

This Week in The Journal

● Cellular/Molecular

With and without the 3 Mints

Angela Ho, Wade Morishita, Deniz Atasoy, Xinran Liu, Katsuhiko Tabuchi, Robert E. Hammer, Robert C. Malenka, and Thomas C. Südhof

(see pages 13089–13101)

You can tell a lot about a protein by what it hangs out with. The three Mints (also called X11-like proteins) bind to multiple synaptic proteins, and knock-out studies have suggested that they may indeed be necessary in synaptic transmission. But different isoforms can complement each other's function; thus, it has been difficult to come to firm conclusions using single knock-outs. This week, Ho et al. deleted the Mints using constitutive and conditional knock-out strategies. Deletion of Mint 1 and 2, the two isoforms specifically expressed in neurons, caused most mice to die at birth. The 20% that survived had ataxia and reduced body weight. In the double knock-outs, whole-cell recording of hippocampal neurons revealed lowered synaptic strength, a twofold decrease in the frequency of miniature EPSCs, and enhanced paired-pulse facilitation, indicative of a presynaptic action of Mint 1 and 2. Similar results were obtained with acute ablation of Mint 1/2/3.

▲ Development/Plasticity/Repair

Born-Again Neurons in Mice and Men

John J. Ohab, Sheila Fleming, Armin Blesch, and S. Thomas Carmichael

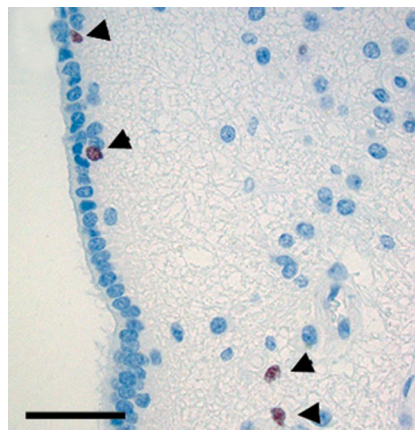
(see pages 13007–13016)

Jadranka Macas, Christian Nern, Karl H. Plate, and Stefan Momma

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Stroke not only causes cell death, but it also stimulates possible recovery through neuronal regeneration in tissues near the infarct, according to two separate studies published this week. Using histological analyses in a large collection of postmor-

tem human brains, Macas et al. found increased numbers of neuronal precursor cells, even in patients of advanced age who had suffered ischemia. Because recent studies have coupled neurogenesis to the formation of new blood vessels, Ohab et al. tested the link in a model of focal stroke in mice. These authors showed that stroke induced the long-distance migration of thousands of newly born neuroblasts from the subventricular zone to peri-infarct cortex. The new cells associated with peri-infarct blood vessels in a region of active vascular remodeling. When Ohab et al. added stromal-derived factor 1 and angiopoietin 1, which are produced by the vasculature, the number of newly formed neurons increased.



Proliferating cells, marked by Ki-67 (arrowheads), were increased close to the lateral ventricular wall in a patient that had a large ipsilateral ischemic stroke 5 d previously. See Macas et al. for details.

■ Behavioral/Systems/Cognitive

Localizing Vocal Emotions

Jane E. Warren, Disa A. Sauter, Frank Eisner, Jade Wiland, M. Alexander Dresner, Richard J. S. Wise, Stuart Rosen, and Sophie K. Scott

(see pages 13067–13075)

The sound of laughter or cheering typically makes us smile or laugh. Warren et al. wanted to know how this happens. A facial expression showing an emotion can produce a so-called “mirror” response or

similar facial expression in an observer. The authors used functional magnetic resonance imaging to determine whether similar mirror responses were also triggered by vocal expressions of emotion. Study participants were asked to listen to human voices conveying positive valence such as amusement and triumph. Listening to these “positive-valence” vocalizations activated specific premotor areas in the left posterior inferior frontal region, an area involved in control of facial movement. The activation was not attributable to facial movement per se. Thus, listening to vocal expressions of emotions appears to automatically engage preparation for orofacial gestures corresponding to the emotional content of the stimulus.

◆ Neurobiology of Disease

Ginkgo biloba and Oligomeric A β in Worms

Yanjue Wu, Zhixin Wu, Peter Butko, Yves Christen, Mary P. Lambert, William L. Klein, Christopher D. Link, and Yuan Luo

(see pages 13102–13113)

Ginkgo biloba, the ancient plant that fed dinosaurs, is widely used in patients with Alzheimer's disease (AD). This week, Wu et al. examined the effects of a standard preparation of plant extract, EGb 761, in *Caenorhabditis elegans*. Nematodes do not express endogenous β amyloid ($A\beta$), the peptide that oligomerizes and form deposits in AD brains. Nonetheless, transgenic expression of $A\beta$ causes striking pathology in *C. elegans*, such as muscle paralysis and problems with chemotaxis, which were alleviated by EGb 761. Rescue of these behaviors was accompanied by a reduction in $A\beta$ oligomers. The beneficial effects of *G. biloba* are thought to result from neuroprotective and antioxidant properties. But in the transgenic *C. elegans*, reducing oxidative stress with the antioxidant L-ascorbic acid was not nearly as effective in suppressing paralysis as EGb 761. Thus, the beneficial effects of the extract may result from block of $A\beta$ oligomerization.

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Cover legend: The cover illustration shows an artistically modified two-photon fluorescence image of a dendrite and a synaptic spine of a cortical layer 2/3 pyramidal neuron. The Ca^{2+} dependence of the synaptic modifications for spike-timing-dependent plasticity induction protocols was investigated in spines on basal dendrites. It was found that the peak Ca^{2+} transient amplitude alone is not sufficient to account for the direction of the change in synaptic efficacy. It determines the magnitude, but the direction of the change is controlled via a metabotropic glutamate receptor-coupled signaling cascade, which acts in conjunction with voltage-dependent calcium channels and phospholipase C as a sequence coincidence detector that detects post-before-presynaptic action potentials resulting in long-term depression. Long term potentiation is induced by activation of NMDA receptors. Thus, presumably two different coincidence detectors in spines control the induction of spike-timing-dependent synaptic plasticity. For more information, see the article by Nevian and Sakmann in the October 25, 2006 issue (pages 11001–11013).

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Yanjue Wu, Zhixin Wu, Peter Butko, Yves Christen, Mary P. Lambert,
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Correction: In Figure 3B of the article “Abnormal Ca^{2+} Dynamics in Transgenic Mice with Neuron-Specific Mitochondrial DNA Defects” by Mie Kubota, Takaaki Kasahara, Takeshi Nakamura, Mizuho Ishiwata, Taeko Miyauchi, and Tadafumi Kato, which appears on pages 12314–12324 of the November 22, 2006 issue the scale bar was incorrectly annotated as 10s. The label of the x-axis should have been labeled 20s instead.

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Increased Generation of Neuronal Progenitors after Ischemic Injury in the Aged Adult Human Forebrain

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The adult human brain retains the capacity to generate new neurons in the hippocampal formation (Eriksson et al., 1998) and neuronal progenitor cells (NPCs) in the forebrain (Bernier et al., 2000), but to what extent it is capable of reacting to injuries, such as ischemia, is not known. We analyzed postmortem tissue from normal and pathological human brain tissue ($n = 54$) to study the cellular response to ischemic injury in the forebrain. We observed that cells expressing the NPC marker polysialylated neural adhesion cell molecule (PSA-NCAM) are continuously generated in the adult human subventricular zone (SVZ) and migrate along the olfactory tracts. These cells were not organized in migrating chains as in the adult rodent rostral migratory stream, and their number was lower in the olfactory tracts of brains from old (56–81 years of age) compared with young (29 + 36 years of age) individuals. Moreover, we show that in brains of patients of advanced age (60–87 years of age), ischemia led to an elevated number of Ki-67-positive cells in the ipsilateral SVZ without concomitant apoptotic cell death. Additionally, ischemia led to an increased number of PSA-NCAM-positive NPCs close to the lateral ventricular walls, compared with brains of comparable age without obvious neuropathologic changes. These results suggest that the adult human brain retains a capacity to respond to ischemic injuries and that this capacity is maintained even in old age.

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Articles

CELLULAR/MOLECULAR

The Voltage-Gated Sodium Channel $\text{Na}_v1.9$ Is an Effector of Peripheral Inflammatory Pain Hypersensitivity

Fumimasa Amaya,¹ Haibin Wang,¹ Michael Costigan,¹ Andrew J. Allchorne,¹ Jon P. Hatcher,² Julie Egerton,² Tania Stean,² Valerie Morisset,² David Grose,³ Martin J. Gunthorpe,² Iain P. Chessell,² Simon Tate,³ Paula J. Green,² and Clifford J. Woolf¹

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We used a mouse with deletion of exons 4, 5, and 6 of the *SCN11A* (sodium channel, voltage-gated, type XI, α) gene that encodes the voltage-gated sodium channel $\text{Na}_v1.9$ to assess its contribution to pain. $\text{Na}_v1.9$ is present in nociceptor sensory neurons that express TRPV1, bradykinin B_2 , and purinergic P2X_3 receptors. In $\text{Na}_v1.9^{-/-}$ mice, the non-inactivating persistent tetrodotoxin-resistant sodium TTXr-Per current is absent, whereas TTXr-Slow is unchanged. TTXs currents are unaffected by the mutation of $\text{Na}_v1.9$. Pain hypersensitivity elicited by intraplantar administration of prostaglandin E_2 , bradykinin, interleukin-1 β , capsaicin, and P2X_3 and P2Y receptor agonists, but not NGF, is either reduced or absent in $\text{Na}_v1.9^{-/-}$ mice, whereas basal thermal and mechanical pain sensitivity is unchanged. Thermal, but not mechanical, hypersensitivity produced by peripheral inflammation (intraplantar complete Freund's adjuvant) is substantially diminished in the null allele mutant mice, whereas hypersensitivity in two neuropathic pain models is unchanged in the $\text{Na}_v1.9^{-/-}$ mice. $\text{Na}_v1.9$ is, we conclude, an effector of the hypersensitivity produced by multiple inflammatory mediators on nociceptor peripheral terminals and therefore plays a key role in mediating peripheral sensitization.

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The Linear Computational Algorithm of Cerebellar Purkinje Cells

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The orchestration of simple motor tasks by the cerebellum results in coordinated movement and the maintenance of balance. The cerebellum integrates sensory and cortical information to generate the signals required for the coordinated execution of simple motor tasks. These signals originate in the firing rate of Purkinje cells, each of which integrates sensory and cortical information conveyed by granule cell synaptic inputs. Given the importance of the granule cell input–Purkinje cell output function for cerebellar computation, this algorithm was determined. Using several stimulation paradigms, including those that mimicked patterns of granule cell activity similar to those observed *in vivo*, we quantified the poststimulus maximum firing rate and number of extra spikes in response to granule cell synaptic input. Both of these parameters linearly encoded the strength of synaptic input when inhibitory synaptic transmission was blocked. This linear algorithm was independent of the location or temporal pattern of synaptic input. With inhibitory synaptic transmission intact, the maximum firing rate, but not the number of extra spikes, encoded the strength of granule cell synaptic input. Furthermore, the maximum firing rate of Purkinje cells linearly encoded the strength of synaptic input whether or not the activation of granule cells resulted

in a pause in Purkinje cell firing. On the basis of the data presented, we propose that Purkinje cells encode the strength of granule cell synaptic input in their maximum firing rate with a linear algorithm.

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AMPA/Kainate Receptors Drive Rapid Output and Precise Synchrony in Olfactory Bulb Granule Cells

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Gamma frequency (30–70 Hz) synchronized oscillatory activity in the olfactory bulb is widely believed to be important for odor detection and discrimination. As in other circuits with “gamma activity,” the activity in the bulb is driven by GABAergic interneurons, specifically a class of axonless cells called granule cells. However, bulb granule cells appear to lack some key mechanistic features that promote rapid synchrony in other circuits, including direct electrical interconnections and dominant actions for fast neurotransmitter receptors. At least under “static” stimulus conditions, granule cells are driven by kinetically slow NMDA receptors. Here, I used patch-clamp recordings in rat olfactory bulb slices to better understand mechanisms that shape granule cell activity under “dynamic” stimulus conditions that mimic a natural odor stimulus. During a 4 Hz patterned stimulation of olfactory nerve afferents, activation of single granule cells was primarily controlled by two classes of AMPA/kainate receptor-mediated synaptic inputs derived from output mitral cells. The rapid kinetics of these receptors, together with inactivation of A-type potassium channels, ensured that granule cells had short spike-response times. Studies in cell pairs, moreover, indicated that excitatory inputs could synchronize granule cells on a rapid time scale (2–5 ms), in turn resulting in phase-locked GABA release onto mitral cells. The precision of granule cell synchrony was controlled by the same biophysical mechanisms that promoted rapid single-cell spiking. These studies demonstrate the mechanistic underpinnings that transform a circuit with slow, uncoupled activity under static conditions into a fast, dynamic circuit operating with high precision under physiological conditions.

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Cell-Surface Actin Binds Plasminogen and Modulates Neurotransmitter Release from Catecholaminergic Cells

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An emerging area of research has documented a novel role for the plasminogen activation system in the regulation of neurotransmitter release. Prohormones, secreted by cells within the sympathoadrenal system, are processed by plasmin to bioactive peptides that feed back to inhibit secretagogue-stimulated release. Catecholaminergic cells of the sympathoadrenal system are prototypic prohormone-secreting cells. Processing of prohormones by plasmin is enhanced in the presence of catecholaminergic cells, and the enhancement requires binding of plasmin(ogen) to cellular receptors. Consequently, modulation of the local cellular fibrinolytic system of catecholaminergic cells results in substantial changes in catecholamine release. However, mechanisms for enhancing prohormone processing and cell-surface molecules mediating the enhancement on catecholaminergic cells have not been investigated. Here we show that plasminogen activation was enhanced >6.5-fold on catecholaminergic cells. Carboxypeptidase B treatment decreased cell-dependent plasminogen activation by ~90%, suggesting that the binding of plasminogen to proteins exposing C-terminal lysines on the cell surface is required to promote plasminogen activation. We identified catecholaminergic plasminogen receptors required for enhancing plasminogen activation, using a novel strategy combining targeted specific proteolysis using carboxypeptidase B with a proteomics approach using two-dimensional gel electrophoresis, radioligand blotting, and tandem mass spectrometry. Two major plasminogen-binding proteins that exposed C-terminal lysines on the cell surface contained amino acid sequences corresponding to β/γ -actin. An anti-actin monoclonal antibody inhibited cell-dependent plasminogen activation and also enhanced nicotine-dependent catecholamine release. Our results suggest that cell-surface-expressed forms of actin bind plasminogen, thereby promoting plasminogen activation and increased prohormone processing leading to inhibition of neurotransmitter release.

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Molecular Dynamics of a Presynaptic Active Zone Protein Studied in Munc13-1–Enhanced Yellow Fluorescent Protein Knock-In Mutant Mice

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GFP (green fluorescent protein) fusion proteins have revolutionized research on protein dynamics at synapses. However, corresponding analyses usually involve protein expression methods that override endogenous regulatory mechanisms, and therefore cause overexpression and temporal or spatial misexpression of exogenous fusion proteins, which may seriously compromise the physiological validity of such experiments. These problems can be circumvented by using knock-in mutagenesis of the endogenous genomic locus to tag the protein of interest with a fluorescent protein. We generated knock-in mice expressing a fusion protein of the presynaptic active zone protein Munc13-1 and enhanced yellow fluorescent protein (EYFP) from the *Munc13-1* locus. Munc13-1–EYFP-containing nerve cells and synapses are functionally

identical to those of wild-type mice. However, their presynaptic active zones are distinctly fluorescent and readily amenable for imaging. We demonstrated the usefulness of these mice by studying the molecular dynamics of Munc13-1-EYFP at individual presynaptic sites. Fluorescence recovery after photobleaching (FRAP) experiments revealed that Munc13-1-EYFP is rapidly and continuously lost from and incorporated into active zones ($\tau_1 \sim 3$ min; $\tau_2 \sim 80$ min). Munc13-1-EYFP steady-state levels and exchange kinetics were not affected by proteasome inhibitors or acute synaptic stimulation, but exchange kinetics were reduced by chronic suppression of spontaneous activity. These experiments, performed in a minimally perturbed system, provide evidence that presynaptic active zones of mammalian CNS synapses are highly dynamic structures. They demonstrate the usefulness of the knock-in approach in general and of Munc13-1-EYFP knock-in mice in particular for imaging synaptic protein dynamics.

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Progressive Deafness and Altered Cochlear Innervation in Knock-Out Mice Lacking Prosaposin

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After a yeast two-hybrid screen identified prosaposin as a potential interacting protein with the nicotinic acetylcholine receptor (nAChR) subunit $\alpha 10$, studies were performed to characterize prosaposin in the normal rodent inner ear. Prosaposin demonstrates diffuse organ of Corti expression at birth, with gradual localization to the inner hair cells (IHCs) and its supporting cells, inner pillar cells, and synaptic region of the outer hair cells (OHCs) and Deiters' cells (DCs) by postnatal day 21 (P21). Microdissected OHC and DC quantitative reverse transcriptase-PCR and immunohistology localizes prosaposin mRNA to DCs and OHCs, and protein predominantly to the apex of the DCs. Subsequent studies in a prosaposin knock-out (KO) ($-/-$) mouse showed intact but slightly reduced hearing through P19, but deafness by P25 and reduced distortion product otoacoustic emissions from P15 onward. Beginning at P12, the prosaposin KO mice showed histologic organ of Corti changes including cellular hypertrophy in the region of the IHC and greater epithelial ridge, a loss of OHCs from cochlear apex, and vacuolization of OHCs. Immunofluorescence revealed exuberant overgrowth of auditory afferent neurites in the region of the IHCs and proliferation of auditory efferent neurites in the region of the tunnel of Corti. IHC recordings from these KO mice showed normal $I-V$ curves and responses to applied acetylcholine. Together, these results suggest that prosaposin helps maintain normal innervation patterns to the organ of Corti. Furthermore, prosaposin's overlapping developmental expression pattern and binding capacity toward the nAChR $\alpha 10$ suggest that $\alpha 10$ may also play a role in this function.

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Genetic Analysis of Mint/X11 Proteins: Essential Presynaptic Functions of a Neuronal Adaptor Protein Family

Angela Ho,¹ Wade Morishita,⁵ Deniz Atasoy,¹ Xinran Liu,¹ Katsuhiko Tabuchi,¹ Robert E. Hammer,^{3,4} Robert C. Malenka,⁵ and Thomas C. Südhof^{1,2,4}

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Mints/X11s are adaptor proteins composed of three isoforms: neuron-specific Mints 1 and 2, and the ubiquitously expressed Mint 3. We have now analyzed constitutive and conditional knock-out mice for all three Mints/X11s. We found that $\sim 80\%$ of mice lacking both neuron-specific Mint isoforms (Mints 1 and 2) die at birth, whereas mice lacking any other combination of Mint isoforms survive normally. The $\sim 20\%$ surviving Mint 1/2 double knock-out mice exhibit a decrease in weight and deficits in motor behaviors. Hippocampal slice electrophysiology uncovered a decline in spontaneous neurotransmitter release, lowered synaptic strength, and enhanced paired-pulse facilitation in Mint-deficient mice, suggesting a decreased presynaptic release probability. Acute ablation of Mint expression in cultured neurons from conditional Mint 1/2/3 triple knock-in mice also revealed a decline in spontaneous release, confirming that deletion of Mints impair presynaptic function. Quantitation of synaptic proteins showed that acute deletion of Mints caused a selective increase in Munc18-1 and Fe65 proteins, and overexpression of Munc18-1 in wild-type neurons also produced a decrease in spontaneous release, suggesting that the interaction of Mints with Munc18-1 may contribute to the presynaptic phenotype observed in Mint-deficient mice. