

This Week in The Journal

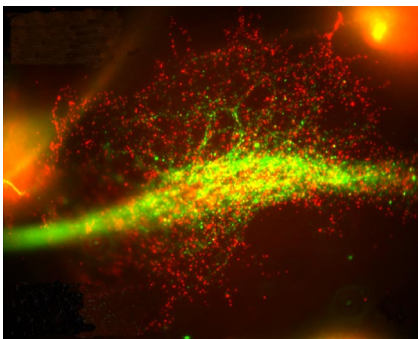
● Cellular/Molecular

Amyloid Precursor Protein Fragments Are Trafficked Separately

Virgil Muresan, Nicholas H. Varvel, Bruce T. Lamb, and Zoia Muresan

(see pages 3565–3578)

Amyloid β is notorious for its hypothesized role in Alzheimer's disease, but the normal function of its parent protein—amyloid precursor protein (APP)—is unknown. APP contains multiple functional domains that, together with effects of APP knock-out, suggest roles in vesicle transport, transcriptional regulation, neurogenesis, neurite outgrowth, and synaptogenesis. But each APP molecule is cleaved by two secretases to create three fragments, and whether the fragments, the full-length protein, or both have important roles is not known. To gain insight into this question, Muresan et al. labeled neurons with antibodies that recognized N-terminal, central (amyloid β), or C-terminal domains of APP. Labeling in neurites was largely nonoverlapping, suggesting the fragments are trafficked along distinct pathways and the full-length molecule is rarely transported out of the soma. Interestingly, phosphorylated C-terminal fragments were the only fragments present in the growth cone periphery, and they were concentrated along the protruding edge in turning growth cones.



Phosphorylated C-terminal fragments of APP (red), localize to the peripheral regions of growth cones, whereas N-terminal fragments (green) are confined to the central portion. See the article by Muresan et al. for details.

▲ Development/Plasticity/Repair

BDNF Is Required for Taste Bud Innervation

Liquan Ma, Grace F. Lopez, and Robin F. Krimm

(see pages 3354–3364)

Gustatory neurons innervate fungiform papillae on the tongue. These taste regions express brain-derived neurotrophic factor (BDNF) during development, suggesting that the precise growth of gustatory axons to their targets depends on BDNF. In support of this hypothesis, Ma et al. report that taste buds were not innervated in BDNF-null mice. Instead, branching of the gustatory nerve increased near the surface of the tongue and axon terminals appeared randomly distributed. After a few days, the number of axon terminals in mutant mice was reduced, but remaining terminals innervated papillae. This suggests that excessive gustatory nerve branching resulted in chance contacts between gustatory nerves and papillae, and that these contacts were maintained while unconnected terminals were pruned. Together with previous studies in which overexpression of BDNF caused inappropriate innervation of the tongue, the results reported here suggest that BDNF is necessary and sufficient for the final steps in guiding gustatory axons to their targets.

■ Behavioral/Systems/Cognitive

Exogenous TRPM8 Expression Allows Selective Neuronal Activation

Nathan C. Peabody, Jascha B. Pohl, Fengqiu Diao, Andrew P. Vreede, David J. Sandstrom, Howard Wang, Paul K. Zelensky, and Benjamin H. White

(see pages 3343–3353)

A new genetic technique that allows selective activation of specific neurons has been developed by Peabody et al. to investigate the neuronal control of wing expansion in *Drosophila*. The authors expressed TRPM8, a cold-sensitive nonselective cation channel, specifically in neurons that endogenously express crustacean cardioactive peptide (CCAP), which are thought to regulate wing expansion. Subsequent cooling shortened the time spent perching by flies that had recently emerged from the pupal case, and it

accelerated the start of abdominal-flexing and air-swallowing behaviors that pump hemolymph into the unfolding wings. This effect was most dramatic in flies that were confined to a small space. Such confinement increased by approximately 20-fold the amount of time normal flies walked around before perching. But in flies expressing TRPM8, this perch-selection phase was reduced to the duration seen in unconfined wild-type flies. These results establish CCAP-expressing neurons as key control points in the production of wing-expansion behaviors.

◆ Neurobiology of Disease

Synaptic Degeneration Occurs Early in EAE

Diego Centonze, Luca Muzio, Silvia Rossi, Francesca Cavasinni, Valentina De Chiara, Alessandra Bergami, Alessandra Musella, Marcello D'Amelio, Virve Cavallucci, Alessandro Martorana, Andrea Bergamaschi, Maria Teresa Cencioni, Adamo Diamantini, Erica Butti, Giancarlo Comi, Giorgio Bernardi, Francesco Cecconi, Luca Battistini, Roberto Furlan, and Gianvito Martino

(see pages 3442–3452)

Multiple sclerosis results from chronic inflammation of the CNS that causes demyelination and axon loss. A similar pathology can be produced in mice by injecting myelin protein fragments into the blood, which induces experimental autoimmune encephalomyelitis. Using this model system, Centonze et al. found that synaptic loss in the striatum is an early event in disease progression. In the presymptomatic stage of the disease, before demyelination or motor deficits were detected, AMPA receptor expression and phosphorylation were increased in striatal neurons, which prolonged the decay phase of spontaneous EPSCs and increased their frequency. This was accompanied by an increase in markers for synaptic degeneration and spine loss. These changes were likely induced by release of tumor necrosis factor by activated microglia and subsequent downregulation of the immediate early gene *Arc/Arg3.1*. Blocking AMPA receptors reduced clinical signs and increased spine density, and therefore may be an effective treatment in multiple sclerosis.

The Journal of Neuroscience

March 18, 2009 • Volume 29 Number 11 • www.jneurosci.org



Cover legend: Gustatory axon bundles innervating a mouse tongue. A scanning electron microscope image was superimposed on an image of the same tongue showing Dil-labeled fiber bundles. This overlay allowed visualization and quantification of innervated and uninnervated taste papillae. In this E16.5 wild-type tongue, most taste papillae are successfully innervated. For more information, see the article by Ma in this issue (pages 3354–3364).

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Articles

CELLULAR/MOLECULAR

Evolutionary Conservation of Vertebrate Blood–Brain Barrier Chemoprotective Mechanisms in *Drosophila*

Fahima Mayer,^{1*} Nasima Mayer,^{1*} Leslie Chinn,² Robert L. Pinsonneault,¹ Deanna Kroetz,² and Roland J. Bainton¹

¹Department of Anesthesia and Perioperative Care, San Francisco General Hospital, and ²Department of Pharmaceutical Chemistry, University of California at San Francisco, San Francisco, California 94143

Pharmacologic remedy of many brain diseases is difficult because of the powerful drug exclusion properties of the blood–brain barrier (BBB). Chemical isolation of the vertebrate brain is achieved through the highly integrated, anatomically compact and functionally overlapping chemical isolation processes of the BBB. These include functions that need to be coordinated between tight diffusion junctions and unidirectionally acting xenobiotic transporters. Understanding of many of these processes has been hampered, because they are not well mimicked by *ex vivo* models of the BBB and have been experimentally difficult and expensive to disentangle in intact rodent models. Here we show that the *Drosophila melanogaster* (*Dm*) humoral/CNS barrier conserves the xenobiotic exclusion properties found in the vertebrate vascular endothelium. We characterize a fly ATP binding cassette (ABC) transporter, Mdr65, that functions similarly to mammalian xenobiotic BBB transporters and show that varying its levels solely in the *Dm* BBB changes the inherent sensitivity of the barrier to cytotoxic pharmaceuticals. Furthermore, we demonstrate orthologous function between Mdr65 and vertebrate ABC transporters by rescuing chemical protection of the *Dm* brain with human MDR1/Pgp. These data indicate that the ancient origins of CNS chemoprotection extend to both conserved molecular means and functionally analogous anatomic spaces that together promote CNS selective drug partition. Thus, *Dm* presents an experimentally tractable system for analyzing physiological properties of the BBB in an intact organism.

