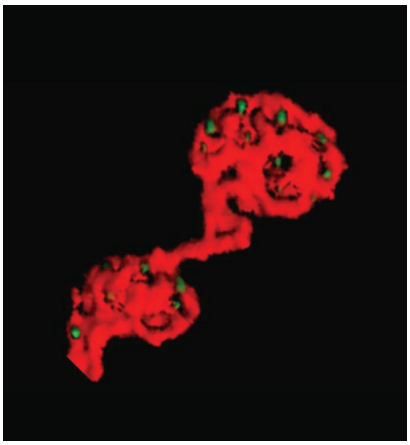
This Week in The Journal

● Cellular/Molecular

Seeing Presynaptic Calcium Channels

Fumiko Kawasaki, Beiyan Zou, Xia Xu, and Richard W. Ordway
(see pages 282–285)

The *Drosophila* gene *cacophony* (*cac*) encodes the α1 subunit of a presynaptic voltage-gated calcium channel. This gene locus was first identified, and named, because of a role in the male courtship song. These channels, activated during an action potential, trigger neurotransmitter release at the fly neuromuscular junction. To visualize their location in live animals, Kawasaki et al. created a transgenic fly in which α1 subunits were tagged with enhanced green fluorescent protein (EGFP). Deletion of the *cac* gene is embryonic lethal, but flies rescued with the transgene had normal viability and EPSCs, confirming that the transgene was functional. CAC1-EGFP was expressed on synaptic boutons at larval neuromuscular junctions in register with postsynaptic active zones. In the adult fly, CAC1-EGFP marked synaptic axonal swellings of 1–2 μm that were spaced at 1–2 μm intervals. The new protein will allow live imaging of calcium channels as well as colocalization of other presynaptic proteins.



The fly neuromuscular junction was labeled with an EGFP-tagged presynaptic calcium channel. See the article by Kawasaki et al. for details.

▲ Development/Plasticity/Repair

Branding Neurons by their Birthdate

Mitsuhiro Hashimoto and Katsuhiko Mikoshiba
(see pages 286–296)

The "inside-out" organization of the developing cerebral cortex makes for a birthdate-specific set of layers. First-born neurons end up in internal layers, whereas younger cells migrate to outer layers. Thus progenitor cells that divide on a given day are expected to give rise to distinct neuronal populations. Studies of the function and morphology of these cohorts requires a time-sensitive labeling method. In this issue, Hashimoto and Mikoshiba used single ventricular injections of a nonreplicating adenoviral vector carrying the *LacZ* gene to pulse-label progenitors in the subventricular zone. Colabeling with bromodeoxyuridine demonstrated that virus could infect and label cells within a 4 hr time window. Distinct cohorts were observed after different injection times. For example, injection on embryonic day 11.5 (E11.5) preferentially labeled subplate and Cajal-Retzius cells, whereas injection on E12.5 labeled neurons in the cortical plate. This approach provides another strategy to examine the molecular processes that determine neuronal fate.

■ Behavioral/Systems/Cognitive

A Cyclic Nucleotide Channelopathy in Two Sisters

Dimitri Tränkner, Herbert Jägle, Susanne Kohl, Eckart Apfelstedt-Sylla, Lindsay T. Sharpe, U. Benjamin Kaupp, Eberhart Zrenner, Reinhard Seifert, and Bernd Wissinger
(see pages 138–147)

Imagine living in a blurry, black-and-white world. Such is the case for those with achromatopsia, a family of disorders that arises from gene mutations in cones. This week, Tränkner et al. describe two sisters with incomplete achromatopsia. They carry two heterozygous mutations of the *CNGA3* gene that encodes the A3 subunit of the cyclic nucleotide-gated channel. The girls showed increased sensitivity to light (photophobia), reduced visual acuity, and color-discrimination defects. One mutation was within an intracellular loop, whereas the other was within the pore region of the channel subunit. When homomeric A3 channels were expressed in HEK293 cells, one mutant was nonfunctional, whereas the pore mutation dramatically altered channel activity. However, coexpression of the pore mutant with the B3 subunit, the expected subunit mixture in intact cells, restored wild-type channel properties except for altered calcium permeability. Thus this channelopathy appears to result from a relatively subtle change in ion flux through the channel.

♦ Neurobiology of Disease

Huntingtin and Vesicle Trafficking

Zheng-Hong Qin, Yumei Wang, Ellen Sapp, Benjamin Cuiffo, Erich Wanker, Michael R. Hayden, Kimberly B. Kegel, Neil Aronin, and Marian DiFiglia
(see pages 269–281)

In Huntington's disease, the huntingtin protein (htt) contains a polyglutamine expansion at its N terminus, leading to selective neuronal dysfunction and death. Expression of mutant htt in mice, or in cells *in vitro*, results in marked neuronal accumulation of insoluble protein aggregates, although their relationship to cell death is disputed. In this week's *Journal*, Qin et al. examined the so-called huntingtin bodies in search of alternate forms of htt. The htt bodies contained an insoluble core with fibrillar htt, but also a shell with soluble htt and several cytosolic proteins including heat shock protein 70 and dynamin. Cells containing htt bodies had impaired internalization of membrane proteins, suggesting that the soluble htt sequestered proteins involved in vesicular trafficking. Deletion of a polyproline region adjacent to the polyglutamate expansion reduced htt bodies and prevented redistribution of cytosolic proteins. Thus abnormal protein interactions might allow soluble forms of mutant htt to deprive the neuron of critical cytoplasmic proteins.

Editorial

New Sections and New Editors

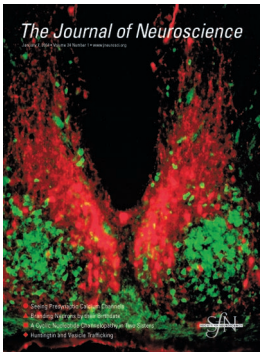
Over the past several years, *The Journal of Neuroscience* has published an increasing number of papers related to neurological disease. Such papers often are multidisciplinary and thus did not fit naturally into one of the existing sections. Thus starting in 2004, authors will have the option to submit to a new section in the *Journal* , entitled Neurobiology of Disease. Authors can make this choice at the time of initial submission. Manuscripts will be handled by the Editor-in-Chief or assigned to the most appropriate Senior Editor. We will also highlight one of these papers in This Week in The Journal (http://www.jneurosci.org/thisweek.shtml), thus further enhancing the visibility of these papers. We hope that this section will quickly become a focal point for outstanding papers addressing the mechanisms underlying disorders of the nervous system.

We also welcome several new editors in 2004. Larry Trussell is now a Senior Editor in Cellular/Molecular Neuroscience. Valina Dawson, David Fitzpatrick, Diane Lipscombe, Chris McBain, and Fred Rieke have recently joined the editorial board as Reviewing Editors. Randy Buckner, Ron Calabrese, and Marie Filbin joined as Reviewing Editors earlier in 2003. A listing of the new Associate Editors will appear soon. I want to extend a special thanks to the Reviewing Editors (Mike Nusbaum, Carol Mason, Kathy Dunlap, and Junying Yuan) and to those Associate Editors who completed their terms in 2003. The diversity, expertise, and dedicated work of the editorial board are the essence of the *Journal*.

Gary Westbrook
Editor-in-Chief

The Journal of Neuroscience

January 7, 2004 • Volume 24 Number 1 www.jneurosci.org



Cover picture: The suprachiasmatic nuclei (SCNs) of the hypothalamus are the master circadian pacemakers in mammals. In Karatsoreos et al., the authors delineate two separate populations of cells in the mouse SCN: one rhythmic, and the other not rhythmic but light responsive. A large proportion of the light-responsive cells contain gastrin-releasing peptide (GRP). On the cover, the image shows the mouse SCN stained for vasopressin (red) and a green fluorescent protein tag on GRP-positive cells (green). For details, see the article by Karatsoreos in this issue (pages 68–75).

Correction: In the article "Two Distinct Subpopulations of Nestin-Positive Cells in Adult Mouse Dentate Gyrus," by Satoshi Fukuda, Fusao Kato, Yusuke Tozuka, Masahiro Yamaguchi, Yusei Miyamoto, and Tatsuhiro Hisatsune, which appeared on pages 9357–9366 of the October 15, 2003 issue, the authors would like to add the citation of the related work by Filippov et al. [Filippov V, Kronenberg G, Pivneva T, Reuter K, Steiner B, Wang LP, Yamaguchi M, Kettenmann H, Kempermann G (2003) Subpopulation of nestin-expressing progenitor cells in the adult murine hippocampus shows electrophysiological and morphological characteristics of astrocytes. Mol Cell Neurosci 22:373–382], which was published during the revision of the article. They have also classified nestin-expressing cells in the adult hippocampal dentate gyrus based on the difference in their morphological and electrophysiological properties.

