bin Wang, Jing Jing Li, Ruishan Wang, Qiyue Deng, Shiyong Diao, Yaomin Chen, Raymond Xu, Eliezer Masliah, Huaxi Xu, Jung-Joon Sung, and Francesca-Fang Liao
- 10995 **Increase in Sodium Conductance Decreases Firing Rate and Gain in Model Neurons**
Tilman J. Kispersky, Jonathan S. Caplan, and Eve Marder
- 11050 **Activation of Aurora-A Is Essential for Neuronal Migration via Modulation of Microtubule Organization**
Takako Takitoh, Kanako Kumamoto, Chen-Chi Wang, Makoto Sato, Shiori Toba, Anthony Wynshaw-Boris, and Shinji Hirotsune
- 11095 **Formation of Golgi-Derived Active Zone Precursor Vesicles**
Christoph Maas, Viviana I. Torres, Wilko D. Altmann, Sergio Leal-Ortiz, Dhananjay Wagh, Ryan T. Terry-Lorenzo, Anna Fejtova, Eckart D. Gundelfinger, Noam E. Ziv, and Craig C. Garner
- 11109 **Methylene Blue Modulates Huntingtin Aggregation Intermediates and Is Protective in Huntington's Disease Models**
Emily Mitchell Sontag, Gregor P. Lotz, Namita Agrawal, Andrew Tran, Rebecca Aron, Guocheng Yang, Mihaela Necula, Alice Lau, Steven Finkbeiner, Charles Glabe, J. Lawrence Marsh, Paul J. Muchowski, and Leslie M. Thompson
- 11132 **Tonic Inhibition Enhances Fidelity of Sensory Information Transmission in the Cerebellar Cortex**
Ian Duguid, Tiago Branco, Michael London, Paul Chadderton, and Michael Häusser
- 11144 **Rabconnectin3 α Promotes Stable Activity of the H⁺ Pump on Synaptic Vesicles in Hair Cells**
Zev Einhorn, Josef G. Trapani, Qianying Liu, and Teresa Nicolson

DEVELOPMENT/PLASTICITY/REPAIR

- 10940 **Fezf2 Regulates Multilineage Neuronal Differentiation through Activating Basic Helix-Loop-Helix and Homeodomain Genes in the Zebrafish Ventral Forebrain**
Nan Yang, Zhiqiang Dong, and Su Guo
- 10949 **Early Development of Electrical Excitability in the Mouse Enteric Nervous System**
Marlene M. Hao, Alan E. Lomax, Sonja J. McKeown, Christopher A. Reid, Heather M. Young, and Joel C. Bornstein
- 10982 **Voltage-Sensitive Dye Imaging Reveals Dynamic Spatiotemporal Properties of Cortical Activity after Spontaneous Muscle Twitches in the Newborn Rat**
David A. McVea, Majid H. Mohajerani, and Timothy H. Murphy

BEHAVIORAL/SYSTEMS/COGNITIVE

- 10797 **Supraspinal Peroxynitrite Modulates Pain Signaling by Suppressing the Endogenous Opioid Pathway**
Joshua W. Little, Zhoumou Chen, Timothy Doyle, Frank Porreca, Mahsa Ghaffari, Leesa Bryant, William L. Neumann, and Daniela Salvemini
- 10819 **Sodium Channel Na_v1.7 Is Essential for Lowering Heat Pain Threshold after Burn Injury**
Shannon D. Shields, Xiaoyang Cheng, Nurcan Üçeyler, Claudia Sommer, Sulayman D. Dib-Hajj, and Stephen G. Waxman
- 10854 **Organization of Cerebral Projections to Identified Cerebellar Zones in the Posterior Cerebellum of the Rat**
Lucia Suzuki, Patrice Coulon, Erika H. Sabel-Goedknecht, and Tom J. H. Ruigrok
- 10870 **How Preparation Changes the Need for Top-Down Control of the Basal Ganglia When Inhibiting Premature Actions**
Sara Jahfari, Frederick Verbruggen, Michael J. Frank, Lourens J. Waldorp, Lorenza Colzato, K. Richard Ridderinkhof, and Birte U. Forstmann
- 10879 **An Essential Role for Histone Deacetylase 4 in Synaptic Plasticity and Memory Formation**
Mi-Sung Kim, M. Waseem Akhtar, Megumi Adachi, Melissa Mahgoub, Rhonda Bassel-Duby, Ege T. Kavalali, Eric N. Olson, and Lisa M. Monteggia
- 10895 **Afferents from Vocal Motor and Respiratory Effectors Are Recruited during Vocal Production in Juvenile Songbirds**
Sarah W. Bottjer and Michelle To
- 10907 **Persistent Spatial Information in the Frontal Eye Field during Object-Based Short-Term Memory**
Kelsey L. Clark, Behrad Noudoost, and Tirin Moore
- 10915 **Hippocampal-Prefrontal Cortical Circuit Mediates Inhibitory Response Control in the Rat**
Yogita Chudasama, Victoria M. Doobay, and Yuchen Liu
- 10925 **A Gradient in Endogenous Rhythmicity and Oscillatory Drive Matches Recruitment Order in an Axial Motor Pool**
Evdokia Menelaou and David L. McLean

- 10961 **Effect of Locomotor Training in Completely Spinalized Cats Previously Submitted to a Spinal Hemisection**
Marina Martinez, Hugo Delivet-Mongrain, Hugues Leblond, and Serge Rossignol
- 11005 **What and Where Information in the Caudate Tail Guides Saccades to Visual Objects**
Shinya Yamamoto, Ilya E. Monosov, Masaharu Yasuda, and Okihide Hikosaka
- 11017 **Neuronal Activity during a Cued Strategy Task: Comparison of Dorsolateral, Orbital, and Polar Prefrontal Cortex**
Satoshi Tsujimoto, Aldo Genovesio, and Steven P. Wise
- 11032 **Role of Dopamine Tone in the Pursuit of Brain Stimulation Reward**
Giovanni Hernandez, Ivan Trujillo-Pisanty, Marie-Pierre Cossette, Kent Conover, and Peter Shizgal
- 11042 **Stress Modulates the Engagement of Multiple Memory Systems in Classification Learning**
Lars Schwabe and Oliver T. Wolf
- 11120 **Long-Range Parallel Processing and Local Recurrent Activity in the Visual Cortex of the Mouse**
Pierre-Olivier Polack and Diego Contreras

NEUROBIOLOGY OF DISEASE

- 10809 **Reactive Astrocytes Overexpress TSPO and Are Detected by TSPO Positron Emission Tomography Imaging**
Sonia Lavis, Martine Guillermer, Anne-Sophie Hérard, Fanny Petit, Marion Delahaye, Nadja Van Camp, Lucile Ben Haim, Vincent Lebon, Philippe Remy, Frédéric Dollé, Thierry Delzescaux, Gilles Bonvento, Philippe Hantraye, and Carole Escartin
- 10833 **Chronic-Pain-Associated Astrocytic Reaction in the Spinal Cord Dorsal Horn of Human Immunodeficiency Virus-Infected Patients**
Yuqiang Shi, Benjamin B. Gelman, Joshua G. Lisinicchia, and Shao-Jun Tang
- 10841 **Schizophrenia-Like Features in Transgenic Mice Overexpressing Human HO-1 in the Astrocytic Compartment**
Wei Song, Hillel Zukor, Shih-Hsiung Lin, Jacob Hascalovici, Adrienne Liberman, Ayda Tavitian, Jeannie Mui, Hojatollah Vali, Xin-Kang Tong, Sanjeev K. Bhardwaj, Lalit K. Srivastava, Edith Hamel, and Hyman M. Schipper
- 10887 **Normal Midbrain Dopaminergic Neuron Development and Function in miR-133b Mutant Mice**
Mary P. Heyer, Amar K. Pani, Richard J. Smeyne, Paul J. Kenny, and Guoping Feng
- 11067 **Enhanced NMDA Receptor-Dependent Thalamic Excitation and Network Oscillations in Stargazer Mice**
Carolyn J. Lacey, Astra Bryant, Julia Brill, and John R. Huguenard
- 11082 **Anti-NMDA Receptor Encephalitis Antibody Binding Is Dependent on Amino Acid Identity of a Small Region within the GluN1 Amino Terminal Domain**
Amy J. Gleichman, Lynn A. Spruce, Josep Dalmau, Steven H. Seeholzer, and David R. Lynch

11157 **Correction:** The article “Transglutaminase Inhibition Protects against Oxidative Stress-Induced Neuronal Death Downstream of Pathological ERK Activation” by Manuela Basso, Jill Berlin, Li Xia, Sama F. Sleiman, Brendan Ko, Renee Haskew-Layton, Eunhee Kim, Marc A. Antonyak, Richard A. Cerione, Siiri E. Iismaa, Dianna Willis, Sunghee Cho, and Rajiv R. Ratan appeared on pages 6561–6569 of the May 9, 2012 issue. A correction for that article appears on page 11157.