The Journal of Neuroscience, March 18, 2009 • 29(11):3538–3550

Disruption of Cdk5-Associated Phosphorylation of Residue Threonine-161 of the δ -Opioid Receptor: Impaired Receptor Function and Attenuated Morphine Antinociceptive Tolerance

Wei-Yan Xie,* Yi He,* Yan-Rui Yang, Ya-Fang Li, Kai Kang, Bao-Ming Xing, and Yun Wang

Neuroscience Research Institute and Department of Neurobiology, The Key Laboratory for Neuroscience of the Ministry of Education and Health, Peking University Health Science Center, Beijing 100083, People's Republic of China

Morphine is the most commonly used and most effective analgesic in the clinic. However, its use is limited by the tolerance. Evidence indicates that the δ -opioid receptor (DOR) is essential for morphine antinociceptive tolerance; however, their underlying mechanisms are poorly understood. Here, we show that cyclin-dependent kinase 5 (Cdk5), activated in morphine antinociceptive tolerance, directly phosphorylates DOR at Thr-161 in DRG neurons. Cdk5 was found to phosphorylate Thr-161 in the second loop of DOR, but not the corresponding residue in the μ -opioid receptor (MOR). Phosphorylation at Thr-161 is required for normal cell surface expression of DOR, and the formation of DOR–MOR heterodimers. Our studies indicated that inhibition of Cdk5 activity or overexpression of a DOR mutant lacking the Cdk5 phosphorylation site displayed relatively low cell surface expression and relatively low abilities to form heterodimers of DOR and MOR; intrathecal delivery of a construct expressing the T161A mutant of DOR attenuated morphine antinociceptive tolerance in rats, suggesting that Thr-161 phosphorylation contributed to Cdk5-mediated morphine antinociceptive tolerance. Furthermore, an engineered Tat fusion-interfering peptide corresponding to the second intracellular loop of DOR (Tat-DOR-2L), reduced the cell surface expression of DOR, disrupted the formation of DOR–MOR heterodimers, and significantly attenuated the development of morphine antinociceptive tolerance after intrathecal injection. The present study indicates that Cdk5-mediated phosphorylation of DOR at Thr-161 plays a crucial role in the development of morphine tolerance and suggests the possibility of targeting DOR phosphorylation at Thr-161 to attenuate morphine antinociceptive tolerance during pain management.

The Journal of Neuroscience, March 18, 2009 • 29(11):3551–3564

The Cleavage Products of Amyloid- β Precursor Protein Are Sorted to Distinct Carrier Vesicles That Are Independently Transported within Neurites

Virgil Muresan,¹ Nicholas H. Varvel,^{2,3} Bruce T. Lamb,^{2,3} and Zoia Muresan¹

¹Department of Pharmacology and Physiology, University of Medicine and Dentistry of New Jersey, New Jersey Medical School, Newark, New Jersey 07103,

²Department of Neurosciences, Lerner Research Institute, The Cleveland Clinic Foundation, Cleveland, Ohio 44195, and ³Department of Neurosciences, Case Western Reserve University, Cleveland, Ohio 44106

The amyloid- β ($A\beta$) precursor protein (APP), a transmembrane protein that undergoes proteolytic cleavage into defined fragments, has been implicated in axonal transport. The proposed role of APP as a vesicle receptor for the microtubule motor kinesin-1 has relevance for the pathogenesis of Alzheimer's disease. Nevertheless, this function, which relies on the transport to the cell periphery of full-length APP rather than its cleavage fragments, remains controversial. Other proposed functions of APP, such as regulating transcription, neurogenesis, cell movement, or neurite growth also rely on APP's presence as a full-length protein at the cell surface, implying that APP cleavage occurs after its transport to the cell periphery. To test this hypothesis, we mapped the localization of various APP epitopes in neurons in culture and in the mouse brain. Surprisingly, epitopes from the N-terminal, C-terminal, and central ($A\beta$) domains of APP each showed a distinct distribution throughout the cell and rarely colocalized. Within neurites, these epitopes were localized to distinct transport vesicles that associated with different sets of microtubules and, occasionally, actin filaments. C-terminal APP fragments were preferentially transported into neurites as phosphorylated forms, entered the lamellipodium and filopodia of growth cones, and concen-

trated in regions of growth cone turning and advancement (unlike the N-terminal and A β fragments). We conclude that, under normal conditions, the proteolytic cleavage of APP primarily occurs before its sorting into axonal transport vesicles and the cleaved fragments segregate into separate vesicle populations that reach different destinations, and thus have different functions.

The Journal of Neuroscience, March 18, 2009 • 29(11):3565–3578

Classification of NPY-Expressing Neocortical Interneurons

Anastassios Karagiannis,^{1,2*} Thierry Gallopin,^{1*} Csaba Dávid,^{3*} Demian Battaglia,^{4,5*} Hélène Geoffroy,¹ Jean Rossier,¹ Elizabeth M. C. Hillman,⁶ Jochen F. Staiger,³ and Bruno Cauli^{1,2}

¹Laboratoire de Neurobiologie et Diversité Cellulaire, Centre National de la Recherche Scientifique (CNRS) Unité Mixte de Recherche (UMR) 7637, Ecole Supérieure de Physique et de Chimie Industrielles, and ²Laboratoire de Neurobiologie des Processus Adaptatifs, Université Pierre et Marie Curie, CNRS UMR 7102, 75005 Paris, France, ³Institute of Anatomy and Cell Biology, Department of Neuroanatomy, Albert-Ludwigs-University Freiburg, D-79001 Freiburg, Germany, ⁴Laboratoire de Neurophysique et Physiologie, Université Paris Descartes, CNRS UMR 8119, 75270 Paris Cedex 06, France, ⁵Bernstein Center for Computational Neuroscience, D-37073 Göttingen, Germany, and ⁶Department of Biomedical Engineering, Columbia University, New York, New York 10027

Neuropeptide Y (NPY) is an abundant neuropeptide of the neocortex involved in numerous physiological and pathological processes. Because of the large electrophysiological, molecular, and morphological diversity of NPY-expressing neurons their precise identity remains unclear. To define distinct populations of NPY neurons we characterized, in acute slices of rat barrel cortex, 200 cortical neurons of layers I–IV by means of whole-cell patch-clamp recordings, biocytin labeling, and single-cell reverse transcriptase-PCR designed to probe for the expression of well established molecular markers for cortical neurons. To classify reliably cortical NPY neurons, we used and compared different unsupervised clustering algorithms based on laminar location and electrophysiological and molecular properties. These classification schemes confirmed that NPY neurons are nearly exclusively GABAergic and consistently disclosed three main types of NPY-expressing interneurons. (1) Neurogliaform-like neurons exhibiting a dense axonal arbor, were the most frequent and superficial, and substantially expressed the neuronal isoform of nitric oxide synthase. (2) Martinotti-like cells characterized by an ascending axon ramifying in layer I coexpressed somatostatin and were the most excitable type. (3) Among fast-spiking and parvalbumin-positive basket cells, NPY expression was correlated with pronounced spike latency. By clarifying the diversity of cortical NPY neurons, this study establishes a basis for future investigations aiming at elucidating their physiological roles.

The Journal of Neuroscience, March 18, 2009 • 29(11):3642–3659

DEVELOPMENT/PLASTICITY/REPAIR

Epithelial-Derived Brain-Derived Neurotrophic Factor Is Required for Gustatory Neuron Targeting during a Critical Developmental Period

Liqun Ma,^{*} Grace F. Lopez,^{*} and Robin F. Krimm

Department of Anatomical Sciences and Neurobiology, University of Louisville School of Medicine, Louisville, Kentucky 40292

Brain-derived neurotrophic factor (BDNF) is expressed in epithelial targets of gustatory neurons (i.e., fungiform papillae) before their innervation, and BDNF overexpression in nontaste regions of the tongue misdirects gustatory axons to these sites, suggesting that BDNF is necessary for gustatory axons to locate and innervate their correct targets during development. To test this hypothesis, we examined the targeting of taste neurons in BDNF-null mice (*bdnf*^{-/-}). Analysis of *bdnf*^{-/-} mice using a combination of DiI labeling and electron microscopy revealed that taste regions were not innervated by gustatory axons. Instead, branching was increased and many nontaste regions were innervated. The increased branching by gustatory axons in these animals was facilitated by neurotrophin 4 (NT4), because branching was virtually eliminated in *bdnf*^{-/-} *nt4*^{-/-} mice. No abnormalities in gustatory innervation patterns and targeting were observed in *nt4*^{-/-} mice. Conditional removal of BDNF selectively in epithelial cells disrupted targeting at the tongue tip, where gene recombination removed *bdnf* by embryonic day 13.5 (E13.5). However, innervation patterns were normal in the midregion and caudal portions of the tongue, where gene recombination did not occur until E14.5. These findings demonstrate that BDNF derived from gustatory epithelia is required for gustatory axons to correctly locate and innervate fungiform papillae. In addition, they show that BDNF-mediated targeting is restricted to a critical period of development, on or before E13.5.

The Journal of Neuroscience, March 18, 2009 • 29(11):3354–3364

Reverse Signaling by Glycosylphosphatidylinositol-Linked *Manduca* Ephrin Requires a Src Family Kinase to Restrict Neuronal Migration *In Vivo*

Thomas M. Coate, Tracy L. Swanson, and Philip F. Copenhaver

Department of Cell and Developmental Biology, Oregon Health & Science University, Portland, Oregon 97239

Reverse signaling via glycosylphosphatidylinositol (GPI)-linked Ephrins may help control cell proliferation and outgrowth within the nervous system, but the mechanisms underlying this process remain poorly understood. In the embryonic enteric nervous system (ENS) of the moth *Manduca sexta*, migratory neurons forming the enteric plexus (EP cells) express a single Ephrin ligand (GPI-linked MsEphrin), whereas adjacent midline cells that are inhibitory to migration express the cognate receptor (MsEph). Knocking down MsEph receptor expression in cultured embryos with antisense morpholino oligonucleotides allowed the EP cells to cross the midline inappropriately, consistent with the model that reverse signaling via MsEphrin mediates a repulsive response in the ENS. Src family kinases have been implicated in reverse

signaling by type-A Ephrins in other contexts, and MsEphrin colocalizes with activated forms of endogenous Src in the leading processes of the EP cells. Pharmacological inhibition of Src within the developing ENS induced aberrant midline crossovers, similar to the effect of blocking MsEphrin reverse signaling. Hyperstimulating MsEphrin reverse signaling with MsEph-Fc fusion proteins induced the rapid activation of endogenous Src specifically within the EP cells, as assayed by Western blots of single embryonic gut explants and by whole-mount immunostaining of cultured embryos. In longer cultures, treatment with MsEph-Fc caused a global inhibition of EP cell migration and outgrowth, an effect that was prevented by inhibiting Src activation. These results support the model that MsEphrin reverse signaling induces the Src-dependent retraction of EP cell processes away from the enteric midline, thereby helping to confine the neurons to their appropriate pathways.

The Journal of Neuroscience, March 18, 2009 • 29(11):3404–3418

NKCC1-Dependent GABAergic Excitation Drives Synaptic Network Maturation during Early Hippocampal Development

Carsten K. Pfeffer,^{1,2} Valentin Stein,³ Damien J. Keating,² Hannes Maier,⁴ Ilka Rinke,³ York Rudhard,² Moritz Hentschke,⁵ Gabriele M. Rune,⁶ Thomas J. Jentsch,^{1,2} and Christian A. Hübner^{2,5,7}

¹Max Delbrück Centrum für Molekulare Medizin (MDC) and Leibniz Institut für Molekulare Pharmakologie (FMP), D-13125 Berlin, Germany, ²Zentrum für Molekulare Neurobiologie Hamburg (ZMNH), Universität Hamburg, D-20251 Hamburg, Germany, ³Max Planck Institut für Neurobiologie, D-82152 Martinsried, Germany, and ⁴Klinik für Hals-, Nasen- und Ohrenheilkunde, ⁵Institut für Humangenetik, ⁶Institut für Anatomie, Universitätsklinikum Hamburg-Eppendorf, D-22529 Hamburg, Germany, and ⁷Institut für Klinische Chemie, Friedrich Schiller Universität Jena, D-07747 Jena, Germany

A high intracellular chloride concentration in immature neurons leads to a depolarizing action of GABA that is thought to shape the developing neuronal network. We show that GABA-triggered depolarization and Ca²⁺ transients were attenuated in mice deficient for the Na–K–2Cl cotransporter NKCC1. Correlated Ca²⁺ transients and giant depolarizing potentials (GDPs) were drastically reduced and the maturation of the glutamatergic and GABAergic transmission in CA1 delayed. Brain morphology, synaptic density, and expression levels of certain developmental marker genes were unchanged. The expression of *lynx1*, a protein known to dampen network activity, was decreased. In mice deficient for the neuronal Cl[−]/HCO₃[−] exchanger AE3, GDPs were also diminished. These data show that NKCC1-mediated Cl[−] accumulation contributes to GABAergic excitation and network activity during early postnatal development and thus facilitates the maturation of excitatory and inhibitory synapses.

The Journal of Neuroscience, March 18, 2009 • 29(11):3419–3430

Specificity and Sufficiency of EphB1 in Driving the Ipsilateral Retinal Projection

Timothy J. Petros,¹ Brikha R. Shrestha,¹ and Carol Mason^{1,2,3}

Departments of ¹Neuroscience, ²Pathology and Cell Biology, and ³Ophthalmology, Columbia University, College of Physicians and Surgeons, New York, New York 10032

At the optic chiasm, retinal ganglion cell (RGC) axons make the decision to either avoid or traverse the midline, a maneuver that establishes the binocular pathways. In mice, the ipsilateral retinal projection arises from RGCs in the peripheral ventrotemporal (VT) crescent of the retina. These RGCs express the guidance receptor EphB1, which interacts with ephrin-B2 on radial glia cells at the optic chiasm to repulse VT axons away from the midline and into the ipsilateral optic tract. However, because VT RGCs express more than one EphB receptor, the sufficiency and specificity of the EphB1 receptor in directing the ipsilateral projection is unclear. In this study, we use *in utero* retinal electroporation to demonstrate that ectopic EphB1 expression can redirect RGCs with a normally crossed projection to an ipsilateral trajectory. Moreover, EphB1 is specifically required for rerouting RGC projections ipsilaterally, because introduction of the highly similar EphB2 receptor is much less efficient in redirecting RGC fibers, even when expressed at higher surface levels. Introduction of EphB1–EphB2 chimeric receptors into RGCs reveals that both extracellular and juxtamembrane domains of EphB1 are required to efficiently convert RGC projections ipsilaterally. Together, these data describe for the first time functional differences between two highly similar Eph receptors at a decision point *in vivo*, with EphB1 displaying unique properties that efficiently drives the uncrossed retinal projection.

The Journal of Neuroscience, March 18, 2009 • 29(11):3463–3474

Regulation of Group I Metabotropic Glutamate Receptor Trafficking and Signaling by the Caveolar/Lipid Raft Pathway

Anna Francesconi, Ranju Kumari, and R. Suzanne Zukin

Dominick P. Purpura Department of Neuroscience, Albert Einstein College of Medicine, Bronx, New York 10461

Endocytic trafficking of neurotransmitter receptors is critical to neuronal signaling and activity-dependent synaptic plasticity. Although the importance of clathrin-mediated endocytosis in receptor trafficking in neurons is well established, the contribution of the caveolar/lipid raft pathway has been little explored. Here, we show that caveolin-1, an adaptor protein that associates with lipid rafts and the main coat protein of caveolae, binds to and colocalizes with metabotropic glutamate receptors 1/5 (mGluR1/5). The interaction with caveolin-1 controls the rate of constitutive mGluR1 internalization, thereby regulating expression of the receptor at the cell surface. Consistent with a role for caveolin-1 in mGluR trafficking, we show that mGluR1/5 associate with lipid rafts in the brain and that their constitutive internalization is mediated, in both heterologous cells and neurons, by caveolar/raft-dependent endocytosis. We further show that caveolin-1 attenuates mGluR1-dependent activation of extracellular signal-regulated kinase (ERK)–mitogen-activated protein kinase (MAPK) signaling, an effect that is abolished in cells expressing mutant mGluR1 lacking intact caveolin binding motifs. Neurons from caveolin-1 knock-out mice show enhanced basal ERK1/2 phosphorylation and prolonged ERK1/2 activation in response to stimulation with DHPG [(*RS*)-3,5-dihydroxyphenylglycine], a group I mGluR-selective agonist. Together, these findings underscore the importance of caveolar rafts in neurons and suggest that this pathway might play an important role in synapse formation and plasticity.