Brief Communications

Nicotinic Enhancement of the Noradrenergic Inhibition of Sleep-Promoting Neurons in the Ventrolateral Preoptic Area

Benoît Saint-Mleux,[1]* Emmanuel Eggermann,[1]* Arnaud Bisetti,[1] Laurence Bayer,[1] Danièle Machard,[1] Barbara E. Jones,[2] Michel Mühlethaler,[1] and Mauro Serafin[1]

[1]Département de Physiologie, Centre Médical Universitaire, 1211 Geneva 4, Switzerland, and [2]Department of Neurology and Neurosurgery, McGill University, Montreal Neurological Institute, Montreal, Quebec, Canada H3A 2B4

According to multiple lines of evidence, neurons in the ventrolateral preoptic area (VLPO) that contain GABA promote sleep by inhibiting neurons of the arousal systems. Reciprocally, transmitters used by these systems, including acetylcholine (ACh) and noradrenaline (NA), exert an inhibitory action on the VLPO neurons. Because nicotine, an agonist of ACh, acts as a potent stimulant, we queried whether it might participate in the cholinergic inhibition of these sleep-promoting cells. Indeed, we found that ACh inhibits the VLPO neurons through a nicotinic, as well as a muscarinic, action. As evident in the presence of atropine, the non-muscarinic component was mimicked by epibatidine, a nonselective nicotinic ACh receptor (nAChR) agonist and was blocked by dihydro-β-erythroidine, a nonselective nAChR antagonist. It was not, however, blocked by methyllycaconitine, a selective antagonist of the α7 subtype, indicating that the action was mediated by non-α7 nAChRs. The nicotinic inhibition was attributed to a presynaptic facilitation of NA release because it persisted in the presence of tetrodotoxin and was blocked by yohimbine and RS 79948, which are both selective antagonists of α2 adrenergic receptors. Sleep-promoting VLPO neurons are thus dually inhibited by ACh through a muscarinic postsynaptic action and a nicotinic presynaptic action on noradrenergic terminals. Such dual complementary actions allow ACh and nicotine to enhance wakefulness by inhibiting sleep-promoting systems while facilitating other wake-promoting systems.

The Journal of Neuroscience, January 7, 2004 • 24(1):63–67

Wnt Signaling Mutants Have Decreased Dentate Granule Cell Production and Radial Glial Scaffolding Abnormalities

Cheng-Ji Zhou, Chunjie Zhao, and Samuel J. Pleasure

Department of Neurology, Programs in Neuroscience and Developmental Biology, University of California, San Francisco, California 94143-0435

LRP6 mutant mice have generalized defects in the Wnt/β-catenin signaling pathway because of the crucial function of LRP6 as a Wnt signaling co-receptor (Pinson et al., 2000). We examined the hippocampal phenotype of single LRP6 mutant mice as well as LRP6/Lef1 double mutant mice. LRP6 mutants had reduced production of dentate granule neurons and abnormalities of the radial glial scaffolding in the forming dentate gyrus. These defects were more severe with the addition of a single Lef1 null allele to an LRP6 null background. Pyramidal cell fields were unaffected in the LRP6, Lef1, or double mutants. The dentate defects were accompanied by decreased numbers of mitotic precursors in the migratory pathway to the dentate and in the displaced proliferative zone in the dentate itself. At earlier gestational ages, there was a reduction in the number of dentate granule cell progenitors in the dentate ventricular zone before the emigration of the earliest differentiated granule neurons and precursors to form the dentate anlage.

The Journal of Neuroscience, January 7, 2004 • 24(1):121–126

Active Zone Localization of Presynaptic Calcium Channels Encoded by the *cacophony* Locus of *Drosophila*

Fumiko Kawasaki, Beiyan Zou, Xia Xu, and Richard W. Ordway

Department of Biology and Genetics Graduate Program, Pennsylvania State University, University Park, Pennsylvania 16802

Presynaptic calcium channels play a central role in chemical synaptic transmission by providing the calcium trigger for evoked neurotransmitter release. These voltage-gated calcium channels are composed of a primary structural subunit, α1, as well as auxiliary β and α2δ subunits. Our previous genetic, molecular, and functional analysis has shown that the *cacophony* (*cac*) gene encodes a primary presynaptic calcium channel α1 subunit in *Drosophila*. Here we report that transgenic expression of a *cac*-encoded α1 subunit fused with enhanced green fluorescent protein efficiently rescues *cac* lethal mutations and allows *in vivo* analysis of calcium channel localization at active zones. The results reported here further characterize the primary role of *cac*-encoded calcium channels in neurotransmitter release. In addition, these studies provide a unique genetic tool for live imaging of functional presynaptic calcium channels *in vivo* and define a molecular marker for immunolocalization of other presynaptic proteins relative to active zones. These findings are expected to facilitate additional analysis of synaptic development and function in this important model system.

The Journal of Neuroscience, January 7, 2004 • 24(1):282–285

Articles

CELLULAR/MOLECULAR

Production and Release of Neuroprotective Tumor Necrosis Factor by $P2X_7$ Receptor-Activated Microglia

Tomohisa Suzuki,[1] Izumi Hide,[1] Katsutoshi Ido,[1] Shinichi Kohsaka,[2] Kazuhide Inoue,[3,4] and Yoshihiro Nakata[1]

[1]Department of Pharmacology, Graduate School of Biomedical Sciences, Hiroshima University, Hiroshima 734-8551, Japan, [2]Department of Neurochemistry, National Institute of Neuroscience, Tokyo 187-8502, Japan, [3]Division of Biosignaling, National Institute of Health Science, Tokyo 158-8501, Japan, and [4]Graduate School of Pharmaceutical Sciences, Kyushu University, Fukuoka 812-8582, Japan

After a brain insult, ATP is released from injured cells and activates microglia. The microglia that are activated in this way then release a range of bioactive substances, one of which is tumor necrosis factor (TNF). The release of TNF appears to be dependent on the $P2X_7$ receptor. The inhibitors 1,4-diamino-2,3-dicyano-1,4-bis[2-amino-phenylthio]butadiene (U0126), anthra[1,9-cd]pyrazol-6(2H)-one (SP600125), and 4-(4-fluorophenyl)-2-(4-methylsulfinylphenyl)-5-(4-pyridyl)IH-imidazole (SB203580), which target MEK (mitogen-activated protein kinase kinase), JNK (c-Jun N-terminal kinase) and p38, respectively, all potently suppress the production of TNF in ATP-stimulated icroglia, whereas the production of TNF mRNA is strongly inhibited by U0126 and SP600125. SB203580 did not affect the ncreased levels of TNF mRNA but did prevent TNF mRNA from accumulating in the cytoplasm. The ATP-provoked activation of JNK and p38 [but not xtracellular signal-regulated kinase (ERK)] could be inhibited by brilliant blue G, a $P2X_7$ receptor blocker, and by genistein and 4-amino-5-(4-chlorophenyl)-7-(*t*-butyl)pyrazolo[3,4-D]pyrimidine, which are general and *src*family-specific tyrosine kinase inhibitors, respectively. Most important, we found that treatment of the microglia in neuron–microglia cocultures with the $P2X_7$ agonist 2′–3′-*O*-(benzoyl-benzoyl) ATP led to significant reductions in glutamate-induced neuronal cell death, and that either TNF-α converting enzyme inhibitor or anti-TNF readily suppressed the protective effect implied by this result. Together, these findings indicate that both ERK and JNK are involved in the regulation of TNF mRNA expression, that p38 is involved in the nucleocytoplasmic transport of TNF mRNA, and that a PTK (protein tyrosine kinase), possibly a member of the *src* family, acts downstream of the $P2X_7$ receptor to activate JNK and p38. Finally, our data suggest that $P2X_7$ receptor-activated microglia protect neurons against glutamate toxicity primarily because they are able to release TNF.

The Journal of Neuroscience, January 7, 2004 • 24(1):1–7

Production of Resurgent Current in $Na_V1.6$-Null Purkinje Neurons by Slowing Sodium Channel Inactivation with β-Pompilidotoxin

Tina M. Grieco[1] and Indira M. Raman[1,2]

[1]Northwestern University Institute for Neuroscience and [2]Department of Neurobiology and Physiology, Northwestern University, Evanston, Illinois 60208

Voltage-gated tetrodotoxin-sensitive sodium channels of Purkinje neurons produce "resurgent" current with repolarization, which results from relief of an open-channel block that terminates current flow at positive potentials. The associated recovery of sodium channels from inactivation is thought to facilitate the rapid firing patterns characteristic of Purkinje neurons. Resurgent current appears to depend primarily on $Na_V1.6$ α subunits, because it is greatly reduced in "*med*" mutant mice that lack $Na_V1.6$. To identify factors that regulate the susceptibility of α subunits to open-channel block, we voltage clamped wild-type and *med* Purkinje neurons before and after slowing conventional inactivation with β-pompilidotoxin (β-PMTX). β-PMTX increased resurgent current in wild-type neurons and induced resurgent current in *med* neurons. In *med* cells, the resurgent component of β-PMTX-modified sodium currents could be selectively abolished by application of intracellular alkaline phosphatase, suggesting that, like in $Na_V1.6$-expressing cells, the open-channel block of $Na_V1.1$ and $Na_V1.2$ subunits is regulated by constitutive phosphorylation. These results indicate that the endogenous blocker exists independently of $Na_V1.6$ expression, and conventional inactivation regulates resurgent current by controlling the extent of open-channel block. In Purkinje cells, therefore, the relatively slow conventional inactivation kinetics of $Na_V1.6$ appear well adapted to carry resurgent current. Nevertheless, $Na_V1.6$ is not unique in its susceptibility to open-channel block, because under appropriate conditions, the non-$Na_V1.6$ subunits can produce robust resurgent currents.

The Journal of Neuroscience, January 7, 2004 • 24(1):35–42

Endocannabinoids Mediate Presynaptic Inhibition of Glutamatergic Transmission in Rat Ventral Tegmental Area Dopamine Neurons through Activation of CB1 Receptors

Miriam Melis,[1,2] Marco Pistis,[1,2] Simona Perra,[1,2] Anna Lisa Muntoni,[1,3] Giuliano Pillolla,[1,2] and Gian Luigi Gessa[1,2]

[1]Centre of Excellence, Neurobiology of Addiction, and [2]B. B. Brodie Department of Neuroscience, University of Cagliari, Monserrato, 09042 Italy, and [3]Consiglio Nazionale delle Ricerche Institute of Neuroscience in Pisa, Section of Cagliari, c/o B. B. Brodie Department of Neuroscience, University of Cagliari, Monserrato, 09042 Italy

The endogenous cannabinoid system has been shown to play a crucial role in controlling neuronal excitability and synaptic transmission. In this study we investigated the effects of a cannabinoid receptor (CB-R) agonist WIN 55,212–2 (WIN) on excitatory synaptic transmission in the rat ventral tegmental area (VTA). Whole-cell patch clamp recordings were performed from VTA dopamine (DA) neurons in an *in vitro* slice preparation. WIN reduced both NMDA and AMPA EPSCs as well as miniature EPSCs (mEPSCs), and increased the paired-pulse ratio, indicating a presynaptic locus of its action. We also found that WIN-induced effects were dose-dependent and mimicked by the CB1-R agonist HU210. Furthermore, two CB1-R antagonists, AM281 and SR141716A, blocked WIN-induced effects, suggesting that WIN modulates excitatory synaptic transmission via activation of CB1-Rs. Our additional finding that both AM281 and SR141716A *per se* increased NMDA EPSCs suggests that endogenous

cannabinoids, released from depolarized postsynaptic neurons, might act retrogradely on presynaptic CB1-Rs to suppress glutamate release. Hence, we report that a type of synaptic modulation, previously termed depolarization-induced suppression of excitation (DSE), is present also in the VTA as a calcium-dependent phenomenon, blocked by both AM281 and SR141716A, and occluded by WIN. Importantly, DSE was partially blocked by the D_2DA antagonist eticlopride and enhanced by the D_2DA agonist quinpirole without changing the presynaptic cannabinoid sensitivity. These results indicate that the two pathways work in a cooperative manner to release endocannabinoids in the VTA, where they play a role as retrograde messengers for DSE via CB1-Rs.
The Journal of Neuroscience, January 7, 2004 • 24(1):53–62

Extracellular Signal-Regulated Kinase 1/2 Is Required for the Induction of Group I Metabotropic Glutamate Receptor-Mediated Epileptiform Discharges

Wangfa Zhao, Riccardo Bianchi, Min Wang, and Robert K. S. Wong
Department of Physiology and Pharmacology, State University of New York Downstate Medical Center, Brooklyn, New York 11203

Transient stimulation of group I metabotropic glutamate receptors (mGluRs) induces persistent prolonged epileptiform discharges in hippocampal slices via a protein synthesis-dependent process. At present, the signaling process underlying the induction of these epileptiform discharges remains unknown. We examined the possible role of extracellular signal-regulated kinases (ERK1 and ERK2) because these kinases can be activated by group I mGluRs, and their activation may regulate gene expression and alter protein synthesis. The group I mGluR agonist (*S*)3,5-dihydroxyphenylglycine (DHPG; 50 μM) induced activation of ERK1/2 in hippocampal slices. 2-(2-Diamino-3-methoxyphenyl-4H-1-benzopyran-4-one (PD98059 (50 μM) a specific inhibitor of mitogen-activated protein kinase kinase (MEK), suppressed ERK1/2 activation by DHPG. PD98059 or another MEK inhibitor, 1,4-diamino-2,3-dicyano-1,4-bis[2-aminophenylthio]butadiene (10 μM), also prevented the induction of the prolonged epileptiform discharges by DHPG. In the presence of ionotropic glutamate receptor inhibitors and tetrodotoxin (blockers), DHPG-induced epileptiform discharges were suppressed, whereas ERK1/2 activation persisted. Protein kinase C inhibitors (2-[1-(3-dimethylaminopropyl)-5-methoxyindol-3-yl]-3-(1H-indol-3-yl) maleimide 1 μM; or chelerythrine, 10 μM) did not prevent the generation of DHPG-induced epileptiform discharges, nor did they suppress the activation of ERK1/2 by DHPG in slices pretreated with the blockers. Genistein (30 μM), a broad-spectrum tyrosine kinase inhibitor, suppressed the DHPG-induced epileptiform discharges and the ERK1/2 activation in the presence of blockers. Induction of DHPG-mediated epileptiform discharges was also suppressed by 4-amino-5-(4-chlorophenyl)-7-(t-butyl)pyrazolo[3,4-D]pyrimidine (10 μM), an Src-family tyrosine kinase inhibitor. The study shows that group I mGluRs activate ERK1/2 through a tyrosine kinase-dependent process and that this activation of ERK1/2 is necessary for the induction of prolonged epileptiform discharges in the hippocampus.
The Journal of Neuroscience, January 7, 2004 • 24(1):76–84

Tetraspanin Protein CD9 Is a Novel Paranodal Component Regulating Paranodal Junctional Formation

Tomoko Ishibashi,[1] Lei Ding,[2] Kazuhiro Ikenaka,[3] Yoshiro Inoue,[2] Kenji Miyado,[4] Eisuke Mekada,[4] and Hiroko Baba[1]
[1]Department of Molecular Neurobiology, School of Pharmacy, Tokyo University of Pharmacy and Life Science, Hachioji 192-0392, Japan, [2]Department of Molecular Neuroanatomy, Hokkaido University School of Medicine, Sapporo 060-8368, Japan, [3]Laboratory of Neural Information, National Institute for Physiological Sciences, Okazaki National Research Institutes, Okazaki 444-8585, Japan, [4] and Department of Cell Biology, Research Institute for Microbial Disease, Osaka University, Suita 565-0871, Japan

The axoglial paranodal junction is essential for the proper localization of ion channels around the node of Ranvier. The integrity of this junction is important for nerve conduction. Although recent studies have made significant progress in understanding the molecular composition of the paranodal junction, it is not known how these membrane components are distributed to the appropriate sites and interact with each other. Here we show that CD9, a member of the tetraspanin family, is present at the paranode. CD9 is concentrated in the paranode as myelination proceeds, but CD9 clusters become diffuse, associated with disruption of the paranode, in cerebroside sulfotransferasedeficient mice. Immunohistochemical and Western blot analysis showed that CD9 is distributed predominantly in the PNS. Ablation of CD9 in mutant mice disrupts junctional attachment at the paranode and alters the paranodal components contactin-associated protein (also known as Paranodin) and neurofascin 155, although the frequency of such abnormalities varies among individuals and individual axons even in the same mouse. Electron micrographs demonstrated that compact myelin sheaths were also affected in the PNS. Therefore, CD9 is a myelin protein important for the formation of paranodal junctions. CD9 also plays a role in the formation of compact myelin in the PNS.
The Journal of Neuroscience, January 7, 2004 • 24(1):96–102

Climbing Fiber Activation of EAAT4 Transporters and Kainate Receptors in Cerebellar Purkinje Cells

Yanhua H. Huang,[1] Margaret Dykes-Hoberg,[2] Kohichi Tanaka,[3] Jeffrey D. Rothstein,[2] and Dwight E. Bergles[1]
Departments of [1]Neuroscience and [2]Neurology, Johns Hopkins University, Baltimore, Maryland 21205, and [3]Laboratory of Molecular Neuroscience, School of Biomedical Science and Medical Research Institute, Tokyo Medical and Dental University, Tokyo, Japan 113-8510

Cerebellar Purkinje cells (PCs) express two glutamate transporters, EAAC1 (EAAT3) and EAAT4; however, their relative contribution to the uptake of glutamate at synapses is not known. We found that glutamate transporter currents recorded at climbing fiber (CF)–PC synapses are absent in mice lacking EAAT4 but unchanged in mice lacking EAAC1, indicating that EAAT4 is preferentially involved in clearing glutamate from CF synapses. However, comparison of CF synaptic currents between wild-type and transporter knock-out mice revealed that ionotropic glutamate receptors are responsible for >40% of the current previously attributed to transporters, indicating that PCs remove <10% of the glutamate released by the CF. The receptors responsible for the nontransporter component accounted for 5% of the CF EPSC, had a slower time course

and lower occupancy than AMPA receptors at CF synapses, and exhibited pharmacological properties consistent with kainate receptors. In GluR5 knock-out mice, this current was dramatically reduced, indicating that CF excitation of PCs involves two distinct classes of ionotropic glutamate receptors, AMPA receptors and GluR5-containing kainate receptors.

The Journal of Neuroscience, January 7, 2004 • 24(1):103–111

Selective Effects of Potassium Elevations on Glutamate Signaling and Action Potential Conduction in Hippocampus

Julian P. Meeks and Steven Mennerick

Departments of Psychiatry and Anatomy and Neurobiology, Washington University School of Medicine, St. Louis, Missouri 63110

High-frequency synaptic transmission is depressed by moderate rises in the extracellular potassium concentration ($[K^+]_o$). Previous reports have indicated that depression of action potential signaling may underlie the synaptic depression. Here, we investigated the specific contribution of K^+-induced action potential changes to synaptic depression. We found that glutamatergic transmission in the hippocampal area CA1 was significantly depressed by 8–10 mM $[K^+]_o$, but that GABAergic transmission remained intact. Riluzole, a drug that slows recovery from inactivation of voltage-gated sodium channels (NaChs), interacts with subthreshold $[K^+]_o$ to depress afferent volleys and EPSCs strongly. Thus, elevated $[K^+]_o$ likely depresses synapses by slowing NaCh recovery from inactivation. It is unclear from previous studies whether $[K^+]_o$-induced action potential depression is caused by changes in initiation, reliability, or waveform. We investigated these possibilities explicitly. $[K^+]_o$-induced afferent volley depression was independent of stimulus strength, suggesting that changes in action potential initiation do not explain $[K^+]_o$-induced depression. Measurements of action potentials from single axons revealed that 8 mM $[K^+]_o$ increased conduction failures in a subpopulation of fibers and depressed action potential amplitude in all fibers. Together, these changes quantitatively account for the afferent volley depression. We estimate that conduction failure explains more than half of the synaptic depression observed at 8 mM $[K^+]_o$, with the remaining depression likely explained by waveform changes. These mechanisms of selective sensitivity of glutamate release to $[K^+]_o$ accumulation represent a unique neuromodulatory mechanism and a brake on runaway excitation.

The Journal of Neuroscience, January 7, 2004 • 24(1):197–206

Gephyrin Is Critical for Glycine Receptor Clustering But Not for the Formation of Functional GABAergic Synapses in Hippocampal Neurons

Sabine Lévi,* Stephen M. Logan,* Kenneth R. Tovar, and Ann Marie Craig

Department of Anatomy and Neurobiology, Washington University School of Medicine, St. Louis, Missouri 63110

The role of the scaffolding protein gephyrin at hippocampal inhibitory synapses is not well understood. A previous study (Kneussel et al., 1999) reported a complete loss of synaptic clusters of the major $GABA_AR$ subunits $\alpha2$ and $\gamma2$ in hippocampal neurons lacking gephyrin. In contrast, we show here that $GABA_AR$ $\alpha2$ and $\gamma2$ subunits do cluster at pyramidal synapses in hippocampal cultures from gephyrin$-/-$ mice, albeit at reduced levels compared with control neurons. Synaptic aggregation of $GABA_AR$ $\alpha1$ on interneurons was identical between the culture types. Furthermore, we recorded miniature IPSCs (mIPSCs) from gephyrin$-/-$ neurons. Although the mean mIPSC amplitude was reduced (by 23%) compared with control, the frequency of these events was unchanged. Cell surface labeling experiments indicated that gephyrin contributes, in part, to aggregation but not to insertion or stabilization of $GABA_AR$ $\alpha2$ and $\gamma2$ in the plasma membrane. Thus, a major gephyrin-independent component of hippocampal inhibitory synapse development must exist. We also report that glycine receptors cluster at GABAergic synapses in a subset of hippocampal interneurons and pyramidal neurons. Unlike $GABA_A$Rs, synaptic clustering of glycine receptors was completely abolished in gephyrin$-/-$ neurons. Finally, artificial extrasynaptic aggregation of $GABA_AR$ was able to redistribute and cocluster gephyrin by a mechanism requiring a neuron-specific modification or intermediary protein. We propose a model of hippocampal inhibitory synapse development in which some $GABA_A$Rs cluster at synapses by a gephyrin-independent mechanism and recruit gephyrin. This clustered gephyrin may then recruit glycine receptors, additional $GABA_A$Rs, and other signal-transducing components.

The Journal of Neuroscience, January 7, 2004 • 24(1):207–217

DEVELOPMENT/PLASTICITY/REPAIR

The Secretory Granule-Associated Protein CAPS2 Regulates Neurotrophin Release and Cell Survival

Tetsushi Sadakata,[1,4] Akira Mizoguchi,[5] Yumi Sato,[1] Ritsuko Katoh-Semba,[6] Mitsunori Fukuda,[2] Katsuhiko Mikoshiba,[3,4] and Teiichi Furuichi[1]

[1]Laboratory for Molecular Neurogenesis, [2]Fukuda Initiative Research Unit, and [3]Laboratory for Developmental Neurobiology, RIKEN Brain Science Institute, Wako, Saitama 351-0198, Japan, [4]Department of Molecular Neurobiology, Institute of Medical Science, The University of Tokyo, Tokyo 108-8639, Japan, [5]Department of Anatomy, School of Medicine, Mie University, Tsu, Mie 514-8507, Japan, and [6]Department of Perinatology, Institute for Developmental Research, Aichi Human Service Center, Kasugai, Aichi 480-0392, Japan

Neurotrophins are key modulators of various neuronal functions, including differentiation, survival, and synaptic plasticity, but the molecules that regulate their secretion are poorly understood. We isolated a clone that is predominantly expressed in granule cells of postnatally developing mouse cerebellum, which turned out to be a paralog of CAPS (Ca^{2+}-dependent activator protein for secretion), and named CAPS2. CAPS2 is enriched on vesicular structures of presynaptic parallel fiber terminals of granule cells connecting postsynaptic spines of Purkinje cell dendrites. Vesicle factions affinity-purified by the CAPS2 antibody from mouse cerebella contained significant

amounts of neurotrophin-3 (NT-3), brain-derived neurotrophic factor (BDNF), and chromogranin B but not marker proteins for synaptic vesicle synaptophysin and synaptotagmin. In cerebellar primary cultures, punctate CAPS2 immunoreactivities are primarily colocalized with those of NT-3 and BDNF and near those of a postsynaptic marker, postsynaptic density-95, around dendritic arborization of Purkinje cells. Exogenously expressed CAPS2 enhanced release of exogenous NT-3 and BDNF from PC12 cells and endogenous NT-3 from cultured granule cells in a depolarization-dependent manner. Moreover, the overexpression of CAPS2 in granule cells promotes the survival of Purkinje cells in cerebellar cultures. Thus, we suggest that CAPS2 mediates the depolarization-dependent release of NT-3 and BDNF from granule cells, leading to regulation in cell differentiation and survival during cerebellar development.