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.

Upregulation of the E3 ligase NEDD4-1 by Oxidative Stress Degrades IGF-1 Receptor Protein in Neurodegeneration

Young-Don Kwak,¹ Bin Wang,¹ Jing Jing Li,¹ Ruishan Wang,¹ Qiyue Deng,² Shiyong Diao,¹ Yaomin Chen,³ Raymond Xu,¹ Eliezer Masliah,⁴ Huaxi Xu,³ Jung-Joon Sung,⁵ and Francesca-Fang Liao¹

¹Department of Pharmacology, University of Tennessee Health Science Center, College of Medicine, Memphis, Tennessee 38163, ²Department of Neurobiology, Third Military Medical University, Chongqing 400038, China, ³Del E. Webb Center for Neuroscience, Aging, and Stem Cell Research, Sanford-Burnham Medical Research Institute, La Jolla, California 92037, ⁴Department of Neuroscience, University of California of San Diego, La Jolla, California 92039, and ⁵Department of Neurology, Seoul National University Hospital, Jongno-gu, Seoul 110-744, Korea

The importance of ubiquitin E3 ligases in neurodegeneration is being increasingly recognized. The crucial role of NEDD4-1 in neural development is well appreciated; however, its role in neurodegeneration remains unexplored. Herein, we report increased NEDD4-1 expression in the degenerated tissues of several major neurodegenerative diseases. Moreover, its expression is upregulated in cultured neurons in response to various neurotoxins, including zinc and hydrogen superoxide, via transcriptional activation likely mediated by the reactive oxygen species (ROS)-responsive FOXM1B. Reduced protein levels of the insulin-like growth factor receptor (IGF-1R β) were observed as a consequence of upregulated NEDD4-1 via the ubiquitin-proteasome system. Overexpression of a familial mutant form of superoxide dismutase 1 (SOD1) (G93A) in neuroblastoma cells resulted in a similar reduction of IGF-1R β protein. This inverse correlation between NEDD4-1 and IGF-1R β was also observed in the cortex and spinal cords of mutant (G93A) SOD1 transgenic mice at a presymptomatic age, which was similarly induced by *in vivo*-administered zinc in wild-type C57BL/6 mice. Furthermore, histochemistry reveals markedly increased NEDD4-1 immunoreactivity in the degenerating/degenerated motor neurons in the lumbar anterior horn of the spinal cord, suggesting a direct causative role for NEDD4-1 in neurodegeneration. Indeed, downregulation of NEDD4-1 by shRNA or overexpression of a catalytically inactive form rescued neurons from zinc-induced cell death. Similarly, neurons with a NEDD4-1 haplotype are more resistant to apoptosis, largely due to expression of higher levels of IGF-1R β . Together, our work identifies a novel molecular mechanism for ROS-upregulated NEDD4-1 and the subsequently reduced IGF-1R β signaling in neurodegeneration.

The Journal of Neuroscience, August 8, 2012 • 32(32):10971–10981

Increase in Sodium Conductance Decreases Firing Rate and Gain in Model Neurons

Tilman J. Kispersky,* Jonathan S. Caplan,* and Eve Marder

Biology Department and Volen Center, Brandeis University, Waltham, Massachusetts 02454-9110

We studied the effects of increased sodium conductance on firing rate and gain in two populations of conductance-based, single-compartment model neurons. The first population consisted of 1000 model neurons with differing values of seven voltage-dependent conductances. In many of these models, increasing the sodium conductance threefold unexpectedly reduced the firing rate and divisively scaled the gain at high input current. In the second population, consisting of 1000 simplified model neurons, we found that enhanced sodium conductance changed the frequency–current (FI) curve in two computationally distinct ways, depending on the firing rate. In these models, increased sodium conductance produced a subtractive shift in the FI curve at low firing rates because the additional sodium conductance allowed the neuron to respond more strongly to equivalent input current. In contrast, at high input current, the increase in sodium conductance resulted in a divisive change in the gain because the increased conductance produced a proportionally larger activation of the delayed rectifier potassium conductance. The control and sodium-enhanced FI curves intersect at a point that delimits two regions in which the same biophysical manipulation produces two fundamentally different changes to the model neuron's computational properties. This suggests a potentially difficult problem for homeostatic regulation of intrinsic excitability.

The Journal of Neuroscience, August 8, 2012 • 32(32):10995–11004

Activation of Aurora-A Is Essential for Neuronal Migration via Modulation of Microtubule Organization

Takako Takitoh,¹ Kanako Kumamoto,¹ Chen-Chi Wang,^{2,4} Makoto Sato,^{2,3,4} Shiori Toba,¹ Anthony Wynshaw-Boris,⁵ and Shinji Hirotsune¹

¹Department of Genetic Disease Research, Osaka City University Graduate School of Medicine, Osaka 545-8585, Japan, ²Division of Cell Biology and Neuroscience, Department of Morphological and Physiological Sciences, Faculty of Medical Sciences, University of Fukui, ³Child Development Research Center, Graduate School of Medical Sciences, University of Fukui, ⁴Research and Education Program for Life Science, University of Fukui, Fukui 910-1193, Japan, and ⁵Department of Pediatrics and Institute for Human Genetics, University of California, San Francisco, School of Medicine, San Francisco, California 94143

Neuronal migration is a critical feature to ensure proper location and wiring of neurons during cortical development. Postmitotic neurons migrate from the ventricular zone into the cortical plate to establish neuronal lamina in an “inside-out” gradient of maturation. Here, we report that the mitotic kinase Aurora-A is critical for the regulation of microtubule organization during neuronal migration via an Aurora-A–NDEL1 pathway in the mouse. Suppression of Aurora-A activity by inhibitors or siRNA resulted in severe impairment of neuronal migration of granular neurons. In addition, *in utero* injection of the Aurora-A kinase-dead mutant provoked defective migration

of cortical neurons. Furthermore, we demonstrated that suppression of Aurora-A impaired microtubule modulation in migrating neurons. Interestingly, suppression of CDK5 by an inhibitor or siRNA reduced Aurora-A activity and NDEL1 phosphorylation by Aurora-A, which led to defective neuronal migration. We found that CDK5RAP2 is a key molecule that mediates functional interaction and is essential for centrosomal targeting of Aurora-A. Our observations demonstrated novel and surprising cross talk between Aurora-A and CDK5 during neuronal migration.

The Journal of Neuroscience, August 8, 2012 • 32(32):11050–11066

Formation of Golgi-Derived Active Zone Precursor Vesicles

Christoph Maas,¹ Viviana I. Torres,¹ Wilko D. Altmann,² Sergio Leal-Ortiz,¹ Dhananjay Wagh,¹ Ryan T. Terry-Lorenzo,¹ Anna Fejtova,² Eckart D. Gundelfinger,² Noam E. Ziv,³ and Craig C. Garner¹

¹Department of Psychiatry and Behavioral Sciences, Nancy Pritzker Laboratory, Stanford University, Palo Alto, California 94304-5485, ²Department of Neurochemistry and Molecular Biology, Leibniz Institute for Neurobiology, Magdeburg D-39118, Germany, and ³Technion Faculty of Medicine and Network Biology Research Laboratories, Haifa 32000, Israel

Vesicular trafficking of presynaptic and postsynaptic components is emerging as a general cellular mechanism for the delivery of scaffold proteins, ion channels, and receptors to nascent and mature synapses. However, the molecular mechanisms leading to the selection of cargos and their differential transport to subneuronal compartments are not well understood, in part because of the mixing of cargos at the plasma membrane and/or within endosomal compartments. In the present study, we have explored the cellular mechanisms of active zone precursor vesicle assembly at the Golgi in dissociated hippocampal neurons of *Rattus norvegicus*. Our studies show that Piccolo, Bassoon, and ELKS2/CAST exit the *trans*-Golgi network on a common vesicle that requires Piccolo and Bassoon for its proper assembly. In contrast, Munc13 and synaptic vesicle proteins use distinct sets of Golgi-derived transport vesicles, while RIM1 α associates with vesicular membranes in a post-Golgi compartment. Furthermore, Piccolo and Bassoon are necessary for ELKS2/CAST to leave the Golgi in association with vesicles, and a core domain of Bassoon is sufficient to facilitate formation of these vesicles. While these findings support emerging principles regarding active zone differentiation, the cellular and molecular analyses reported here also indicate that the Piccolo-Bassoon transport vesicles leaving the Golgi may undergo further changes in protein composition before arriving at synaptic sites.

The Journal of Neuroscience, August 8, 2012 • 32(32):11095–11108

Methylene Blue Modulates Huntingtin Aggregation Intermediates and Is Protective in Huntington's Disease Models

Emily Mitchell Sontag,^{1,2,6} Gregor P. Lotz,^{7,8} Namita Agrawal,³ Andrew Tran,⁶ Rebecca Aron,^{7,8} Guocheng Yang,^{7,8} Mihaela Necula,⁴ Alice Lau,² Steven Finkbeiner,^{7,8,10,11,12,13} Charles Glabe,^{4,6} J. Lawrence Marsh,³ Paul J. Muchowski,^{7,8,9,10} and Leslie M. Thompson^{1,2,5,6}

Departments of ¹Biological Chemistry, ²Psychiatry and Human Behavior, ³Developmental and Cell Biology, ⁴Molecular Biology and Biochemistry, and ⁵Neurobiology and Behavior and ⁶Institute for Memory Impairments and Neurological Disorders, University of California, Irvine, California 92697, ⁷Gladstone Institute of Neurological Disease, San Francisco, California 94158, Departments of ⁸Neurology and ⁹Biochemistry and Biophysics and ¹⁰Taube-Koret Center for Huntington's Disease Research, University of California, San Francisco, California 94158, ¹¹Medical Scientist Training Program and ¹²Neuroscience Program, University of California, San Francisco, California 94141, and ¹³Department of Physiology, University of California, San Francisco, California 94143

Huntington's disease (HD) is a devastating neurodegenerative disorder with no disease-modifying treatments available. The disease is caused by expansion of a CAG trinucleotide repeat and manifests with progressive motor abnormalities, psychiatric symptoms, and cognitive decline. Expression of an expanded polyglutamine repeat within the Huntingtin (Htt) protein impacts numerous cellular processes, including protein folding and clearance. A hallmark of the disease is the progressive formation of inclusions that represent the culmination of a complex aggregation process. Methylene blue (MB) has been shown to modulate aggregation of amyloidogenic disease proteins. We investigated whether MB could impact mutant Htt-mediated aggregation and neurotoxicity. MB inhibited recombinant protein aggregation *in vitro*, even when added to preformed oligomers and fibrils. MB also decreased oligomer number and size and decreased accumulation of insoluble mutant Htt in cells. In functional assays, MB increased survival of primary cortical neurons transduced with mutant Htt, reduced neurodegeneration and aggregation in a *Drosophila melanogaster* model of HD, and reduced disease phenotypes in R6/2HD modeled mice. Furthermore, MB treatment also promoted an increase in levels of BDNF RNA and protein *in vivo*. Thus, MB, which is well tolerated and used in humans, has therapeutic potential for HD.

The Journal of Neuroscience, August 8, 2012 • 32(32):11109–11119

Tonic Inhibition Enhances Fidelity of Sensory Information Transmission in the Cerebellar Cortex

Ian Duguid, Tiago Branco,* Michael London,* Paul Chadderton, and Michael Häusser

Wolfson Institute for Biomedical Research and Department of Neuroscience, Physiology and Pharmacology, University College London, London WC1E 6BT, United Kingdom

Tonic inhibition is a key regulator of neuronal excitability and network function in the brain, but its role in sensory information processing remains poorly understood. The cerebellum is a favorable model system for addressing this question as granule cells, which form the input layer of the cerebellar cortex, permit high-resolution patch-clamp recordings *in vivo*, and are the only neurons in the cerebellar cortex that express the $\alpha 6\delta$ -containing GABA_A receptors mediating tonic inhibition. We investigated how tonic

inhibition regulates sensory information transmission in the rat cerebellum by using a combination of intracellular recordings from granule cells and molecular layer interneurons *in vivo*, selective pharmacology, and *in vitro* dynamic clamp experiments. We show that blocking tonic inhibition significantly increases the spontaneous firing rate of granule cells while only moderately increasing sensory-evoked spike output. In contrast, enhancing tonic inhibition reduces the spike probability in response to sensory stimulation with minimal effect on the spontaneous spike rate. Both manipulations result in a reduction in the signal-to-noise ratio of sensory transmission in granule cells and of parallel fiber synaptic input to downstream molecular layer interneurons. These results suggest that under basal conditions the level of tonic inhibition *in vivo* enhances the fidelity of sensory information transmission through the input layer of the cerebellar cortex.

The Journal of Neuroscience, August 8, 2012 • 32(32):11132–11143

Rabconnectin3 α Promotes Stable Activity of the H⁺ Pump on Synaptic Vesicles in Hair Cells

Zev Einhorn, Josef G. Trapani, Qianyong Liu, and Teresa Nicolson

Oregon Hearing Research Center and Vollum Institute, Howard Hughes Medical Institute, Oregon Health and Science University, Portland, Oregon 97239

Acidification of synaptic vesicles relies on the vacuolar-type ATPase (V-ATPase) and provides the electrochemical driving force for neurotransmitter exchange. The regulatory mechanisms that ensure assembly of the V-ATPase holoenzyme on synaptic vesicles are unknown. Rabconnectin3 α (Rbc3 α) is a potential candidate for regulation of V-ATPase activity because of its association with synaptic vesicles and its requirement for acidification of intracellular compartments. Here, we provide the first evidence for a role of Rbc3 α in synaptic vesicle acidification and neurotransmission. In this study, we characterized mutant alleles of *rbc3 α* isolated from a large-scale screen for zebrafish with auditory/vestibular defects. We show that Rbc3 α is localized to basal regions of hair cells in which synaptic vesicles are present. To determine whether Rbc3 α regulates V-ATPase activity, we examined the acidification of synaptic vesicles and localization of the V-ATPase in hair cells. In contrast to wild-type hair cells, we observed that synaptic vesicles had elevated pH, and a cytosolic subunit of the V-ATPase was no longer enriched in synaptic regions of mutant hair cells. As a consequence of defective acidification of synaptic vesicles, afferent neurons in *rbc3 α* mutants had reduced firing rates and reduced accuracy of phase-locked action potentials in response to mechanical stimulation of hair cells. Collectively, our data suggest that Rbc3 α modulates synaptic transmission in hair cells by promoting V-ATPase activity in synaptic vesicles.

The Journal of Neuroscience, August 8, 2012 • 32(32):11144–11156

DEVELOPMENT/PLASTICITY/REPAIR

Fezf2 Regulates Multilineage Neuronal Differentiation through Activating Basic Helix-Loop-Helix and Homeodomain Genes in the Zebrafish Ventral Forebrain

Nan Yang,^{1,2} Zhiqiang Dong,² and Su Guo²

¹Institute of Genetics, School of Life Sciences, Fudan University, Shanghai 200433, People's Republic of China, and ²Department of Bioengineering and Therapeutic Sciences, Programs in Human Genetics and Biological Sciences, University of California, San Francisco, San Francisco, California 94143-2811

Transcription factors of the *achaete-scute* and *atonal* bHLH proneural gene family play important roles in neuronal differentiation. They are also involved in neuronal subtype specification through collaboration with homeodomain (HD) transcription factors. However, concerted regulation of these genes and in turn progenitor fate toward distinct lineages within the developing vertebrate brain is not well understood. Fezf2 is an evolutionarily conserved zinc finger protein important for monoaminergic neuronal development in zebrafish. Here, we show that Fezf2 is also critical for GABAergic neuronal fate and investigate how a single transcription factor regulates the identity of multiple neuronal lineages in the developing ventral forebrain. First, our genetic analyses reveal the requirement of the *achaete-scute*-like genes *ascl1a* and *1b* in serotonergic and GABAergic neuron development, but they are dispensable for the specification of dopaminergic neurons, which is dependent on the *atonal*-like gene *neurog1*. Second, the expression of *fezf2*, *ascl1a/1b*, and *neurog1* demarcates distinct progenitor subpopulations, where *fezf2* is required for activating but not maintaining the expression of bHLH genes. Third, Fezf2 is required to activate HD genes *otpb* and *dlx2*, which are involved in dopaminergic and GABAergic neuronal development, respectively. Finally, we uncover that Fezf2 is sufficient to increase dopaminergic neuronal numbers but not serotonergic or GABAergic lineages. Together, these findings reveal new mechanisms by which multilineage differentiation is coordinately regulated by a single transcription factor in the vertebrate ventral forebrain.

The Journal of Neuroscience, August 8, 2012 • 32(32):10940–10948

Early Development of Electrical Excitability in the Mouse Enteric Nervous System

Marlene M. Hao,¹ Alan E. Lomax,⁴ Sonja J. McKeown,¹ Christopher A. Reid,³ Heather M. Young,¹ and Joel C. Bornstein²

¹Departments of Anatomy and Neuroscience and ²Physiology, University of Melbourne, and ³Florey Neuroscience Institutes, Melbourne, Victoria 3010, Australia, and ⁴Department of Biomedical and Molecular Sciences, Queen's University, Kingston, Ontario K7L 3N6, Canada

Neural activity is integral to the development of the enteric nervous system (ENS). A subpopulation of neural crest-derived cells expresses pan-neuronal markers at early stages of ENS development (at E10.5 in the mouse). However, the electrical activity of these cells has not been previously characterized, and it is not known whether all cells expressing neuronal markers are capable of firing action potentials (APs). In this study, we examined the activity of “neuron”-like cells (expressing pan-neuronal markers or with neuronal morphology) in the gut of E11.5 and E12.5 mice using whole-cell patch-clamp electrophysiology and compared them to the activity of neonatal and adult enteric neurons. Around 30–40% of neuron-like cells at E11.5 and E12.5 fired APs, some of which were very similar to those of adult enteric neurons. All APs were sensitive to tetrodotoxin (TTX), indicating that they were driven by voltage-gated Na⁺ currents. Expression of mRNA encoding several voltage-gated Na⁺ channels by the E11.5 gut was detected using RT-PCR. The density of voltage-gated Na⁺ currents increased from E11.5 to neonates. Immature active responses, mediated in part by TTX- and lidocaine-insensitive channels, were observed in most cells at E11.5 and E12.5, but not in P0/P1 or adult neurons. However, some cells expressing neuronal markers at E11.5

or E12.5 did not exhibit an active response to depolarization. Spontaneous depolarizations resembling excitatory postsynaptic potentials were observed at E12.5. The ENS is one of the earliest parts of the developing nervous system to exhibit mature forms of electrical activity.
The Journal of Neuroscience, August 8, 2012 • 32(32):10949–10960

Voltage-Sensitive Dye Imaging Reveals Dynamic Spatiotemporal Properties of Cortical Activity after Spontaneous Muscle Twitches in the Newborn Rat

David A. McVea,^{1,2} Majid H. Mohajerani,^{1,2} and Timothy H. Murphy^{1,2}

¹Department of Psychiatry and ²Brain Research Center, University of British Columbia at Vancouver, Vancouver, British Columbia, Canada V6T 1Z3

Spontaneous activity in the developing brain contributes to its maturation, but how this activity is coordinated between distinct cortical regions and whether it might reflect developing sensory circuits is not well understood. Here, we address this question by imaging the spread and synchronization of cortical activity using voltage-sensitive dyes (VSDs) in the developing rat *in vivo*. In postnatal day 4–6 rats ($n = 10$), we collected spontaneous changes in VSD signal that reflect underlying membrane potential changes over a large craniotomy (50 mm²) that encompassed both the sensory and motor cortices of both hemispheres. Bursts of depolarization that occurred approximately once every 12 s were preceded by spontaneous twitches of the hindlimbs and/or tail. The close association with peripheral movements suggests that these bursts may represent a slow component of spindle bursts, a prominent form of activity in the developing somatosensory cortex. Twitch-associated cortical activity was synchronized between subregions of somatosensory cortex, which reflected the synchronized twitching of the limbs and tail. This activity also spread asymmetrically, toward the midline of the brain. We found that the spatial and temporal structure of such spontaneous cortical bursts closely matched that of sensory-evoked activity elicited via direct stimulation of the periphery. These data suggest that spontaneous cortical activity provides a recurring template of functional cortical circuits within the developing cortex and could contribute to the maturation of integrative connections between sensory and motor cortices.

The Journal of Neuroscience, August 8, 2012 • 32(32):10982–10994

BEHAVIORAL/SYSTEMS/COGNITIVE

Supraspinal Peroxynitrite Modulates Pain Signaling by Suppressing the Endogenous Opioid Pathway

Joshua W. Little,¹ Zhoumou Chen,¹ Timothy Doyle,¹ Frank Porreca,² Mahsa Ghaffari,³ Leesa Bryant,¹ William L. Neumann,³ and Daniela Salvemini¹

¹Saint Louis University School of Medicine, Saint Louis, Missouri 63104, ²University of Arizona College of Medicine, Tucson, Arizona 85724, and ³Southern Illinois University Edwardsville School of Pharmacy, Edwardsville, Illinois 62026

Peroxynitrite (PN, ONOO[−]) is a potent oxidant and nitrating agent that contributes to pain through peripheral and spinal mechanisms, but its supraspinal role is unknown. We present evidence here that PN in the rostral ventromedial medulla (RVM) is essential for descending nociceptive modulation in rats during inflammatory and neuropathic pain through PN-mediated suppression of opioid signaling. Carrageenan-induced thermal hyperalgesia was associated with increased 3-nitrotyrosine (NT), a PN biomarker, in the RVM. Furthermore, intra-RVM microinjections of the PN decomposition catalyst Fe(III)-5,10,15,20-tetrakis(*N*-methyl-pyridinium-4-yl)porphyrin (FeTMPyP⁵⁺) dose-dependently reversed this thermal hyperalgesia. These effects of FeTMPyP⁵⁺ were abrogated by intra-RVM naloxone, implicating potential interplay between PN and opioids. In support, we identified NT colocalization with the endogenous opioid enkephalin (ENK) in the RVM during thermal hyperalgesia, suggesting potential *in situ* interactions. To address the functional significance of such interactions, we exposed methionine-enkephalin (MENK) to PN and identified the major metabolite, 3-nitrotyrosine-methionine-sulfoxide (NSO)-MENK, using liquid chromatography-mass spectrometry. Next, we isolated, purified, and tested NSO-MENK for opioid receptor binding affinity and analgesic effects. Compared to MENK, this NSO-MENK metabolite lacked appreciable binding affinity for δ , μ , and κ opioid receptors. Intrathecal injection of NSO-MENK in rats did not evoke antinociception, suggesting that PN-mediated chemical modifications of ENK suppress opioid signaling. When extended to chronic pain, intra-RVM FeTMPyP⁵⁺ produced naloxone-sensitive reversal of mechanical allodynia in rats following chronic constriction injury of the sciatic nerve. Collectively, our data reveal the central role of PN in RVM descending facilitation during inflammatory and neuropathic pain potentially through anti-opioid activity.

The Journal of Neuroscience, August 8, 2012 • 32(32):10797–10808

Sodium Channel Na_v1.7 Is Essential for Lowering Heat Pain Threshold after Burn Injury

Shannon D. Shields,^{1,2} Xiaoyang Cheng,^{1,2} Nurcan Üçeyler,³ Claudia Sommer,³ Sulayman D. Dib-Hajj,^{1,2} and Stephen G. Waxman^{1,2}

¹Department of Neurology and Center for Neuroscience and Regeneration Research, Yale University School of Medicine, New Haven, Connecticut 06520,

²Rehabilitation Research Center, Veterans Affairs Connecticut Healthcare System, West Haven, Connecticut 06516, and ³Department of Neurology, University of Würzburg, 97080 Würzburg, Germany

Marked hypersensitivity to heat and mechanical (pressure) stimuli develop after a burn injury, but the neural mechanisms underlying these effects are poorly understood. In this study, we establish a new mouse model of focal second-degree burn injury to investigate the molecular and cellular basis for burn injury-induced pain. This model features robust injury-induced behavioral effects and tissue-specific altered cytokine profile, but absence of glial activation in spinal dorsal horn. Three voltage-gated sodium channels, Na_v1.7, Na_v1.8, and Na_v1.9, are preferentially expressed in peripheral somatosensory neurons of the dorsal root ganglia (DRGs) and have been implicated in injury-induced neuronal hyperexcitability. Using knock-out mice, we provide evidence that Na_v1.7 selectively contributes to burn-induced hypersensitivity to heat, but

not mechanical, stimuli. After burn model injury, wild-type mice display increased sensitivity to heat stimuli, and a normally non-noxious warm stimulus induces activity-dependent Fos expression in spinal dorsal horn neurons. Strikingly, both effects are absent in Na_v1.7 conditional knock-out (cKO) mice. Furthermore, burn injury increases density and shifts activation of tetrodotoxin-sensitive currents in a hyperpolarized direction, both pro-excitatory properties, in DRG neurons from wild-type but not Na_v1.7 cKO mice. We propose that, in sensory neurons damaged by burn injury to the hindpaw, Na_v1.7 currents contribute to the hyperexcitability of sensory neurons, their communication with postsynaptic spinal pain pathways, and behavioral thresholds to heat stimuli. Our results offer insights into the molecular and cellular mechanisms of modality-specific pain signaling, and suggest Na_v1.7-blocking drugs may be effective in burn patients.