The Journal of Neuroscience, March 18, 2009 • 29(11):3590–3602

Characterization of the Decision Network for Wing Expansion in *Drosophila* Using Targeted Expression of the TRPM8 Channel

Nathan C. Peabody, Jascha B. Pohl, Fengqiu Diao, Andrew P. Vreede, David J. Sandstrom, Howard Wang, Paul K. Zelensky, and Benjamin H. White

Laboratory of Molecular Biology, National Institute of Mental Health, National Institutes of Health, Bethesda, Maryland 20892

After emergence, adult flies and other insects select a suitable perch and expand their wings. Wing expansion is governed by the hormone bursicon and can be delayed under adverse environmental conditions. How environmental factors delay bursicon release and alter perch selection and expansion behaviors has not been investigated in detail. Here we provide evidence that in *Drosophila* the motor programs underlying perch selection and wing expansion have different environmental dependencies. Using physical manipulations, we demonstrate that the decision to perch is based primarily on environmental valuations and is incrementally delayed under conditions of increasing perturbation and confinement. In contrast, the all-or-none motor patterns underlying wing expansion are relatively invariant in length regardless of environmental conditions. Using a novel technique for targeted activation of neurons, we show that the highly stereotyped wing expansion motor patterns can be initiated by stimulation of N_{CCAP} , a small network of central neurons that regulates the release of bursicon. Activation of this network using the cold-sensitive rat TRPM8 channel is sufficient to trigger all essential behavioral and somatic processes required for wing expansion. The delay of wing expansion under adverse circumstances thus couples an environmentally sensitive decision network to a command-like network that initiates a fixed action pattern. Because N_{CCAP} mediates environmentally insensitive ecdysis-related behaviors in *Drosophila* development before adult emergence, the study of wing expansion promises insights not only into how networks mediate behavioral choices, but also into how decision networks develop.

The Journal of Neuroscience, March 18, 2009 • 29(11):3343–3353

Rapid Synaptic Depression Explains Nonlinear Modulation of Spectro-Temporal Tuning in Primary Auditory Cortex by Natural Stimuli

Stephen V. David, Nima Mesgarani, Jonathan B. Fritz, and Shihab A. Shamma

Institute for Systems Research, University of Maryland, College Park, Maryland 20742

In this study, we explored ways to account more accurately for responses of neurons in primary auditory cortex (A1) to natural sounds. The auditory cortex has evolved to extract behaviorally relevant information from complex natural sounds, but most of our understanding of its function is derived from experiments using simple synthetic stimuli. Previous neurophysiological studies have found that existing models, such as the linear spectro-temporal receptive field (STRF), fail to capture the entire functional relationship between natural stimuli and neural responses. To study this problem, we compared STRFs for A1 neurons estimated using a natural stimulus, continuous speech, with STRFs estimated using synthetic ripple noise. For about one-third of the neurons, we found significant differences between STRFs, usually in the temporal dynamics of inhibition and/or overall gain. This shift in tuning resulted primarily from differences in the coarse temporal structure of the speech and noise stimuli. Using simulations, we found that the stimulus dependence of spectro-temporal tuning can be explained by a model in which synaptic inputs to A1 neurons are susceptible to rapid nonlinear depression. This dynamic reshaping of spectro-temporal tuning suggests that synaptic depression may enable efficient encoding of natural auditory stimuli.

The Journal of Neuroscience, March 18, 2009 • 29(11):3374–3386

Segregated Populations of Hippocampal Principal CA1 Neurons Mediating Conditioning and Extinction of Contextual Fear

Natalie C. Tronson,¹ Christina Schrick,¹ Yomayra F. Guzman,¹ Kyu Hwan Huh,¹ Deepak P. Srivastava,² Peter Penzes,² Anita L. Guedea,¹ Can Gao,¹ and Jelena Radulovic¹

¹Department of Psychiatry and Behavioral Sciences, The Asher Center for the Study and Treatment of Depressive Disorders, and ²Department of Physiology, Feinberg School of Medicine, Northwestern University, Chicago, Illinois 60611

Learning processes mediating conditioning and extinction of contextual fear require activation of several key signaling pathways in the hippocampus. Principal hippocampal CA1 neurons respond to fear conditioning by a coordinated activation of multiple protein kinases and immediate early genes, such as *cFos*, enabling rapid and lasting consolidation of contextual fear memory. The extracellular signal-regulated kinase (Erk) additionally acts as a central mediator of fear extinction. It is not known however, whether these molecular events take place in overlapping or nonoverlapping neuronal populations. By using mouse models of conditioning and extinction of fear, we set out to determine the time course of *cFos* and Erk activity, their cellular overlap, and regulation by afferent cholinergic input from the medial septum. Analyses of *cFos*⁺ and *pErk*⁺ cells by immunofluorescence revealed predominant nuclear activation of either protein during conditioning and extinction of fear, respectively. Transgenic *cFos-LacZ* mice were further used to label *in vivo* *Fos*⁺ hippocampal cells during conditioning followed by *pErk* immunostaining after extinction. The results showed that these signaling molecules were activated in segregated populations of hippocampal principal neurons. Furthermore, immunotoxin-induced lesions of medial septal neurons, providing cholinergic input into the hippocampus, selectively abolished Erk activation and extinction of fear without affecting *cFos* responses and conditioning. These results demonstrate that extinction mechanisms based on Erk signaling involve a specific population of CA1 principal neurons distinctively regulated by afferent cholinergic input from the medial septum.

The Journal of Neuroscience, March 18, 2009 • 29(11):3387–3394

Environmental Enrichment Restores Memory Functioning in Mice with Impaired IL-1 Signaling via Reinstatement of Long-Term Potentiation and Spine Size Enlargement

Inbal Goshen,¹ Avi Avital,³ Tirzah Kreisel,¹ Tamar Licht,⁴ Menahem Segal,⁵ and Raz Yirmiya¹

¹Department of Psychology, The Hebrew University, Jerusalem 91905, Israel, ²Department of Psychology and ³The Center for Psychobiological Research, The Max Stern Yezreel Valley College, Emek Yezreel 19300, Israel, ⁴Department of Molecular Biology, The Hebrew University–Hadassah Medical School, Jerusalem 91120, Israel, and ⁵Department of Neurobiology, Weizmann Institute of Science, Rehovot 76100, Israel

Environmental enrichment (EE) was found to facilitate memory functioning and neural plasticity in normal and neurologically impaired animals. However, the ability of this manipulation to rescue memory and its biological substrate in animals with specific genetically based deficits in these functions has not been extensively studied. In the present study, we investigated the effects of EE in two mouse models of impaired memory functioning and plasticity. Previous research demonstrated that mice with a deletion of the receptor for the cytokine interleukin-1 (IL-1rKO), and mice with CNS-specific transgenic over-expression of the IL-1 receptor antagonist (IL-1raTG) display impaired hippocampal memory and long-term potentiation (LTP). We report here a corrective effect of EE on spatial and contextual memory in IL-1rKO and IL-1raTG mice and reveal two mechanisms for this beneficial effect: Concomitantly with their disturbed memory functioning, LTP in IL-1rKO mice that were raised in a regular environment is impaired, and their dendritic spine size is reduced. Both of these impairments were corrected by environmental enrichment. No deficiencies in neurogenesis or hippocampal BDNF and vascular endothelial growth factor secretion were found in IL-1rKO mice that were raised in a regular environment, and both of these variables were increased to a similar degree in enriched IL-1rKO and wild-type mice. These findings suggest that exposure to an enriched environment may be beneficial for individuals with impaired learning and memory related to genetic impairments of IL-1 signaling (and possibly other genetic causes), by reversing impairments in dentate gyrus LTP and spine size and by promoting neurogenesis and trophic factors secretion.