The Journal of Neuroscience, January 7, 2004 • 24(1):43–52

Nitric Oxide Is a Physiological Inhibitor of Neurogenesis in the Adult Mouse Subventricular Zone and Olfactory Bulb

Bernardo Moreno-López, Carmen Romero-Grimaldi, José Angel Noval, Maribel Murillo-Carretero, Esperanza R. Matarredona, and Carmen Estrada

Área de Fisiología, Facultad de Medicina, Universidad de Cádiz, 11003 Cádiz, Spain

The subventricular zone of the rodent brain retains the capacity of generating new neurons in adulthood. The newly formed neuroblasts migrate rostrally toward the olfactory bulb, where they differentiate as granular and periglomerular interneurons. The reported presence of differentiated neurons expressing the neuronal isoform of nitric oxide synthase (NOS) in the periphery of the neurogenic region and the organization of their varicose axons as a network in which the precursors are immersed raised the hypothesis that endogenous nitric oxide (NO) may participate in the control of neurogenesis in the subventricular zone. Systemic administration of the NOS inhibitors N^{ω}-nitro-L-arginine methyl ester or 7-nitroindazole to adult mice produced a dose- and time-dependent increase in the number of mitotic cells in the subventricular zone, rostral migratory stream, and olfactory bulb, but not in the dentate gyrus of the hippocampus, without affecting apoptosis. In the subventricular zone, this effect was exerted selectively on a precursor subpopulation expressing nestin but not neuronal or glial cell-specific proteins. In addition, in the olfactory bulb, analysis of maturation markers in the newly generated neurons indicated that chronic NOS inhibition caused a delay in neuronal differentiation. Postmitotic cell survival and migration were not affected when NO production was impaired. Our results suggest that NO, produced by nitrergic neurons in the adult mouse subventricular zone and olfactory bulb, exerts a negative control on the size of the undifferentiated precursor pool and promotes neuronal differentiation.

The Journal of Neuroscience, January 7, 2004 • 24(1):85–95

Transient Electrical Coupling Delays the Onset of Chemical Neurotransmission at Developing Synapses

Theresa M. Szabo,[1] Donald S. Faber,[1] and Mark J. Zoran[2]

[1]Department of Neuroscience, Albert Einstein College of Medicine, Yeshiva University, Bronx, New York 10465, and [2]Department of Biology, Texas A & M University, College Station, Texas 77843

The formation and subsequent elimination of electrical coupling between neurons has been demonstrated in many developing vertebrate and invertebrate nervous systems. The relationship between the disappearance of electrical synaptic connectivity and the appearance of chemical neurotransmission is not well understood. We report here that identified motoneurons from the snail *Helisoma* formed transient electrical and chemical connections during regeneration both *in vivo* and *in vitro*. Electrical connections that formed *in vivo* were strongest by day 2 and no longer detectable by day 7. During elimination of this electrical connection, an inhibitory chemical connection from 110 onto 19 formed. This sequence of synaptic development was recapitulated in cell culture with a similar time course. The relationship between the appearance of transient electrical coupling and its possible effects on the subsequent chemical synaptogenesis were examined by reducing transient intercellular coupling. Trophic factor-deprived medium resulted in a 66% reduction in coupling coefficient. In these conditions, the unidirectional chemical connection formed readily; in contrast, chemical synaptogenesis was delayed in cell pairs exposed to trophic factors where transient electrical coupling was strong. Dye coupling and synaptic vesicle cycling studies supported electrophysiological results. Exposure to cholinergic antagonists, curare and hexamethonium bromide, which block chemical neurotransmission in these synapses, resulted in prolonged maintenance of the electrical connection. These studies demonstrated an inverse relationship between chemical and electrical connectivity at early stages of synaptic development and suggest a dynamic interaction between these forms of neuronal communication as adult neural networks are constructed or regenerated.

The Journal of Neuroscience, January 7, 2004 • 24(1):112–120

Functional and Genomic Changes in the Mouse Ocular Motor System in Response to Light Deprivation from Birth

Colleen A. McMullen, Francisco H. Andrade, and John S. Stahl

Department of Neurology, Case Western Reserve University and University Hospitals of Cleveland, Cleveland, Ohio 44106

Previous studies have suggested that abnormal visual experience early in life induces ocular motor abnormalities. The purpose of this study was to determine how visual deprivation alters the function and gene expression profile of the ocular motor system in mice. We measured the effect of dark rearing on eye movements, gene expression in the oculomotor nucleus, and contractility of isolated extraocular muscles. *In vivo* eye movement recordings showed decreased gains for optokinetic and vestibulo-ocular reflexes, confirming an effect of dark rearing on overall ocular motor function. Saccade peak velocities were preserved, however, arguing that the quantitative changes in these reflexes were not secondary to limitations in force generation. Using microarrays and quantitative PCR, we found that dark rearing shifted the oculomotor nucleus transcriptome to a state of delayed/arrested development. The expression of 132 genes was altered by dark rearing; these genes fit in various functional categories (signal transduction, transcription/translation control, metabolism, synaptic function, cytoskeleton), and some were known to be associated with neuronal development and

plasticity. Extraocular muscle contractility was impaired by dark rearing to a greater extent than expected from the *in vivo* ocular motility studies: changes included decreased force and shortening speed and evidence of abnormal excitability. The results indicate that normal development of the mouse ocular motor system and its muscles requires visual experience. The transcriptional pattern of arrested development may indicate that vision is required to establish the adult pattern, but it also may represent the plastic response of oculomotor nuclei to abnormal extraocular muscles.

The Journal of Neuroscience, January 7, 2004 • 24(1):161–169

Spatial and Temporal Properties of Visual Responses in the Thalamus of the Developing Ferret

Colin J. Akerman, Matthew S. Grubb, and Ian D. Thompson

University Laboratory of Physiology, Oxford University, Oxford OX1 3PT, United Kingdom

Spatiotemporal patterning of neural activity is thought to influence the development of connections in the visual pathway. This patterning can arise spontaneously or through sensory experience. Here, we use a combination of natural and simple stimuli to investigate which elements of the visual environment modulate the earliest responses in the primary visual pathway of developing ferrets. Recordings were made during the first 2 weeks of visual responsiveness, which, in the ferret, overlaps with the period that the eyelids have not yet opened. Even when the eyelids are closed, both thalamic and cortical activity was found to be temporally modulated under conditions of natural visual stimulation. The modulations correlated with temporal changes in stimulus contrast but also reflected spatial structure in the visual scene. Simple stimuli were used to show that early responses to naturalistic stimuli are influenced by the localization and structure of through-the-eyelid receptive fields. The early visual responses were also characterized by substantial variability in the ability of the cells to detect stimuli of different duration and different intensity, in a temporally precise manner. These temporal and spatial properties should constrain how plasticity mechanisms interpret naturally patterned activity.

The Journal of Neuroscience, January 7, 2004 • 24(1):170–182

The Role of AMPA Receptor Gating in the Development of High-Fidelity Neurotransmission at the Calyx of Held Synapse

Indu Joshi, Shahira Shokralla, Paul Titis, and Lu-Yang Wang

The Program for Brain and Behavioral Research and Division of Neurology, The Hospital for Sick Children and Department of Physiology, University of Toronto, Toronto, Ontario, Canada M5G, 1X8

During early postnatal development of auditory synapses, the decay time course of AMPA receptor (AMPAR) EPSCs accelerates markedly, but the mechanisms underlying this process remain uncertain. Using the developing calyx of Held synapse in the mouse auditory brainstem, we have examined presynaptic and postsynaptic elements that may regulate decay kinetics of AMPAR EPSCs We found that the decay time kinetics was voltage dependent in both immature and mature synapses, being slower at positive potentials than negative potentials. By recording evoked miniature events in extracellular Ca^{2+} or Sr^{2+}, we revealed a significant decrease in decay time constants of EPSCs as maturation progresses. On the basis of internal and external polyamine block of AMPAR EPSCs and immunohistochemistry assays with subunit-specific antibodies, we demonstrated that the glutamate receptor (GluR) 2 subunit is virtually absent at all developmental ages. Antibody staining patterns suggest a gradual shift in subunit composition from GluR1- to GluR3/4-dominant phenotypes. Kinetic analyses of deactivation, desensitization, and recovery from desensitization in outside-out patches in response to ultrafast application of glutamate lend supportive evidence that such a shift in the gating phenotype likely accounts for the accelerated time course throughout development. Finally, by pharmacologically manipulating AMPAR gating and using simulated EPSCs to evoke action potentials, we demonstrated that rapid decay kinetics of AMPAR EPSCs is essential for this synapse to accommodate high-frequency firing without compromising spike amplitude. Hence, developmental alterations in the subunit composition likely dictate changes in the time course of AMPAR EPSCs and play an indispensable role in the refinement of high-fidelity neurotransmission at the calyx of Held synapse.

The Journal of Neuroscience, January 7, 2004 • 24(1):183–196

Persistent Progenitors at the Retinal Margin of *ptc*+/− Mice

Ala Moshiri and Thomas A. Reh

Neurobiology and Behavior Program, Department of Biological Structure, University of Washington, School of Medicine, Seattle, Washington 98195

The hedgehog signaling pathway is a key regulator of neural development, affecting both proliferation and differentiation of neural progenitors. Sonic hedgehog (Shh) is a mitogenic factor for retinal progenitors *in vitro*. To determine whether this signaling system is important *in vivo* for regulating retinal progenitor proliferation, we analyzed mice with a single functional allele of the Shh receptor *patched* (*ptc*). We found that *ptc*+/− mice had increased numbers of neural progenitors at every stage of retinal development that we examined. In addition, these mice had persistent progenitors at the retinal margin for up to 3 months of age, reminiscent of the ciliary marginal zone of lower vertebrates. To test whether the progenitors at the retinal margin of *ptc*+/− mice could be induced to regenerate retinal neurons in response to damage, we bred *ptc*+/− mice onto a retinal degeneration background (pro23his rhodopsin transgenic) and labeled newly generated cells with combined immunohistochemistry for bromodeoxyuridine and retinal neuron and photoreceptor-specific markers. We found newly generated neurons and photoreceptors at the retinal margin in *ptc*+/−; pro23his mice. We propose that the Shh pathway may act as a regulator of both prenatal and postnatal retinal growth.