The Journal of Neuroscience, August 8, 2012 • 32(32):10819–10832

Organization of Cerebral Projections to Identified Cerebellar Zones in the Posterior Cerebellum of the Rat

Lucia Suzuki,¹ Patrice Coulon,² Erika H. Sabel-Goedknecht,¹ and Tom J. H. Ruigrok¹

¹Department of Neuroscience, Erasmus University Medical Center Rotterdam, 3000CA Rotterdam, The Netherlands, and ²Institut des Neurosciences de la Timone, CNRS and Aix-Marseille Université, 13385 Marseille Cedex 05, France

The cerebrocerebellar connection makes use of two of the largest fiber tracts in the mammalian brain, i.e., the cerebral and medial cerebellar peduncles. Neuroanatomical approaches aimed to elucidate the organization of this important connection have been hindered by its multisynaptic nature, the complex organization of its components, and the dependency of conventional tracers on precisely placed injections. To overcome these problems, we used rabies virus (RV) as a retrograde transneuronal tracer. RV was injected simultaneously with cholera toxin β subunit (CTb) into selected areas of the cerebellar cortex of 18 male Wistar rats. A survival time of 48–50 h resulted in first- and second-order labeling of RV in combination with first-order labeling of CTb. The distribution of CTb-labeled neurons in the inferior olive established the zonal identity of the injection site. In this way, it was possible to examine the cortical distribution of neurons from which disynaptic cerebrocerebellar projections to specific cerebellar loci originate.

The results show that this distribution covaries with the identity of the injected cerebellar lobule. More subtle changes were present when different zones of the same lobule were injected. The C1 zone of lobule VIII receives a more prominent projection from the somatosensory cortex compared with the C2/D zones. The laterally positioned D zones receive information from more rostral regions of the cerebral cortex. The vermis of lobule VII receives a prominent input from the retrosplenial and orbitofrontal cortices. Different injection sites also result in differences in laterality of the connections.

The Journal of Neuroscience, August 8, 2012 • 32(32):10854–10869

How Preparation Changes the Need for Top–Down Control of the Basal Ganglia When Inhibiting Premature Actions

Sara Jahfari,^{1,2} Frederick Verbruggen,³ Michael J. Frank,⁴ Lourens J. Waldorp,¹ Lorenza Colzato,⁵ K. Richard Ridderinkhof,^{1,2} and Birte U. Forstmann^{1,2,6}

¹Department of Psychology and ²Cognitive Science Center Amsterdam, University of Amsterdam, 1018 XA Amsterdam, The Netherlands, ³School of Psychology, University of Exeter, Exeter EX4 4QG, United Kingdom, ⁴Cognitive, Linguistic and Psychological Sciences, Brown University, Providence, Rhode Island 02912-1821, ⁵Institute for Psychological Research, University of Leiden, 2333 AK Leiden, The Netherlands, and ⁶Max Planck Institute for Human Cognitive and Brain Sciences, 04103 Leipzig, Germany

Goal-oriented signals from the prefrontal cortex gate the selection of appropriate actions in the basal ganglia. Key nodes within this fronto-basal ganglia action regulation network are increasingly engaged when one anticipates the need to inhibit and override planned actions. Here, we ask how the advance preparation of action plans modulates the need for fronto-subcortical control when a planned action needs to be withdrawn. Functional magnetic resonance imaging data were collected while human participants performed a stop task with cues indicating the likelihood of a stop signal being sounded. Mathematical modeling of go trial responses suggested that participants attained a more cautious response strategy when the probability of a stop signal increased. Effective connectivity analysis indicated that, even in the absence of stop signals, the proactive engagement of the full control network is tailored to the likelihood of stop trial occurrence. Importantly, during actual stop trials, the strength of fronto-subcortical projections was stronger when stopping had to be engaged reactively compared with when it was proactively prepared in advance. These findings suggest that fronto-basal ganglia control is strongest in an unpredictable environment, where the prefrontal cortex plays an important role in the optimization of reactive control. Importantly, these results further indicate that the advance preparation of action plans reduces the need for reactive fronto-basal ganglia communication to gate voluntary actions.

The Journal of Neuroscience, August 8, 2012 • 32(32):10870–10878

An Essential Role for Histone Deacetylase 4 in Synaptic Plasticity and Memory Formation

Mi-Sung Kim,^{1*} M. Waseem Akhtar,^{2*} Megumi Adachi,² Melissa Mahgoub,² Rhonda Bassel-Duby,¹ Ege T. Kavalali,³ Eric N. Olson,¹ and Lisa M. Monteggia²

Departments of ¹Molecular Biology, ²Psychiatry, and ³Neuroscience, University of Texas Southwestern Medical Center, Dallas, Texas 75390

Histone deacetylases (HDACs), a family of enzymes involved in epigenetic regulation, have been implicated in the control of synaptic plasticity, as well as learning and memory. Previous work has demonstrated administration of pharmacological HDAC inhibitors, primarily those targeted to class I HDACs, enhance learning and memory as well as long-term potentiation. However, a detailed understanding of the role of class II HDACs in these processes remains elusive. Here, we show that selective loss of *Hdac4* in brain results in impairments in hippocampal-dependent learning and memory and long-term synaptic plasticity. In contrast, loss of *Hdac5* does not impact

learning and memory demonstrating unique roles in brain for individual class II HDACs. These findings suggest that HDAC4 is a crucial positive regulator of learning and memory, both behaviorally and at the cellular level, and that inhibition of *Hdac4* activity may have unexpected detrimental effects to these processes.
The Journal of Neuroscience, August 8, 2012 • 32(32):10879–10886

Afferents from Vocal Motor and Respiratory Effectors Are Recruited during Vocal Production in Juvenile Songbirds

Sarah W. Bottjer and Michelle To

Section of Neurobiology, University of Southern California, Los Angeles, California 90089-2520

Learned behaviors require coordination of diverse sensory inputs with motivational and motor systems. Although mechanisms underlying vocal learning in songbirds have focused primarily on auditory inputs, it is likely that sensory inputs from vocal effectors also provide essential feedback. We investigated the role of somatosensory and respiratory inputs from vocal effectors of juvenile zebra finches (*Taeniopygia guttata*) during the stage of sensorimotor integration when they are learning to imitate a previously memorized tutor song. We report that song production induced expression of the immediate early gene product Fos in trigeminal regions that receive hypoglossal afferents from the tongue and syrinx (the main vocal organ). Furthermore, unilateral lesion of hypoglossal afferents greatly diminished singing-induced Fos expression on the side ipsilateral to the lesion, but not on the intact control side. In addition, unilateral lesion of the vagus reduced Fos expression in the ipsilateral nucleus of the solitary tract in singing birds. Lesion of the hypoglossal nerve to the syrinx greatly disrupted vocal behavior, whereas lesion of the hypoglossal nerve to the tongue exerted no obvious disruption and lesions of the vagus caused some alterations to song behavior. These results provide the first functional evidence that somatosensory and respiratory feedback from peripheral effectors is activated during vocal production and conveyed to brainstem regions. Such feedback is likely to play an important role in vocal learning during sensorimotor integration in juvenile birds and in maintaining stereotyped vocal behavior in adults.

The Journal of Neuroscience, August 8, 2012 • 32(32):10895–10906

Persistent Spatial Information in the Frontal Eye Field during Object-Based Short-Term Memory

Kelsey L. Clark,¹ Behrad Noudoost,¹ and Tirin Moore^{1,2}

¹Department of Neurobiology and ²Howard Hughes Medical Institute, Stanford University School of Medicine, Stanford, California 94305

Spatial attention is known to gate entry into visual short-term memory, and some evidence suggests that spatial signals may also play a role in binding features or protecting object representations during memory maintenance. To examine the persistence of spatial signals during object short-term memory, the activity of neurons in the frontal eye field (FEF) of macaque monkeys was recorded during an object-based delayed match-to-sample task. In this task, monkeys were trained to remember an object image over a brief delay, regardless of the locations of the sample or target presentation. FEF neurons exhibited visual, delay, and target period activity, including selectivity for sample location and target location. Delay period activity represented the sample location throughout the delay, despite the irrelevance of spatial information for successful task completion. Furthermore, neurons continued to encode sample position in a variant of the task in which the matching stimulus never appeared in their response field, confirming that FEF maintains sample location independent of subsequent behavioral relevance. FEF neurons also exhibited target-position-dependent anticipatory activity immediately before target onset, suggesting that monkeys predicted target position within blocks. These results show that FEF neurons maintain spatial information during short-term memory, even when that information is irrelevant for task performance.