The Journal of Neuroscience, March 18, 2009 • 29(11):3395–3403

Phase Encoding in the Mauthner System: Implications in Left–Right Sound Source Discrimination

Shennan A. Weiss, Thomas Preuss,* and Donald S. Faber*

Dominick P. Purpura Department of Neuroscience, Albert Einstein College of Medicine, Bronx, New York 10461

The paired teleost Mauthner (M)-cells and their associated network serve as an excellent system to study the biophysical basis of decision making. In teleosts, an abrupt sound evokes an M-spike, triggering a C-start escape that is usually directed away from a sound source. The response latency is minimized by electrical synapses between auditory afferents and the M-cell lateral dendrite. Here, we demonstrate that the electrical synapses also mediate phase encoding. Ramped sound pressure waves (150–250 Hz) evoked electrotonic postsynaptic potentials in the M-cell locked to two diametrically opposed phase angles that were frequency dependent but intensity independent. Phase encoding was also evident at the behavioral level underwater, because the stimuli evoked directional C-starts with an onset that was phase locked to the sound wave. In interneurons inhibitory to the M-cell, these same stimuli also evoked phase-locked electrotonic postsynaptic potentials and action potentials. The resulting chemical and electrical (i.e., field effect) inhibitions functioned tonically and phasically, respectively. Phase encoding could be important in underwater sound source localization, which is thought to require a neural computation involving a phase comparison between the pressure and the directional particle motion components of sound. This computation may be implemented by an interplay between phase-dependent afferent excitation and feedforward inhibition that activates the appropriate M-cell and directs the C-start away from the sound source.

The Journal of Neuroscience, March 18, 2009 • 29(11):3431–3441

A Specific Role of the Human Hippocampus in Recall of Temporal Sequences

Hanne Lehn,¹ Hill-Aina Steffenach,¹ Niels M. van Strien,¹ Dick J. Veltman,³ Menno P. Witter,² and Asta K. Häberg^{1,4}

¹Department of Circulation and Medical Imaging and ²Department of Neuroscience, Kavli Institute for Systems Neuroscience and Center for the Biology of Memory, Norwegian University of Science and Technology, 7489 Trondheim, Norway, ³Department of Psychiatry, VU University Medical Center and Academic Medical Center, 1007 MB Amsterdam, The Netherlands, and ⁴Department of Medical Imaging, St. Olavs Hospital, 7006 Trondheim, Norway

There is a growing interest in how temporal order of episodic memories is represented within the medial temporal lobe (MTL). Animal studies suggest that the hippocampal formation (HF) is critical for retrieving the temporal order of past experiences. However, human imaging studies that have tested recency discrimination between pairs of previously encoded items have generally failed to report HF activation. We hypothesized that recalling a naturalistic sequence of past events would be particularly sensitive to HF function, attributable to greater involvement of associative processes. To test this prediction, we let subjects watch a novel movie and later, during functional magnetic resonance imaging, asked them to rearrange and “replay” scenes from the movie in correct order. To identify areas specifically involved in retrieval of temporal order, we used a control condition where subjects logically inferred the order of scenes from the same movie. Extensive MTL activation was observed during sequence recall. Activation within the right HF was specifically related to retrieval of temporal order and correlated positively with accuracy of sequence recall. Also, the bilateral parahippocampal cortex responded to retrieval of temporal order, but the activation here was not related to performance. Our study is the first to unequivocally demonstrate that correct sequence recall depends on HF.

The Journal of Neuroscience, March 18, 2009 • 29(11):3475–3484

Evidence for a Proprioception-Based Rapid On-Line Error Correction Mechanism for Hand Orientation during Reaching Movements in Blind Subjects

Nadia Gosselin-Kessiby,^{1,4} John F. Kalaska,^{1,3,4} and Julie Messier^{2,5}

¹Département de Physiologie, ²Département de Kinésiologie, ³Groupe de Recherche en Sciences Neurologiques (Canadian Institutes of Health Research), ⁴Groupe de Recherche sur le Système Nerveux Central (Fonds de la Recherche en Santé du Québec), and ⁵Centre de Recherche Institut Universitaire de Gériatrie de Montréal, Université de Montréal, Montréal, Québec, Canada H3C 3J7

The contribution of visual experience to the perception and sensorimotor control of spatial orientation of the hand was investigated in blind subjects. In “orientation-matching” tasks, subjects aligned a match handle held in their right hand to a target handle held in their left hand and fixed in different orientations, with both arms outstretched. In “letter-posting” task 1, the same subjects reached out and simultaneously oriented their right hand to insert the match handle into a target slot fixed in the same range of orientations. Orientations were signaled proprioceptively by a reference handle held in the left hand. Final hand orientation errors were smaller when blind subjects simultaneously reached out and rotated their hand to insert the match handle into the target slot in letter-posting task 1 than when they held their arm extended and aligned the handles in the orientation-matching task. In letter-posting task 2, blind subjects first aligned their hand to the orientation of the target and then subsequently reached to the target with the instruction to not change hand orientation during reaching. Despite the instruction, subjects showed a reduction in absolute hand orientation error from the beginning to the end of the reach. In all tasks, performance of blind subjects was very similar to that of blindfolded normally sighted subjects. These findings provide the first evidence of an automatic on-line error-correction mechanism for hand orientation guided only by proprioceptive inputs during reaching in blind subjects, and reveal that the on-line mechanism does not depend on prior visual experience.

The Journal of Neuroscience, March 18, 2009 • 29(11):3485–3496

Nuclear Factor κ B Signaling Regulates Neuronal Morphology and Cocaine Reward

Scott J. Russo,¹ Matthew B. Wilkinson,^{1*} Michelle S. Mazei-Robison,^{1*} David M. Dietz,¹ Ian Maze,¹ Vaishnav Krishnan,² William Renthal,² Ami Graham,² Shari G. Birnbaum,² Thomas A. Green,² Bruce Robison,² Alan Lesselyong,² Linda I. Perrotti,² Carlos A. Bolaños,² Arvind Kumar,² Michael S. Clark,³ John F. Neumaier,³ Rachael L. Neve,⁴ Asha L. Bhakar,⁵ Philip A. Barker,⁵ and Eric J. Nestler¹

¹Fishberg Department of Neuroscience, Mount Sinai School of Medicine, New York, New York 10029, ²Department of Psychiatry, University of Texas Southwestern Medical Center, Dallas, Texas 75390, ³Harborview Medical Center, University of Washington, Seattle, Washington 98104, ⁴Department of Brain and Cognitive Sciences, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, and ⁵Montreal Neurological Institute, McGill University, Montreal, Quebec, Canada H3A 2B4

Although chronic cocaine-induced changes in dendritic spines on nucleus accumbens (NAc) neurons have been correlated with behavioral sensitization, the molecular pathways governing these structural changes, and their resulting behavioral effects, are poorly understood. The transcription factor, nuclear factor κ B (NF κ B), is rapidly activated by diverse stimuli and regulates expression of many genes known to maintain cell structure. Therefore, we evaluated the role of NF κ B in regulating cocaine-induced dendritic spine changes on medium spiny neurons of the NAc and the rewarding effects of cocaine. We show that chronic cocaine induces NF κ B-dependent transcription in the NAc of NF κ B-Lac transgenic mice. This induction of NF κ B activity is accompanied by increased expression of several NF κ B genes, the promoters of which show chromatin modifications after chronic cocaine exposure consistent with their transcriptional activation. To study the functional significance of this induction, we used viral-mediated gene transfer to express either a constitutively active or dominant-negative mutant of Inhibitor of κ B kinase (IKKca or IKKdn), which normally activates NF κ B signaling, in the NAc. We found that activation of NF κ B by IKKca increases the number of dendritic spines on NAc neurons, whereas inhibition of NF κ B by IKKdn decreases basal dendritic spine number and blocks the increase in dendritic spines after chronic cocaine. Moreover, inhibition of NF κ B blocks the rewarding effects of cocaine and the ability of previous cocaine exposure to increase an animal's preference for cocaine. Together, these studies establish a direct role for NF κ B pathways in the NAc to regulate structural and behavioral plasticity to cocaine.