The Journal of Neuroscience, January 7, 2004 • 24(1):229–237

Neurite Outgrowth by the Alternatively Spliced Region of Human Tenascin-C Is Mediated by Neuronal $\alpha7\beta1$ Integrin

Mary Lynn T. Mercado,* Alam Nur-e-Kamal,* Hsing-Yin Liu,* Stephane R. Gross,* Reza Movahed, and Sally Meiners
Department of Pharmacology, University of Medicine and Dentistry of New Jersey, Robert Wood Johnson Medical School, Piscataway, New Jersey 08854

The region of tenascin-C containing only alternately spliced fibronectin type-III repeat D (fnD) increases neurite outgrowth by itself and also as part of tenascin-C. We previously localized the active site within fnD to an eight amino acid sequence unique to tenascin-C, VFDNFVLK, and showed that the amino acids FD and FV are required for activity. The purpose of this study was to identify the neuronal receptor that interacts with VFDNFVLK and to investigate the hypothesis that FD and FV are important for receptor binding. Function-blocking antibodies against both $\alpha7$ and $\beta1$ integrin subunits were found to abolish VFDNFVLK-mediated process extension from cerebellar granule neurons. VFDNFVLK but not its mutant, VSPNGSLK, induced clustering of neuronal $\beta1$ integrin immunoreactivity. This strongly implicates FD and FV as important structural elements for receptor activation. Moreover, biochemical experiments revealed an association of the $\alpha7\beta1$ integrin with tenascin-C peptides containing the VFDNFVLK sequence but not with peptides with alterations in FD and/or FV. These findings are the first to provide evidence that the $\alpha7\beta1$ integrin mediates a response to tenascin-C and the first to demonstrate a functional role for the $\alpha7\beta1$ integrin receptor in CNS neurons.
The Journal of Neuroscience, January 7, 2004 • 24(1):238–247

Neuronal Birthdate-Specific Gene Transfer with Adenoviral Vectors

Mitsuhiro Hashimoto[1] and Katsuhiko Mikoshiba[1,2]
[1]Laboratory for Developmental Neurobiology, RIKEN Brain Science Institute, 2-1 Hirosawa, Wako-shi, Saitama 351-0198, Japan, and [2]Department of Molecular Neurobiology, The Institute of Medical Science, The University of Tokyo, Tokyo 108-8639, Japan

The multilayered structure of the cerebral cortex has been studied in detail. Early-born neurons migrate into the inner layer and late-born neurons migrate into more superficial layers, thus establishing an inside-out gradient. The progenitor cells appear to acquire layer-specific properties at the time of neuronal birth; however, the molecular mechanisms of cell-fate acquisition are still unclear, because it has been difficult to identify a cohort of birthdate-related progenitor cells. Using replication-defective adenoviral vectors, we successfully performed "pulse gene transfer" into progenitor cells in a neuronal birthdate-specific manner. When adenoviral vectors were injected into the midbrain ventricle of mouse embryos between embryonic day 10.5 (E10.5) and E14.5, the adenoviral vectors introduced a foreign gene into a specific cohort of birthdate-related progenitor cells. The virally infected cohorts developed normally into cortical neurons and formed the canonical cortical layers in an inside-out manner. This technique allows us to distinguish a cohort of birthdate-related progenitor cells from other progenitor cells with different birthdates and to introduce a foreign gene into specific subsets of cortical layers by performing adenoviral injection at specific times. This adenovirus-meditated gene transfer technique will enable us to examine the properties of each subset of progenitor cells that share the same neuronal birthdate.
The Journal of Neuroscience, January 7, 2004 • 24(1):286–296

BEHAVIORAL/SYSTEMS/COGNITIVE

Dopamine D1/D5 Receptor Modulates State-Dependent Switching of Soma-Dendritic Ca^{2+} Potentials via Differential Protein Kinase A and C Activation in Rat Prefrontal Cortical Neurons

Clint E. Young and Charles R. Yang
Neuroscience Discovery, Eli Lilly & Company, Lilly Corporate Center, Indianapolis, Indiana 46285-0510

To determine the nature of dopamine modulation of dendritic Ca^{2+} signaling in layers V–VI prefrontal cortex (PFC) neurons, whole-cell Ca^{2+} potentials were evoked after blockade of Na^+ and K^+ channels. Soma-dendritic Ca^{2+} spikes evoked by suprathreshold depolarizing pulses, which could be terminated by superimposed brief intrasomatic hyperpolarizing pulses, are blocked by the L-type Ca^{2+} channel antagonist nimodipine (1 μM). The D1/D5 receptor agonist dihydrexidine (DHX) (0.01–10 μM; 5 min) or R-(+)SKF81291 (10 μM) induced a prolonged (>30 min) dose-dependent peak suppression of these Ca^{2+} spikes. This effect was dependent on $[Ca^{2+}]_i$- and protein kinase C (PKC)-dependent mechanisms because $[Ca^{2+}]_i$ chelation by BAPTA or inhibition of PKC by bisindolylmaleimide (BiM1), but not inhibition of $[Ca^{2+}]_i$ release with heparin or Xestospongin C, prevented the D1-mediated suppression of Ca^{2+} spikes. Depolarizing pulses subthreshold to activating a Ca^{2+} spike evoked a nimodipine-sensitive Ca^{2+} "hump" potential. D1/D5 stimulation induced an *N*-[2-((*o*-bromocinamyl)amino)ethyl]-5-isoquinolinesulfonamide (H-89)- or internal PKA inhibitory peptide$_{[5-24]}$-sensitive (PKA-dependent) transient (~7 min) potentiation of the hump potential to full Ca^{2+} spike firing. Furthermore, application of DHX in the presence of the PKC inhibitor BiM1 or internal PKC inhibitory peptide$_{[19-36]}$ resulted in persistent firing of full Ca^{2+} spike bursts, suggesting that a D1/D5–PKA mechanism switches subthreshold Ca^{2+} hump potential to fire full Ca^{2+} spikes, which are eventually turned off by a D1/D5–Ca^{2+}-dependent PKC mechanism. This depolarizing state-dependent, D1/D5-activated, bi-directional switching of soma-dendritic L-type Ca^{2+} channels via PKA-dependent potentiation and PKC-dependent suppression may provide spatiotemporal regulation of synaptic integration and plasticity in PFC.
The Journal of Neuroscience, January 7, 2004 • 24(1):8–23

Preterm Fetal Hypoxia–Ischemia Causes Hypertonia and Motor Deficits in the Neonatal Rabbit: A Model for Human Cerebral Palsy?

atthew Derrick,[1] Ning Ling Luo,[3] Joanne C. Bregman,[1] Tamas Jilling,[1] Xinhai Ji,[1] Kara Fisher,[1] Candece L. Gladson,[2] Douglas J. Beardsley,[3] Geoffrey Murdoch,[4] Stephen A. Back,[3,5]* and Sidhartha Tan[1]*

[1]Department of Pediatrics, Northwestern University, and Evanston Northwestern Healthcare, Evanston, Illinois 60201, [2]Department of Neuro-Pathology, University of Alabama at Birmingham, Birmingham, Alabama 35243, and Departments of [3]Pediatrics, [4]Pathology, and [5]Neurology, Oregon Health Sciences University, Portland, Oregon 97201

Prenatal hypoxia–ischemia to the developing brain has been strongly implicated in the subsequent development of the hypertonic motor deficits of cerebral palsy (CP) in premature and full-term infants who present with neonatal encephalopathy. Despite the enormous impact of CP, there is no animal model that reproduces the hypertonia and motor disturbances of this disorder. We report a rabbit model of *in utero* placental insufficiency, in which hypertonia is accompanied by marked abnormalities in motor control. Preterm fetuses (67–70% gestation) were subjected to sustained global hypoxia. The dams survived and gave spontaneous birth. At postnatal day 1, the pups that survived were subjected to a battery of neurobehavioral tests developed specifically for these animals, and the tests were videotaped and scored in a masked manner. Newborn pups of hypoxic groups displayed significant impairment in multiple tests of spontaneous locomotion, reflex motor activity, and the coordination of suck and swallow. Increased tone of the limbs at rest and with active flexion and extension were observed in the survivors of the preterm insult.