The Journal of Neuroscience, August 8, 2012 • 32(32):10907–10914

Hippocampal-Prefrontal Cortical Circuit Mediates Inhibitory Response Control in the Rat

Yogita Chudasama, Victoria M. Doobay, and Yuchen Liu

Department of Psychology, McGill University, Montreal, Quebec H3A 1B1, Canada

We investigated the interdependent function of the ventral hippocampus (vHC) and ventral prefrontal cortex (vPFC) in visuospatial attention and inhibitory control using a disconnection lesion approach. Rats were trained, and several aspects of their cognitive performance tested on the 5-choice reaction time task. The animals were prepared with unilateral lesions of both the vPFC and vHC, either in the same hemisphere (“ipsilateral”) or in opposite hemispheres (“disconnection”). The disconnection lesion led to both impulsive and compulsive behavior. This deficit is reminiscent of the effects of either bilateral vPFC or bilateral vHC lesions on the same task and is thought to reflect the bihemispheric disruption of the hippocampal-prefrontal circuit. With ipsilateral lesions, behavioral deficits were transient or absent altogether, suggesting that the intact hemisphere was able to exert near normal levels of behavioral control. These behavioral effects were observed in the absence of any changes to visual attention, speed of response, or general motivation. This study provides evidence that optimal inhibitory control of behavior draws upon the functional interaction between the vHC and vPFC.

The Journal of Neuroscience, August 8, 2012 • 32(32):10915–10924

A Gradient in Endogenous Rhythmicity and Oscillatory Drive Matches Recruitment Order in an Axial Motor Pool

Evdokia Menelaou and David L. McLean

Department of Neurobiology, Northwestern University, Evanston, Illinois 60208

The rhythmic firing behavior of spinal motoneurons is a function of their electrical properties and synaptic inputs. However, the relative contribution of endogenous versus network-based rhythmogenic mechanisms to locomotion is unclear. To address this issue, we have recorded from identified motoneurons and compared their current-evoked firing patterns to network-driven ones in the larval zebrafish (*Danio rerio*). Zebrafish axial motoneurons are recruited topographically from the bottom of the spinal cord up. Here, we have explored differences in the morphology of axial motoneurons, their electrical properties, and their synaptic drive, to reveal how they match the topographic pattern of recruitment. More ventrally located “secondary” motoneurons generate bursts of action potentials in response to constant current steps, demonstrating a strong inherent rhythmogenesis. The membrane potential oscillations underlying bursting behavior occur in the normal frequency range of swimming. In contrast, more dorsal secondaries chatter in response to current, while the most dorsally distributed “primary” motoneurons all fire tonically. We find that systematic variations in excitability and endogenous rhythmicity are inversely related to the level of oscillatory synaptic drive within the entire axial motor pool. Specifically, bursting cells exhibit the least amount of drive, while tonic cells exhibit the most. Our data suggest that increases in swimming frequency are accomplished by the recruitment of axial motoneurons that progressively rely on instructive synaptic drive to shape their oscillatory activity appropriately. Thus, within the zebrafish spinal cord, there are differences in the relative contribution of endogenous versus network-based rhythms to locomotion and these vary predictably according to order of recruitment.

The Journal of Neuroscience, August 8, 2012 • 32(32):10925–10939

Effect of Locomotor Training in Completely Spinalized Cats Previously Submitted to a Spinal Hemisection

Marina Martinez,^{1,2} Hugo Delivet-Mongrain,¹ Hugues Leblond,¹ and Serge Rossignol^{1,2}

¹Groupe de Recherche sur le Système Nerveux Central (Fonds de la Recherche en Santé du Québec), Université de Montréal, Département de Physiologie and ²SensoriMotor Rehabilitation Research Team of the Canadian Institute for Health Research, Montreal, Québec H3T 1J4, Canada

After a spinal hemisection in cats, locomotor plasticity occurring at the spinal level can be revealed by performing, several weeks later, a complete spinalization below the first hemisection. Using this paradigm, we recently demonstrated that the hemisection induces durable changes in the symmetry of locomotor kinematics that persist after spinalization. Can this asymmetry be changed again in the spinal state by interventions such as treadmill locomotor training started within a few days after the spinalization? We performed, in 9 adult cats, a spinal hemisection at thoracic level 10 and then a complete spinalization at T13, 3 weeks later. Cats were not treadmill trained during the hemispinal period. After spinalization, 5 of 9 cats were not trained and served as control while 4 of 9 cats were trained on the treadmill for 20 min, 5 d a week for 3 weeks. Using detailed kinematic analyses, we showed that, without training, the asymmetrical state of locomotion induced by the hemisection was retained durably after the subsequent spinalization. By contrast, training cats after spinalization induced a reversal of the left/right asymmetries, suggesting that new plastic changes occurred within the spinal cord through locomotor training. Moreover, training was shown to improve the kinematic parameters and the performance of the hindlimb on the previously hemisected side. These results indicate that spinal locomotor circuits, previously modified by past experience such as required for adaptation to the hemisection, can remarkably respond to subsequent locomotor training and improve bilateral locomotor kinematics, clearly showing the benefits of locomotor training in the spinal state.

The Journal of Neuroscience, August 8, 2012 • 32(32):10961–10970

What and Where Information in the Caudate Tail Guides Saccades to Visual Objects

Shinya Yamamoto,^{1,2} Ilya E. Monosov,¹ Masaharu Yasuda,¹ and Okihide Hikosaka¹

¹Laboratory of Sensorimotor Research, National Eye Institute, National Institutes of Health, Bethesda, Maryland 20892, and ²System Neuroscience, Human Technology Research Institute, National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba 305-8568, Japan

We understand the world by making saccadic eye movements to various objects. However, it is unclear how a saccade can be aimed at a particular object, because two kinds of visual information, what the object is and where it is, are processed separately in the dorsal and ventral visual cortical pathways. Here, we provide evidence suggesting that a basal ganglia circuit through the tail of the monkey caudate nucleus (CDt) guides such object-directed saccades. First, many CDt neurons responded to visual objects depending on where and what the objects were. Second, electrical stimulation in the CDt induced saccades whose directions matched the preferred directions of neurons at the stimulation site. Third, many CDt neurons increased their activity before saccades directed to the preferred objects and directions of the neurons in a free-viewing condition. Our results suggest that CDt neurons receive both “what” and “where” information and guide saccades to visual objects.

The Journal of Neuroscience, August 8, 2012 • 32(32):11005–11016

Neuronal Activity during a Cued Strategy Task: Comparison of Dorsolateral, Orbital, and Polar Prefrontal Cortex

Satoshi Tsujimoto,¹ Aldo Genovesio,² and Steven P. Wise³

¹Developmental Cognitive Neuroscience Laboratory, Graduate School of Human Development and Environment, Kobe University, Kobe 657-8501, Japan,

²Department of Physiology and Pharmacology, Sapienza, University of Rome, 00185 Rome, Italy, ³Olschefske Institute for Neurobiology of Knowledge, Potomac, Maryland 20854

We compared neuronal activity in the dorsolateral (PFDl), orbital (PFO), and polar (PFp) prefrontal cortex as monkeys performed three tasks. In two tasks, a cue instructed one of two strategies: stay with the previous response or shift to the alternative. Visual stimuli served as cues in one of these tasks; in the other, fluid rewards did so. In the third task, visuospatial cues instructed each response. A delay period followed each cue. As reported previously, PFDl encoded strategies (stay or shift) and responses (left or right) during the cue and delay periods, while PFO encoded strategies and PFp encoded neither strategies nor responses; during the feedback period, all three areas encoded responses, but not strategies. Four novel findings emerged from the present analysis. (1) The strategy encoded by PFDl and PFO cells during the cue and delay periods was modality specific. (2) The response encoded by PFDl cells was task and modality specific during the cue period, but during the delay and feedback periods it became task and modality general. (3) Although some PFDl and PFO cells responded to or anticipated rewards, we could rule out reward effects for most strategy- and response-related activity. (4) Immediately before feedback, only PFp signaled responses that were correct according to the cued strategy; after feedback, only PFO signaled the response that had been made, whether correct or incorrect. These signals support a role in generating responses by PFDl, assigning outcomes to choices by PFO, and assigning outcomes to cognitive processes by PFp.