The Journal of Neuroscience, March 18, 2009 • 29(11):3529–3537

Adrenergic and Noradrenergic Innervation of the Midbrain Ventral Tegmental Area and Retrorubral Field: Prominent Inputs from Medullary Homeostatic Centers

Carlos A. Mejías-Aponte,¹ Candice Drouin,² and Gary Aston-Jones³

¹Department of Neurobiology and Anatomy, Drexel University College of Medicine, Philadelphia, Pennsylvania 19129, ²Department of Psychiatry, University of Pennsylvania, Philadelphia, Pennsylvania 19104-6100, and ³Department of Neurosciences, Medical University of South Carolina, Charleston, South Carolina 29425

Adrenergic agents modulate the activity of midbrain ventral tegmental area (VTA) neurons. However, the sources of noradrenergic and adrenergic inputs are not well characterized. Immunostaining for dopamine β -hydroxylase revealed fibers within dopamine (DA) neuron areas, with the highest density in the retrorubral field (A8 cell group), followed by the VTA (A10 cell group), and very few fibers within substantia nigra compacta. A less dense, but a similar pattern of fibers was also found for the epinephrine marker, phenylethanolamine *N*-methyl transferase. Injection of the retrograde tracer wheat germ agglutinin-*apo* (inactivated) horseradish peroxidase conjugated to colloidal gold, or cholera toxin subunit b, revealed that the noradrenergic innervation of the A10 and A8 regions arise primarily from A1, A2, A5, and locus ceruleus neurons. Selective lesions of the ventral noradrenergic bundle confirmed a prominent innervation from A1 and A2 areas. Retrogradely labeled epinephrine

neurons were found mainly in the C1 area. The identification of medullary noradrenergic and adrenergic afferents to DA neuron areas indicates new pathways for visceral-related inputs to reward-related areas in the midbrain.

The Journal of Neuroscience, March 18, 2009 • 29(11):3613–3626

Behavioral and Neural Changes after Gains and Losses of Conditioned Reinforcers

Hyojung Seo and Daeyeol Lee

Department of Neurobiology, Yale University School of Medicine, New Haven, Connecticut 06510

Human behaviors can be more powerfully influenced by conditioned reinforcers, such as money, than by primary reinforcers. Moreover, people often change their behaviors to avoid monetary losses. However, the effect of removing conditioned reinforcers on choices has not been explored in animals, and the neural mechanisms mediating the behavioral effects of gains and losses are not well understood. To investigate the behavioral and neural effects of gaining and losing a conditioned reinforcer, we trained rhesus monkeys for a matching pennies task in which the positive and negative values of its payoff matrix were realized by the delivery and removal of a conditioned reinforcer. Consistent with the findings previously obtained with non-negative payoffs and primary rewards, the animal's choice behavior during this task was nearly optimal. Nevertheless, the gain and loss of a conditioned reinforcer significantly increased and decreased, respectively, the tendency for the animal to choose the same target in subsequent trials. We also found that the neurons in the dorsomedial frontal cortex, dorsal anterior cingulate cortex, and dorsolateral prefrontal cortex often changed their activity according to whether the animal earned or lost a conditioned reinforcer in the current or previous trial. Moreover, many neurons in the dorsomedial frontal cortex also signaled the gain or loss occurring as a result of choosing a particular action as well as changes in the animal's behaviors resulting from such gains or losses. Thus, primate medial frontal cortex might mediate the behavioral effects of conditioned reinforcers and their losses.

The Journal of Neuroscience, March 18, 2009 • 29(11):3627–3641

NEUROBIOLOGY OF DISEASE

Dynamic Changes in Presynaptic and Axonal Transport Proteins Combined with Striatal Neuroinflammation Precede Dopaminergic Neuronal Loss in a Rat Model of AAV α -Synucleinopathy

Chee Yeun Chung,^{1,2,3} James B. Koprach,^{1,2,3} Hasan Siddiqi,^{1,3} and Ole Isacson^{1,2,3}

¹Neuroregeneration Laboratories, Harvard Medical School, McLean Hospital, Belmont, Massachusetts 02478, ²Harvard Neurodiscovery Center, Boston, Massachusetts 02114, and ³Morris K. Udall Parkinson's Disease Research Center of Excellence, Belmont, Massachusetts 02478

Little is known about key pathological events preceding overt neuronal degeneration in Parkinson's disease (PD) and α -synucleinopathy. Recombinant adeno-associated virus 2-mediated delivery of mutant (A53T) human α -synuclein into the substantia nigra (SN) under a neuron-specific synapsin promoter resulted in protracted neurodegeneration with significant dopaminergic (DA) neuron loss by 17 weeks. As early as 4 weeks, there was an increase in a dopamine metabolite, DOPAC and histologically, DA axons in the striatum were dystrophic with degenerative bulbs. Before neuronal loss, significant changes were identified in levels of proteins relevant to synaptic transmission and axonal transport in the striatum and the SN. For example, striatal levels of rabphilin 3A and syntaxin were reduced. Levels of anterograde transport motor proteins (KIF1A, KIF1B, KIF2A, and KIF3A) were decreased in the striatum, whereas retrograde motor proteins (dynein, dynamin, and dynactin1) were increased. In contrast to reduced levels in the striatum, KIF1A and KIF2A levels were elevated in the SN. There were dramatic changes in cytoskeletal protein levels, with actin levels increased and α - γ -tubulin levels reduced. In addition to these alterations, a neuroinflammatory response was observed at 8 weeks in the striatum, but not in the SN, demonstrated by increased levels of Iba-1, activated microglia and increased levels of proinflammatory cytokines, including IL-1 β , IFN- γ and TNF- α . These results demonstrate that changes in proteins relevant to synaptic transmission and axonal transport coupled with neuroinflammation, precede α -synuclein-mediated neuronal death. These findings can provide ideas for antecedent biomarkers and presymptomatic interventions in PD.

The Journal of Neuroscience, March 18, 2009 • 29(11):3365–3373

Inflammation Triggers Synaptic Alteration and Degeneration in Experimental Autoimmune Encephalomyelitis

Diego Centonze,^{1,2*} Luca Muzio,^{5*} Silvia Rossi,^{1,2} Francesca Cavasinni,⁵ Valentina De Chiara,^{1,2} Alessandra Bergami,⁵ Alessandra Musella,^{1,2} Marcello D'Amelio,³ Virve Cavallucci,³ Alessandro Martorana,¹ Andrea Bergamaschi,⁵ Maria Teresa Cencioni,⁴ Adamo Diamantini,⁴ Erica Butti,⁵ Giancarlo Comi,⁵ Giorgio Bernardi,^{1,2} Francesco Cecconi,³ Luca Battistini,⁴ Roberto Furlan,⁵ and Gianvito Martino⁵

¹Neurologic Clinic, Department of Neuroscience, Tor Vergata University, 00133 Rome, Italy, ²Laboratory of Experimental Neurology, ³Laboratory of Molecular Neuroembryology, and ⁴Neuroimmunology Unit, Santa Lucia Foundation at the Centro Europeo per la Ricerca sul Cervello, 00143 Rome, Italy, and ⁵Neuroimmunology Unit, Institute of Experimental Neurology, San Raffaele Scientific Institute, 20132 Milan, Italy

Neurodegeneration is the irremediable pathological event occurring during chronic inflammatory diseases of the CNS. Here we show that, in experimental autoimmune encephalomyelitis (EAE), a mouse model of multiple sclerosis, inflammation is capable in enhancing glutamate transmission in the striatum and in promoting synaptic

degeneration and dendritic spine loss. These alterations occur early in the disease course, are independent of demyelination, and are strongly associated with massive release of tumor necrosis factor- α from activated microglia. CNS invasion by myelin-specific blood-borne immune cells is the triggering event, and the downregulation of the early gene *Arc/Arg3.1*, leading to the abnormal expression and phosphorylation of AMPA receptors, represents a culminating step in this cascade of neurodegenerative events. Accordingly, EAE-induced synaptopathy subsided during pharmacological blockade of AMPA receptors. Our data establish a link between neuroinflammation and synaptic degeneration and calls for early neuroprotective therapies in chronic inflammatory diseases of the CNS.