Histopathological studies identified a distinct pattern of acute injury to subcortical motor pathways that involved the basal ganglia and thalamus. Persistent injury to the caudate putamen and thalamus at P1 was significantly correlated with hypertonic motor deficits in the hypoxic group. Antenatal hypoxia–ischemia at preterm gestation results in hypertonia and abnormalities in motor control. These findings provide a unique behavioral model to define mechanisms and sequelae of perinatal brain injury from antenatal hypoxia–ischemia.

The Journal of Neuroscience, January 7, 2004 • 24(1):24–34

Phenotype Matters: Identification of Light-Responsive Cells in the Mouse Suprachiasmatic Nucleus

Ilia N. Karatsoreos,[1] Lily Yan,[1] Joseph LeSauter,[3] and Rae Silver[1,2,3]

Departments of [1]Psychology and [2]Anatomy and Cell Biology, Columbia University, New York, New York 10027, and [3]Department of Psychology, Barnard College of Columbia University, New York, New York 10027

The suprachiasmatic nucleus (SCN) of the hypothalamus is the neural locus of the circadian clock. To explore the organization of the SCN, two strains of transgenic mice, each bearing a jellyfish green fluorescent protein (GFP) reporter, were used. In one, GFP was driven by the promoter region of the mouse *Period1* gene (*mPer1*) (*Per1*::GFP mouse), whereas in the other, GFP was inserted in the promoter region of calbindin-D_{28K}–bacterial artificial chromosome (*CalB*::GFP mouse). In the latter mouse, GFP-containing SCN cells are immunopositive for gastrin-releasing peptide. In both mouse lines, light-induced *Per1* mRNA and Fos are localized to the SCN subregion containing gastrin-releasing peptide. Double-label immunohistochemistry reveals that most gastrin-releasing peptide cells (~70%) contain Fos after a brief light pulse. To determine the properties of SCN cells in this light-responsive region, we examined the expression of rhythmic *Period* genes and proteins. Gastrin-releasing peptide-containing cells do not express detectable rhythms in these key components of the molecular circadian clock. The results support the view that the mammalian SCN is composed of functionally distinct cell groups, of which some are light induced and others are rhythmic with respect to clock gene expression. Furthermore, the findings suggest that gastrin-releasing peptide is a potential mediator of intercellular communication between light-induced and oscillator cells within the SCN.

The Journal of Neuroscience, January 7, 2004 • 24(1):68–75

Input-Specific Modulation of Neurotransmitter Release in the Lateral Horn of the Spinal Cord via Adenosine Receptors

Ruth E. Brooke, Jim Deuchars, and Susan A. Deuchars

School of Biomedical Sciences, University of Leeds, Leeds LS2 9NQ, United Kingdom

Activation of adenosine $A2_A$ receptors ($A2_A$Rs) in the CNS produces a variety of neuromodulatory actions dependent on the region and preparation examined. In autonomic regions of the spinal cord, A1R activation decreases excitatory synaptic transmission, but the effects of $A2_A$R stimulation are unknown. We sought to determine the location and function of the $A2_A$Rs in the thoracic spinal cord, focusing on the intermediolateral cell column (IML). $A2_A$R immunoreactivity was observed throughout the gray matter, with particularly dense immunostaining in regions containing sympathetic preganglionic neurons (SPNs), namely, the IML and intercalated nucleus. Electron microscopy revealed $A2_A$R immunoreactivity within presynaptic terminals and in postsynaptic structures in the IML. To study the functional relevance of these $A2_A$Rs, visualized whole-cell patch-clamp recordings were made from electrophysiologically identified SPNs and interneurons within the IML. The $A2_A$R agonist c2-[*p*-(carboxyethyl)phenethylamino]-5′-*N*-ethylcarboxyamidoadenosine (CGS 21680) had no significant effect on EPSPs but increased the amplitude of IPSPs elicited by stimulation of the lateral funiculus. These effects were attributable to activation of presynaptic $A2_A$Rs because CGS 21680 application altered the paired pulse ratio. Furthermore, neurons in the IML that have IPSPs increased via $A2_A$R activation also receive excitatory inputs that are inhibited by A1R activation. These data show that activating $A2_A$Rs increase inhibitory but not excitatory transmission onto neurons in the IML. Simultaneous activation of A1Rs and $A2_A$Rs therefore could facilitate inhibition of the postsynaptic neuron, leading to an overall reduction of sympathetic nervous activity.

The Journal of Neuroscience, January 7, 2004 • 24(1):127–137

Molecular Basis of an Inherited Form of Incomplete Achromatopsia

Dimitri Tränkner,[1]* Herbert Jägle,[2,4]* Susanne Kohl,[3,4]* Eckart Apfelstedt-Sylla,[4] Lindsay T. Sharpe,[2,4,5] U. Benjamin Kaupp,[1] Eberhart Zrenner,[4] Reinhard Seifert,[1] and Bernd Wissinger[3,4]

[1]Institut für Biologische Informationsverarbeitung, Forschungszentrum Jülich, 52425 Jülich, Germany, [2]Psychophysisches Labor, [3]Molekulargenetisches Labor, and [4]Abteilung für Pathophysiologie des Sehens und Neuro-Ophthalmologie, Universitäts-Augenklinik, 72076 Tübingen, Germany, and [5]Department of Psychology, School of Biology, University of Newcastle upon Tyne, Newcastle upon Tyne NE2 4HH, United Kingdom

Mutations in the genes encoding the CNGA3 and CNGB3 subunits of the cyclic nucleotide-gated (CNG) channel of cone photoreceptors have been associated with autosomal recessive achromatopsia. Here we analyze the molecular basis of achromatopsia in two siblings with residual cone function. Psychophysical and electroretinographic analyses show that the light sensitivity of the cone system is lowered, and the signal transfer from cones to secondary neurons is perturbed. Both siblings carry two mutant *CNGA3* alleles that give rise to channel subunits with different single-amino acid substitutions. Heterologous expression revealed that only one mutant forms functional channels, albeit with grossly altered properties, including changes in Ca^{2+} blockage and permeation. Surprisingly, coexpression of this mutant subunit with CNGB3 rescues the channel phenotype, except for the Ca^{2+} interaction. We argue that these alterations are responsible for the perturbations in light sensitivity and synaptic transmission.
The Journal of Neuroscience, January 7, 2004 • 24(1):138–147

The Impact of Suppressive Surrounds on Chromatic Properties of Cortical Neurons

Samuel G. Solomon, Jonathan W. Peirce, and Peter Lennie

Center for Neural Science, New York University, New York, New York 10003

Stimulation of the suppressive surround of a cortical neuron affects the responsivity and tuning of the classical receptive field (CRF) on several stimulus dimensions. In V1 and V2 of macaques prepared for acute electrophysiological experiments, we explored the chromatic sensitivity of the surround and its influence on the chromatic tuning of the CRF. We studied receptive fields of single neurons with patches of drifting grating of optimal spatial frequency and orientation and variable size, modulated along achromatic or isoluminant color directions. The responses of most neurons declined as the patch was enlarged beyond the optimal size (surround suppression). In V1 the suppression evoked by isoluminant gratings was less than one-half that evoked by achromatic gratings. Consequently, many cells were most sensitive to achromatic modulation when patches just covered the CRF but were most sensitive to isoluminant modulation when patches were enlarged to cover the suppressive surround. Non-oriented neurons that were strongly chromatically opponent generally lacked suppressive surrounds. In V2 most neurons showed equal surround suppression from isoluminant gratings and achromatic gratings. This makes the relative sensitivity of V2 neurons to achromatic and isoluminant gratings mainly independent of the size of the grating. We also measured the chromatic properties of the CRF in the presence of differently colored surrounds. In neither V1 nor V2 did the surround alter the chromatic tuning of the CRF. Cortical mechanisms sensitive to chromatic contrast seem to provide little input to the suppressive surrounds of V1 neurons but substantial input to those of V2 neurons.
The Journal of Neuroscience, January 7, 2004 • 24(1):148–160

Neural Substrates Mediating Human Delay and Trace Fear Conditioning

David C. Knight,[1] Dominic T. Cheng,[1] Christine N. Smith,[1] Elliot A. Stein,[2] and Fred J. Helmstetter[1,2]

[1]Department of Psychology, University of Wisconsin–Milwaukee, Milwaukee, Wisconsin 53201, and [2]Department of Psychiatry, Medical College of Wisconsin, Milwaukee, Wisconsin 53226