The Journal of Neuroscience, August 8, 2012 • 32(32):11017–11031

Role of Dopamine Tone in the Pursuit of Brain Stimulation Reward

Giovanni Hernandez,^{1,2} Ivan Trujillo-Pisanty,¹ Marie-Pierre Cossette,¹ Kent Conover,¹ and Peter Shizgal¹

¹Centre for Research in Behavioural Neurobiology/FRQS Groupe de recherche en Neurobiologie Comportementale, Concordia University, Montréal, Québec, Canada, H4B 1R6, and ²Department of Anatomy and Neurobiology, University of Maryland School of Medicine, Baltimore, Maryland 21201

Dopaminergic neurons contribute to intracranial self-stimulation (ICSS) and other reward-seeking behaviors, but it is not yet known where dopaminergic neurons intervene in the neural circuitry underlying reward pursuit or which psychological processes are involved. In rats working for electrical stimulation of the medial forebrain bundle, we assessed the effect of GBR-12909 (1-[2-[bis(4-fluorophenyl)-methoxy]ethyl]-4-[3-phenylpropyl]piperazine), a specific blocker of the dopamine transporter. Operant performance was measured as a function of the strength and cost of electrical stimulation. GBR-12909 increased the opportunity cost most subjects were willing to pay for a reward of a given intensity. However, this effect was smaller than that produced by a regimen of cocaine administration that drove similar increases in nucleus accumbens (NAc) dopamine levels in unstimulated rats. Delivery of rewarding stimulation to drug-treated rats caused an additional increase in dopamine concentration in the NAc shell in cocaine-treated, but not GBR-12909-treated, rats. These behavioral and neurochemical differences may reflect blockade of the norepinephrine transporter by cocaine but not by GBR-12909. Whereas the effect of psychomotor stimulants on ICSS has long been attributed to dopaminergic action at early stages of the reward pathway, the results reported here imply that increased dopamine tone boosts reward pursuit by acting at or beyond the output of the circuitry that temporally and spatially summates the output of the directly stimulated neurons underlying ICSS. The observed enhancement of reward seeking could be attributable to a decrease in the value of competing behaviors, a decrease in subjective effort costs, or an increase in reward-system gain.

The Journal of Neuroscience, August 8, 2012 • 32(32):11032–11041

Stress Modulates the Engagement of Multiple Memory Systems in Classification Learning

Lars Schwabe and Oliver T. Wolf

Department of Cognitive Psychology, Ruhr-University Bochum, 44780 Bochum, Germany

Learning and memory are supported by anatomically and functionally distinct systems. Recent research suggests that stress may alter the contributions of multiple memory systems to learning, yet the underlying mechanism in the human brain remains completely unknown. Using event-related functional magnetic resonance imaging, we asked in the present experiment whether stress may modulate the engagement of hippocampus-based “declarative” and striatum-based “procedural” memory systems during classification learning in humans and what brain mechanisms are involved in this effect. We found that stress reduced declarative knowledge about the learning task and changed the used learning strategy from a single-cue-based declarative strategy to a multicue-based procedural strategy, whereas learning performance per se remained unaffected by stress. Neuroimaging revealed that hippocampal activity correlated positively with task performance in the control condition, whereas striatal activity correlated with performance in the stress condition. After stress, hippocampal activity was reduced and even negatively correlated with learning performance. These findings show for the first time that stress alters the engagement of multiple memory systems in the human brain. Stress impaired the hippocampus-dependent system and allowed the striatum to control behavior. The shift toward “procedural” learning after stress appears to rescue task performance, whereas attempts to engage the “declarative” system disrupt performance.

The Journal of Neuroscience, August 8, 2012 • 32(32):11042–11049

Long-Range Parallel Processing and Local Recurrent Activity in the Visual Cortex of the Mouse

Pierre-Olivier Polack and Diego Contreras

Department of Neuroscience, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19106

The transfer of visual information from the primary visual cortex (V1) to higher order visual cortices is an essential step in visual processing. However, the dynamics of activation of visual cortices is poorly understood. In mice, several extrastriate areas surrounding V1 have been described. Using voltage-sensitive dye imaging *in vivo*, we determined the spatiotemporal dynamics of the activity evoked in the visual cortex by simple stimuli. Independently of precise areal boundaries, we found that V1 activation is rapidly followed by the depolarization of three functional groups of higher order visual areas organized retinotopically. After this sequential activation, all four regions were simultaneously active for most of the response. Concomitantly with the parallel processing of the visual input, the activity initiated retinotopically and propagated quickly and isotropically within each region. The size of this activation by local recurrent activity, which extended beyond the initial retinotopic response, was dependent on the intensity of the stimulus. Moreover the difference in the spatiotemporal dynamic of the response to dark and bright stimuli suggested the dominance in the mouse of the ON pathway. Our results suggest that the cortex integrates visual information simultaneously through across-area parallel and within-area serial processing.

The Journal of Neuroscience, August 8, 2012 • 32(32):11120–11131

NEUROBIOLOGY OF DISEASE

Reactive Astrocytes Overexpress TSPO and Are Detected by TSPO Positron Emission Tomography Imaging

Sonia Lavis, ¹ Martine Guillermier, ^{1*} Anne-Sophie Hérard, ^{1*} Fanny Petit, ^{1*} Marion Delahaye, ¹ Nadja Van Camp, ¹ Lucile Ben Haim, ¹ Vincent Lebon, ¹ Philippe Remy, ^{1,2} Frédéric Dollé, ³ Thierry Delzescaux, ¹ Gilles Bonvento, ¹ Philippe Hantraye, ¹ and Carole Escartin ¹

¹Commissariat à l'Energie Atomique et aux Energies Alternatives (CEA), Département des Sciences du Vivant (DSV), Institut d'Imagerie Biomédicale, MIRCen and CNRS, URA2210 (I2BM), 92260 Fontenay-aux-Roses, France, ²Neurology Department, Henri Mondor University Hospital, 94000 Créteil, France, and ³CEA, DSV, I2BM, Service Hospitalier Frédéric Joliot, 91400 Orsay, France

Astrocytes and microglia become reactive under most brain pathological conditions, making this neuroinflammation process a surrogate marker of neuronal dysfunction. Neuroinflammation is associated with increased levels of translocator protein 18 kDa (TSPO) and binding sites for TSPO ligands. Positron emission tomography (PET) imaging of TSPO is thus commonly used to monitor neuroinflammation in preclinical and clinical studies. It is widely considered that TSPO PET signal reveals reactive microglia, although a few studies suggested a potential contribution of reactive astrocytes. Because astrocytes and microglia play very different roles, it is crucial to determine whether reactive astrocytes can also overexpress TSPO and yield to a detectable TSPO PET signal *in vivo*. We used a model of selective astrocyte activation through lentiviral gene transfer of the cytokine ciliary neurotrophic factor (CNTF) into the rat striatum, in the absence of neurodegeneration. CNTF induced an extensive activation of astrocytes, which overexpressed GFAP and become hypertrophic, whereas microglia displayed minimal increase in reactive markers. Two TSPO radioligands, [¹⁸F]DPA-714 [*N,N*-diethyl-2-(2-(4-(2-[¹⁸F]fluoroethoxy)phenyl)-5,7-dimethylpyrazolo[1,5-*a*]pyrimidin-3-yl)acetamide)] and [¹¹C]SSR180575 (7-chloro-*N,N*-dimethyl-5-[¹¹C]methyl-4-oxo-3-phenyl-3,5-dihydro-4*H*-pyridazino[4,5-*b*]indole-1-acetamide), showed a significant binding in the lenti-CNTF-injected striatum that was saturated and displaced by PK11195 [*N*-methyl-*N*-(1-methylpropyl)-1-(2-chlorophenyl)-isoquinoline-3-carboxamide]. The volume of radioligand binding matched the GFAP immunopositive volume. TSPO mRNA levels were significantly increased, and TSPO protein was overexpressed by CNTF-activated astrocytes. We show that reactive astrocytes overexpress TSPO, yielding to a significant and selective binding of TSPO radioligands. Therefore, caution must be used when interpreting TSPO PET imaging in animals or patients because reactive astrocytes can contribute to the signal in addition to reactive microglia.

The Journal of Neuroscience, August 8, 2012 • 32(32):10809–10818

Chronic-Pain-Associated Astrocytic Reaction in the Spinal Cord Dorsal Horn of Human Immunodeficiency Virus-Infected Patients

Yuqiang Shi, ¹ Benjamin B. Gelman, ^{1,2} Joshua G. Lisinichia, ² and Shao-Jun Tang ¹

Departments of ¹Neuroscience and Cell Biology and ²Pathology, University of Texas Medical Branch, Galveston, Texas 77555

Studies with animal models have suggested that reaction of glia, including microglia and astrocytes, critically contributes to the development and maintenance of chronic pain. However, the involvement of glial reaction in human chronic pain is unclear. We performed analyses to compare the glial reaction profiles in the spinal dorsal horn (SDH) from three cohorts of sex- and age-matched human postmortem tissues: (1) HIV-negative patients, (2) HIV-positive patients without chronic pain, and (3) HIV patients with chronic pain. Our results indicate that the expression levels of CD11b and Iba1, commonly used for labeling microglial cells, did not differ in the three patient groups. However, GFAP and S100 β , often used for labeling astrocytes, were specifically upregulated in the SDH of the “pain-positive” HIV patients but not in the “pain-negative” HIV patients. In addition, proinflammatory cytokines, TNF α and IL-1 β , were specifically increased in the SDH of pain-positive HIV patients. Furthermore, proteins in the MAPK signaling pathway, including pERK, pCREB and c-Fos, were also upregulated in the SDH of pain-positive HIV patients. Our findings suggest that reaction of astrocytes in the SDH may play a role during the maintenance phase of HIV-associated chronic pain.