The Journal of Neuroscience, March 18, 2009 • 29(11):3442–3452

Amyloid β -Induced Neuronal Hyperexcitability Triggers Progressive Epilepsy

Rimante Minkeviciene,^{1*} Sylvain Rheims,^{3*} Marton B. Dobszay,^{5*} Misha Zilberter,⁵ Jarmo Hartikainen,¹ Livia Fülöp,⁶ Botond Penke,⁷ Yuri Zilberter,^{4,5} Tibor Harkany,^{5,8} Asla Pitkänen,^{1,2} and Heikki Tanila^{1,2}

¹A. I. Virtanen Institute, University of Kuopio, and ²Department of Neurology, Kuopio University Hospital, FIN-70211 Kuopio, Finland, ³Faculté de Sciences de Luminy, Aix Marseille Université, and ⁴Institut National de la Santé et de la Recherche Médicale, Institut de Neurobiologie de la Méditerranée U901, F-13000 Marseille, France, ⁵Division of Molecular Neurobiology, Department of Medical Biochemistry and Biophysics, Karolinska Institutet, Stockholm, S-17177 Stockholm, Sweden, ⁶Department of Medical Chemistry and ⁷Supramolecular and Nanostructured Materials Research Group of the Hungarian Academy of Science, University of Szeged, H-6720 Szeged, Hungary, ⁸Institute of Medical Sciences, College of Life Sciences & Medicine, University of Aberdeen, Aberdeen, AB25 2ZD, United Kingdom

Alzheimer's disease is associated with an increased risk of unprovoked seizures. However, the underlying mechanisms of seizure induction remain elusive. Here, we performed video-EEG recordings in mice carrying mutant human *APP^{swe}* and *PS1^{DE9}* genes (*APdE9* mice) and their wild-type littermates to determine the prevalence of unprovoked seizures. In two recording episodes at the onset of amyloid β ($A\beta$) pathogenesis (3 and 4.5 months of age), at least one unprovoked seizure was detected in 65% of *APdE9* mice, of which 46% had multiple seizures and 38% had a generalized seizure. None of the wild-type mice had seizures. In a subset of *APdE9* mice, seizure phenotype was associated with a loss of calbindin-D28k immunoreactivity in dentate granular cells and ectopic expression of neuropeptide Y in mossy fibers. In *APdE9* mice, persistently decreased resting membrane potential in neocortical layer 2/3 pyramidal cells and dentate granule cells underpinned increased network excitability as identified by patch-clamp electrophysiology. At stimulus strengths evoking single-component EPSPs in wild-type littermates, *APdE9* mice exhibited decreased action potential threshold and burst firing of pyramidal cells. Bath application (1 h) of $A\beta$ 1–42 or $A\beta$ 25–35 (proto-)fibrils but not oligomers induced significant membrane depolarization of pyramidal cells and increased the activity of excitatory cell populations as measured by extracellular field recordings in the juvenile rodent brain, confirming the pathogenic significance of bath-applied $A\beta$ (proto-)fibrils. Overall, these data identify fibrillar $A\beta$ as a pathogenic entity powerfully altering neuronal membrane properties such that hyperexcitability of pyramidal cells culminates in epileptiform activity.

The Journal of Neuroscience, March 18, 2009 • 29(11):3453–3462

Cellular Plasticity for Group I mGluR-Mediated Epileptogenesis

Riccardo Bianchi, Shih-Chieh Chuang, Wangfa Zhao, Steven R. Young, and Robert K. S. Wong

The Robert F. Furchgott Center for Neural and Behavioral Science and Department of Physiology and Pharmacology, State University of New York Downstate Medical Center, Brooklyn, New York 11203

Stimulation of group I metabotropic glutamate receptors (mGluRs) by the agonist (*S*)-dihydroxyphenylglycine in the hippocampus transforms normal neuronal activity into prolonged epileptiform discharges. The conversion is long lasting in that epileptiform discharges persist after washout of the inducing agonist and serves as a model of epileptogenesis. The group I mGluR model of epileptogenesis took on special significance because epilepsy associated with fragile X syndrome (FXS) may be caused by excessive group I mGluR signaling. At present, the plasticity mechanism underlying the group I mGluR-mediated epileptogenesis is unknown. $I_{mGluR(V)}$, a voltage-gated cationic current activated by group I mGluR agonists in CA3 pyramidal cells in the hippocampus, is a possible candidate. $I_{mGluR(V)}$ activation is associated with group I mGluR agonist-elicited epileptiform discharges. For $I_{mGluR(V)}$ to play a role in epileptogenesis, long-term activation of the current must occur after group I mGluR agonist exposure or synaptic stimulation. We observed that $I_{mGluR(V)}$, once induced by group I mGluR agonist stimulation in CA3 pyramidal cells, remained undiminished for hours after agonist washout. In slices prepared from FXS model mice, repeated stimulation of recurrent CA3 pyramidal cell synapses, effective in eliciting mGluR-mediated epileptiform discharges, also induced long-lasting $I_{mGluR(V)}$ in CA3 pyramidal cells. Similar to group I mGluR-mediated prolonged epileptiform discharges, persistent $I_{mGluR(V)}$ was no longer observed in preparations pretreated with inhibitors of tyrosine kinase, of extracellular signal-regulated kinase 1/2, or of mRNA protein synthesis. The results indicate that $I_{mGluR(V)}$ is an intrinsic plasticity mechanism associated with group I mGluR-mediated epileptogenesis.

The Journal of Neuroscience, March 18, 2009 • 29(11):3497–3507

Diffusion-Weighted Magnetic Resonance Imaging Reversal by Gene Knockdown of Matrix Metalloproteinase-9 Activities in Live Animal Brains

Christina H. Liu,^{1,2} Zerong You,³ Charnng-Ming Liu,^{1,2} Young R. Kim,² Michael J. Whalen,³ Bruce R. Rosen,² and Philip K. Liu^{1,2}

¹Laboratory for Transcript Targeting, Imaging and Repair, ²A. A. Martinos Center for Biomedical Imaging, Department of Radiology, and ³Department of Pediatrics, Massachusetts General Hospital, Charlestown, Massachusetts 02129

The involvement of matrix metalloproteinase-9 (MMP-9) activities in the development of abnormal water diffusion in the brain after cardiac arrest is not fully understood. We used magnetic resonance imaging to determine the correlation between MMP-9 activity and the mechanism of abnormal water diffusion after global cerebral ischemia

(GCI)-induced brain damage in C57black6 mice. We induced GCI in mice by occluding both carotid arteries for 60 min, then allowing reperfusion. We labeled a short DNA that targets mmp-9 mRNA activity [phosphorothioate-modified oligodeoxynucleotide (sODN)-mmp9] or a control probe without intracellular target (sODN-Ran) with iron-based MR contrast agent [superparamagnetic iron oxide nanoparticle (SPION)-mmp9 or SPION-Ran] or fluorescein isothiocyanate (FITC)-sODN-mmp9 or FITC-sODN-Ran; we then delivered these probes by intracerebroventricular infusion or intraperitoneal injection within 3 h of reperfusion. At low dose (120 pmol/kg) the SPION-mmp9 probe was retained at significant levels in the striatum and cortex of living brains 10 h after GCI. Probe retention was validated by similar elevation of mmp-9 mRNA and antigens in postmortem samples taken from regions that exhibited GCI-induced hyperintensity in diffusion-weighted imaging, and a significant reduction in apparent diffusion coefficient (rADC, $p = 0.0006$, $n = 12$). At a higher dose (120 nmol/kg), the FITC-sODN-mmp9 probe revealed significant knockdown of MMP-9 activity, per zymography, and a reversal of striatal rADC ($p = 0.004$, $n = 6$). These observations were not duplicated in the control group. We conclude that expression of mmp-9 mRNA is associated with abnormal ADC after GCI.

The Journal of Neuroscience, March 18, 2009 • 29(11):3508–3517

P2X4-Receptor-Mediated Synthesis and Release of Brain-Derived Neurotrophic Factor in Microglia Is Dependent on Calcium and p38-Mitogen-Activated Protein Kinase Activation

Tuan Trang,^{1,2,3} Simon Beggs,^{1,2,3} Xiang Wan,^{1,2,3} and Michael W. Salter^{1,2,3}

¹Program in Neurosciences & Mental Health, Hospital for Sick Children, Toronto, Ontario, Canada M5G 1X8, ²Department of Physiology, University of Toronto, Toronto, Ontario, Canada M5S 1A8, and ³University of Toronto Centre for the Study of Pain, Toronto, Ontario, Canada M5G 1X8

Microglia in the dorsal horn of the spinal cord are increasingly recognized as being crucial in the pathogenesis of pain hypersensitivity after injury to a peripheral nerve. It is known that P2X4 purinoceptors (P2X4Rs) cause the release of brain-derived neurotrophic factor (BDNF) from microglia, which is necessary for maintaining pain hypersensitivity after nerve injury. However, there is a critical gap in understanding how activation of microglial P2X4Rs leads to the release of BDNF. Here, we show that stimulating P2X4Rs with ATP evokes a biphasic release of BDNF from microglia: an early phase occurs within 5 min, whereas a late phase peaks 60 min after ATP stimulation. Concomitant with the late phase of release is an increased level of BDNF within the microglia. Both phases of BDNF release and the accumulation within the microglia are dependent on extracellular Ca^{2+} . The late phase of BDNF release and accumulation, but not the early phase of release, are suppressed by inhibiting transcription and translation, indicating that activation of P2X4R causes an initial release of a pre-existing pool of BDNF followed by an increase in *de novo* synthesis of BDNF. The release of BDNF is abolished by inhibiting SNARE (soluble N-ethylmaleimide-sensitive factor attachment protein receptor)-mediated exocytosis. Furthermore, we find that the P2X4R-evoked release and synthesis of BDNF are dependent on activation of p38-mitogen-activated protein kinase (MAPK). Together, our findings provide a unifying mechanism for pain hypersensitivity after peripheral nerve injury through P2X4R-evoked increase in Ca^{2+} and activation of p38-MAPK leading to the synthesis and exocytotic release of BDNF from microglia.

The Journal of Neuroscience, March 18, 2009 • 29(11):3518–3528

Specific Loss of Brain ABCA1 Increases Brain Cholesterol Uptake and Influences Neuronal Structure and Function

Joanna M. Karasinska,¹ Franz Rinninger,² Dieter Lütjohann,³ Piers Ruddle,¹ Sonia Franciosi,¹ Janine K. Kruit,¹ Roshni R. Singaraja,¹ Veronica Hirsch-Reinshagen,¹ Jianjia Fan,¹ Liam R. Brunham,¹ Nagat Bissada,¹ Rajasekhar Ramakrishnan,⁴ Cheryl L. Wellington,¹ John S. Parks,⁵ and Michael R. Hayden¹

¹Centre for Molecular Medicine and Therapeutics, University of British Columbia, Vancouver, British Columbia, V5Z 4H4 Canada, ²University Hospital Hamburg Eppendorf, 20246 Hamburg, Germany, ³Institute of Clinical Chemistry and Pharmacology, University of Bonn, 53127 Bonn, Germany, ⁴Division of Biostatistics, Columbia University College of Physicians and Surgeons, New York, New York 10032, and ⁵Department of Pathology, Section on Lipid Sciences, Wake Forest University School of Medicine, Winston-Salem, North Carolina 27157

The expression of the cholesterol transporter ATP-binding cassette transporter A1 (ABCA1) in the brain and its role in the lipidation of apolipoproteins indicate that ABCA1 may play a critical role in brain cholesterol metabolism. To investigate the role of ABCA1 in brain cholesterol homeostasis and trafficking, we characterized mice that specifically lacked ABCA1 in the CNS, generated using the Cre/loxP recombination system. These mice showed reduced plasma high-density lipoprotein (HDL) cholesterol levels associated with decreased brain cholesterol content and enhanced brain uptake of esterified cholesterol from plasma HDL. Increased levels of HDL receptor SR-BI in brain capillaries and apolipoprotein A-I in brain and CSF of mutant mice were evident. Cholesterol homeostasis changes were mirrored by disturbances in motor activity and sensorimotor function. Changes in synaptic ultrastructure including reduced synapse and synaptic vesicle numbers were observed. These data show that ABCA1 is a key regulator of brain cholesterol metabolism and that disturbances in cholesterol transport in the CNS are associated with structural and functional deficits in neurons. Moreover, our findings also demonstrate that specific changes in brain cholesterol metabolism can lead to alterations in cholesterol uptake from plasma to brain.

The Journal of Neuroscience, March 18, 2009 • 29(11):3579–3589

Macrophage-Mediated Degradation of β -Amyloid via an Apolipoprotein E Isoform-Dependent Mechanism

Lingzhi Zhao,^{1*} Suizhen Lin,^{1*} Kelly R. Bales,¹ Valentina Gelfanova,² Deanna Koger,¹ Cynthia DeLong,¹ John Hale,² Feng Liu,¹ Jesse M. Hunter,¹ and Steven M. Paul¹

¹Neuroscience Discovery Research and ²Integrative Biology, Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Indiana 46285

Recent studies suggest that bone marrow-derived macrophages can effectively reduce β -amyloid ($A\beta$) deposition in brain. To further elucidate the mechanisms by which macrophages degrade $A\beta$, we cultured murine macrophages on top of $A\beta$ plaque-bearing brain sections from transgenic mice expressing PDAPP [human amyloid precursor protein (APP) with the APP_{717V>F} mutation driven by the platelet-derived growth factor promoter]. Using this *ex vivo* assay, we found that macrophages from wild-type mice very efficiently degrade both soluble and insoluble $A\beta$ in a time-dependent manner and markedly eliminate thioflavine-S positive amyloid deposits. Because macrophages express and secrete apolipoprotein E (apoE), we compared the efficiency of $A\beta$ degradation by macrophages prepared from apoE-deficient mice or mice expressing human apoE2, apoE3, or apoE4. Macrophages expressing apoE2 were more efficient at degrading $A\beta$ than apoE3-expressing, apoE4-expressing, or apoE-deficient macrophages. Moreover, macrophage-induced degradation of $A\beta$ was effectively blocked by an anti-apoE antibody and receptor-associated protein, an antagonist of the low-density lipoprotein (LDL) receptor family, suggesting involvement of LDL receptors. Measurement of matrix metalloproteinase-9 (MMP-9) activity in the media from human apoE-expressing macrophages cocultured with $A\beta$ -containing brain sections revealed greater levels of MMP-9 activity in apoE2-expressing than in either apoE3- or apoE4-expressing macrophages. Differences in MMP-9 activity appear to contribute to the isoform-specific differences in $A\beta$ degradation by macrophages. These apoE isoform-dependent effects of macrophages on $A\beta$ degradation suggest a novel “peripheral” mechanism for $A\beta$ clearance from brain that may also, in part, explain the isoform-dependent effects of apoE in determining the genetic risk for Alzheimer’s disease.

The Journal of Neuroscience, March 18, 2009 • 29(11):3603–3612

The Cause of the Imbalance in the Neuronal Network Leading to Seizure Activity Can Be Predicted by the Electrographic Pattern of the Seizure Onset

Anatol Bragin,^{1,3} Avetis Azizyan,¹ Joyel Almajano,¹ and Jerome Engel Jr^{1,2,3}

Departments of ¹Neurology and ²Neurobiology, and ³The Brain Research Institute, David Geffen School of Medicine at the University of California, Los Angeles, Los Angeles, California 90095

This study investigates the temporal dynamics of ictal electrical activity induced by injection of the GABA_A receptor antagonist bicuculline, and the glutamate agonist kainic acid, into the CA3 area of hippocampus. Experiments were conducted in freely moving adult Wistar rats implanted with microelectrodes in multiple brain areas. Wide-band electrical activity (0.1–3000 Hz) was recorded, and the latency of seizure onset as well as the pattern of electrical activity were investigated for each drug. The latencies between injection and the occurrence of first epileptiform events were 3.93 ± 2.76 (\pm STD) min for bicuculline and 6.37 ± 7.66 min for kainic acid, suggesting the existence of powerful seizure-suppressive mechanisms in the brain. Bicuculline evoked high-amplitude rhythmic epileptiform events at the site of injection which resembled interictal EEG spikes and rapidly propagated to adjacent and remote brain areas. Kainic acid evoked a completely different pattern with a gradual increase in the amplitude of 30–80 Hz activity. Whereas there was strong temporal correlation between EEG events at the site of bicuculline injection and discharges in distant areas, much less correlation was seen with kainic acid injection. Both patterns were followed by generalized ictal EEG discharges and behavioral seizures. Our results illustrate that the same area of the brain can trigger seizures with different electrographic patterns. The knowledge of the network mechanisms underlying these two distinct electrographic patterns might be helpful in designing differential strategies for preventing seizure occurrence.

The Journal of Neuroscience, March 18, 2009 • 29(11):3660–3671