Previous functional magnetic resonance imaging (fMRI) studies with human subjects have explored the neural substrates involved in forming associations in Pavlovian fear conditioning. Most of these studies used delay procedures, in which the conditioned stimulus (CS) and unconditioned stimulus (UCS) coterminate. Less is known about brain regions that support trace conditioning, a procedure in which an interval of time (trace interval) elapses between CS termination and UCS onset. Previous work suggests significant overlap in the neural circuitry supporting delay and trace fear conditioning, although trace conditioning requires recruitment of additional brain regions. In the present event-related fMRI study, skin conductance and continuous measures of UCS expectancy were recorded concurrently with whole-brain blood oxygenation level-dependent (BOLD) imaging during direct comparison of delay and trace discrimination learning. Significant activation was observed within the visual cortex for all CSs. Anterior cingulate and medial thalamic activity reflected associative learning common to both delay and trace procedures. Activations within the supplementary motor area (SMA), frontal operculum, middle frontal gyri, and inferior parietal lobule were specifically associated with trace interval processing. The hippocampus displayed BOLD signal increases early in training during all conditions; however, differences were observed in hippocampal response magnitude related to the accuracy of predicting UCS presentations. These results demonstrate overlapping patterns of activation within the anterior cingulate, medial thalamus, and visual cortex during delay and trace procedures, with additional recruitment of the hippocampus, SMA, frontal operculum, middle frontal gyrus, and inferior parietal lobule during trace conditioning. These data suggest that the hippocampus codes temporal information during trace conditioning, whereas brain regions supporting working memory processes maintain the CS–UCS representation during the trace interval.
The Journal of Neuroscience, January 7, 2004 • 24(1):218–228

Widespread Thalamic Terminations of Fibers Arising in the Superficial Medullary Dorsal Horn of Monkeys and Their Relation to Calbindin Immunoreactivity

Alessandro Graziano and Edward G. Jones
Center for Neuroscience, University of California Davis, Davis, California 95616

The relay of pain fibers from the spinal and medullary dorsal horn in the thalamus has become a controversial issue. This study analyzed the relationship of fibers arising in lamina I to nuclei in and around the caudal pole of the ventral posterior nuclear complex and especially to a zone of calbindin-dense immunoreactivity (VMpo) identified by some authors as the sole thalamic relay for these fibers. We show that the densest zone of calbindin immunoreactivity is part of a more extensive, calbindin-immunoreactive region that lies well within the medial tip of the ventral posterior medial nucleus (VPM), as delineated by other staining methods, and prove that the use of different anti-calbindin antibodies cannot account for differences in interpretations of the organization of the posterior thalamic region. By combining immunocytochemical staining with anterograde tracing from injections involving lamina I, we demonstrate widespread fiber terminations that are not restricted to the calbindin-rich medial tip of VPM and show that the lamina I arising fibers are not themselves calbindin immunoreactive. This study disproves the existence of VMpo as an independent thalamic pain nucleus or as a specific relay in the ascending pain system.
The Journal of Neuroscience, January 7, 2004 • 24(1):248–256

NEUROBIOLOGY OF DISEASE

Neuroprotective Function of the PGE_2 EP2 Receptor in Cerebral Ischemia

Louise McCullough,[1] Liejun Wu,[1] Norman Haughey,[1] Xibin Liang,[1] Tracey Hand,[1] Qian Wang,[1] Richard M. Breyer,[3] and Katrin Andreasson[1,2]
Departments of [1]Neurology and [2]Neuroscience, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205, and [3]Department of Medicine, Division of Nephrology, Vanderbilt University School of Medicine, Nashville, Tennessee 37232

The cyclooxygenases COX-1 and COX-2 catalyze the first committed step of prostaglandin synthesis from arachidonic acid. Previous studies in rodent stroke models have shown that the inducible COX-2 isoform promotes neuronal injury, and the administration of COX-2 inhibitors reduces infarct volume. We investigated the function of PGE_2, a principal prostaglandin product of COX-2 enzymatic activity, in neuronal survival in cerebral ischemia. PGE_2 exerts its downstream effects by signaling through a class of four distinct G-protein-coupled EP receptors (for E-prostanoid: EP1, EP2, EP3, and EP4) that have divergent effects on cAMP and phosphoinositol turnover and different anatomical distributions in brain. The EP2 receptor subtype is abundantly expressed in cerebral cortex, striatum, and hippocampus, and is positively coupled to cAMP production. *In vitro* studies of dispersed neurons and organotypic hippocampal cultures demonstrated that activation of the EP2 receptor was neuroprotective in paradigms of NMDA toxicity and oxygen glucose deprivation. Pharmacologic blockade of EP2 signaling by inhibition of protein kinase A activation reversed this protective effect, suggesting that EP2-mediated neuroprotection is dependent on cAMP signaling. In the middle cerebral artery occlusion–reperfusion model of transient forebrain ischemia, genetic deletion of the EP2 receptor significantly increased cerebral infarction in cerebral cortex and subcortical structures. These studies indicate that activation of the PGE_2 EP2 receptor can protect against excitotoxic and anoxic injury in a cAMP-dependent manner. Taken together, these data suggest a novel mechanism of neuroprotection mediated by a dominant PGE_2 receptor subtype in brain that may provide a target for therapeutic intervention.
The Journal of Neuroscience, January 7, 2004 • 24(1):257–268

Huntingtin Bodies Sequester Vesicle-Associated Proteins by a Polyproline-Dependent Interaction

Zheng-Hong Qin,[1] Yumei Wang,[1] Ellen Sapp,[1] Benjamin Cuiffo,[1] Erich Wanker,[3] Michael R. Hayden,[4] Kimberly B. Kegel,[1] Neil Aronin,[2] and Marian DiFiglia[1]
[1]Laboratory of Cellular Neurobiology, Massachusetts General Hospital and Harvard Medical School, Charlestown, Massachusetts 02129, [2]Departments of Medicine and Cell Biology, University of Massachusetts Medical Center, Worcester, Massachusetts 01655, [3]Max-Planck-Institut for Molecular Genetics D-14195 Berlin, Germany, and [4]Molecular Medicine and Therapeutics, University of British Columbia, Vancouver, British Columbia V5Z 4H4, Canada

Polyglutamine expansion in the N terminus of huntingtin (htt) causes selective neuronal dysfunction and cell death by unknown mechanisms. Truncated htt expressed *in vitro* produced htt immunoreactive cytoplasmic bodies (htt bodies). The fibrillar core of the mutant htt body resisted protease treatment and contained cathepsin D, ubiquitin, and heat shock protein (HSP) 40. The shell of the htt body was composed of globules 14–34 nm in diameter and was protease sensitive. HSP70, proteasome, dynamin, and the htt binding partners htt interacting protein 1 (HIP1), SH3-containing Grb2-like protein (SH3GL3), and 14.7K-interacting protein were reduced in their normal location and redistributed to the shell. Removal of a series of prolines adjacent to the polyglutamine region in htt blocked formation of the shell of the htt body and redistribution of dynamin, HIP1, SH3GL3, and proteasome to it. Internalization of transferrin was impaired in cells that formed htt bodies. In cortical neurons of Huntington's disease patients with early stage pathology, dynamin immunoreactivity accumulated in cytoplasmic bodies. Results suggest that accumulation of a nonfibrillar form of mutant htt in the cytoplasm contributes to neuronal dysfunction by sequestering proteins involved in vesicle trafficking.
The Journal of Neuroscience, January 7, 2004 • 24(1):269–281

MOVING?

If you are a Society for Neuroscience member, please direct all renewals, new subscriptions, address changes, and subscription inquiries to:

Membership Department
Society for Neuroscience
11 Dupont Circle, N.W.
Suite 500
Washington, D.C. 20036
Phone: 202-462-6688
E-mail: membership@sfn.org

If you have an institutional or individual nonmember subscription, please direct all renewals, new subscriptions, address changes, and subscription inquiries to:

The Journal of Neuroscience
Subscription Services
Society for Neuroscience
11 Dupont Circle, N.W.
Suite 500
Washington, D.C. 20036
Phone: 202-462-6688
E-mail: jn@sfn.org

POST-DOCTORAL FELLOWSHIPS IN ALCOHOL ABUSE AND ADDICTIONS RESEARCH AT BROWN UNIVERSITY CENTER FOR ALCOHOL AND ADDICTION STUDIES

The Center for Alcohol and Addiction Studies at Brown University is recruiting for fellows in two associated post-doctoral fellowship training programs, one funded by NIAAA in alcohol abuse and addictions and one funded by NIDA in substance abuse. The training programs provide postdoctoral research training for biomedical, behavioral, social scientists and health care professionals who wish to conduct high quality research in the early intervention and treatment of alcohol and other drug problems. Areas of expertise in the Fellowship include pharmacotherapy, neurobiology and genetics of Alcohol and Substance Dependence, and animal models of alcohol and drug reinforcement, consumption, sensitization and conditioned reward. For further information/ application go to: http://www.caas.brown.edu