The Journal of Neuroscience, August 8, 2012 • 32(32):10833–10840

Schizophrenia-Like Features in Transgenic Mice Overexpressing Human HO-1 in the Astrocytic Compartment

Wei Song,¹ Hillel Zukor,^{1,2} Shih-Hsiung Lin,^{1,2} Jacob Hascalovici,^{1,2} Adrienne Liberman,¹ Ayda Tavitian,^{1,2} Jeannie Mui,⁴ Hojatollah Vali,^{4,5} Xin-Kang Tong,³ Sanjeev K. Bhardwaj,⁶ Lalit K. Srivastava,⁶ Edith Hamel,^{2,3} and Hyman M. Schipper^{1,2}

¹Lady Davis Institute, Jewish General Hospital, Montreal, Quebec H3T 1E2, Canada, ²Department of Neurology and Neurosurgery, McGill University, and ³Laboratory of Cerebrovascular Research, Montreal Neurological Institute, Montreal, Quebec H3A 2B4, Canada, ⁴Facility for Electron Microscopy Research and ⁵Department of Anatomy and Cell Biology, Faculty of Medicine, McGill University, Montreal, Quebec H3A 2B2, Canada, and ⁶Douglas Hospital and Department of Psychiatry, McGill University, Montreal, Quebec H4H 1R3, Canada

Delineation of key molecules that act epigenetically to transduce diverse stressors into established patterns of disease would facilitate the advent of preventive and disease-modifying therapeutics for a host of neurological disorders. Herein, we demonstrate that selective overexpression of the stress protein heme oxygenase-1 (HO-1) in astrocytes of novel GFAP.HMOX1 transgenic mice results in subcortical oxidative stress and mitochondrial damage/autophagy; diminished neuronal reelin content (males); induction of Nurr1 and Pitx3 with attendant suppression of their targeting miRNAs, 145 and 133b; increased tyrosine hydroxylase and α -synuclein expression with downregulation of the targeting miR-7b of the latter; augmented dopamine and serotonin levels in basal ganglia; reduced D₁ receptor binding in nucleus accumbens; axodendritic pathology and altered hippocampal cytoarchitectonics; impaired neurovascular coupling; attenuated prepulse inhibition (males); and hyperkinetic behavior. The GFAP.HMOX1 neurophenotype bears resemblances to human schizophrenia and other neurodevelopmental conditions and implicates glial HO-1 as a prime transducer of inimical (endogenous and environmental) influences on the development of monoaminergic circuitry. Containment of the glial HO-1 response to noxious stimuli at strategic points of the life cycle may afford novel opportunities for the effective management of human neurodevelopmental and neurodegenerative conditions.

The Journal of Neuroscience, August 8, 2012 • 32(32):10841–10853

Normal Midbrain Dopaminergic Neuron Development and Function in miR-133b Mutant Mice

Mary P. Heyer,^{1,2} Amar K. Pani,³ Richard J. Smeyne,³ Paul J. Kenny,² and Guoping Feng^{1,4}

¹Department of Neurobiology, Duke University Medical Center, Durham, North Carolina 27710, ²Laboratory of Behavioral and Molecular Neuroscience, Department of Molecular Therapeutics, The Scripps Research Institute Florida, Jupiter, Florida 33458, ³Department of Developmental Neurobiology, St. Jude Children's Research Hospital, Memphis, Tennessee 38105, and ⁴McGovern Institute for Brain Research, Department of Brain and Cognitive Sciences, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

Midbrain dopaminergic (mDA) neurons control movement and emotion, and their degeneration leads to motor and cognitive defects in Parkinson's disease (PD). miR-133b is a conserved microRNA that is thought to regulate mDA neuron differentiation by targeting Pitx3, a transcription factor required for appropriate development of mDA substantia nigra neurons. Moreover, miR-133b has been found to be depleted in the midbrain of PD patients. However, the function of miR-133b in the intact midbrain has not been determined. Here we show that miR-133b null mice have normal numbers of mDA neurons during development and aging. Dopamine levels are unchanged in the striatum, while expression of dopaminergic genes, including Pitx3, is also unaffected. Finally, motor coordination and both spontaneous and psychostimulant-induced locomotion are unaltered in miR-133b null mice, suggesting that miR-133b does not play a significant role in mDA neuron development and maintenance *in vivo*.

The Journal of Neuroscience, August 8, 2012 • 32(32):10887–10894

Enhanced NMDA Receptor-Dependent Thalamic Excitation and Network Oscillations in Stargazer Mice

Carolyn J. Lacey, Astra Bryant, Julia Brill, and John R. Huguenard

Department of Neurology and Neurological Sciences, Stanford University School of Medicine, Stanford, California 94305

Disturbances in corticothalamic circuitry can lead to absence epilepsy. The reticular thalamic nucleus (RTN) plays a pivotal role in that it receives excitation from cortex and thalamus and, when strongly activated, can generate excessive inhibitory output and epileptic thalamocortical oscillations that depend on postinhibitory rebound. Stargazer (stg) mice have prominent absence seizures resulting from a mutant form of the AMPAR auxiliary protein stargazin. Reduced AMPAR excitation in RTN has been demonstrated previously in stg, yet the mechanisms leading from RTN hypoexcitation to epilepsy are unknown and unexpected because thalamic epileptiform oscillatory activity requires AMPARs. We demonstrate hyperexcitability in stg thalamic slices and further characterize the various excitatory inputs to RTN using electrical stimulation and laser scanning photostimulation. Patch-clamp recordings of spontaneous and evoked EPSCs in RTN neurons demonstrate reduced amplitude and increased duration of the AMPAR component with an increased amplitude NMDAR component. Short 200 Hz stimulus trains evoked a gradual approximately threefold increase in NMDAR EPSCs compared with single stimuli in wild-type (WT), indicating progressive NMDAR recruitment, whereas in stg cells, NMDAR responses were nearly maximal with single stimuli. Array tomography revealed lower synaptic, but higher perisynaptic, AMPAR density in stg RTN. Increasing NMDAR activity via reduced $[Mg^{2+}]_o$ in WT phenocopied the thalamic hyperexcitability observed in stg, whereas changing $[Mg^{2+}]_o$ had no effect on stg slices. These findings suggest that, in stg, a trafficking defect in synaptic AMPARs in RTN cells leads to a compensatory increase in synaptic NMDARs and enhanced thalamic excitability.

The Journal of Neuroscience, August 8, 2012 • 32(32):11067–11081

Anti-NMDA Receptor Encephalitis Antibody Binding Is Dependent on Amino Acid Identity of a Small Region within the GluN1 Amino Terminal Domain

Amy J. Gleichman,^{1,2} Lynn A. Spruce,³ Josep Dalmau,^{1,4} Steven H. Seeholzer,³ and David R. Lynch^{1,2}

¹Department of Neurology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania 19104, ²Division of Pediatrics and

³Protein and Proteomics Core, Children's Hospital of Philadelphia, Philadelphia, Pennsylvania 19104, and ⁴Institució Catalana de Recerca i Estudis

Avançats (ICREA) at Institut d'Investigació Biomèdica August Pi i Sunyer (IDIBAPS), Service of Neurology, Hospital Clínic, University of Barcelona, 08036 Barcelona, Spain

Anti-NMDA receptor (NMDAR) encephalitis is a newly identified autoimmune disorder that targets NMDARs, causing severe neurological symptoms including hallucinations, psychosis, and seizures, and may result in death (Dalmau et al., 2008). However, the exact epitope to which these antibodies bind is unknown. A clearly defined antigenic region could provide more precise testing, allow for comparison of immunogenicity between patients to explore potential clinically relevant variations, elucidate the functional effects of antibodies, and make patients' antibodies a more effective tool with which to study NMDAR function. Here, we use human CSF to explore the antigenic region of the NMDAR. We created a series of mutants within the amino terminal domain of GluN1 that change patient antibody binding in transfected cells in stereotyped ways. These mutants demonstrate that the N368/G369 region of GluN1 is crucial for the creation of immunoreactivity. Mass spectrometry experiments show that N368 is glycosylated in transfected cells and rat brain regions; however, this glycosylation is not directly required for epitope formation. Mutations of residues N368/G369 change the closed time of the receptor in single channel recordings; more frequent channel openings correlates with the degree of antibody staining, and acute antibody exposure prolongs open time of the receptor. The staining pattern of mutant receptors is similar across subgroups of patients, indicating consistent immunogenicity, although we have identified one region that has a variable role in epitope formation. These findings provide tools for detailed comparison of antibodies across patients and suggest an interaction between antibody binding and channel function.

The Journal of Neuroscience, August 8, 2012 • 32(32):11082–11094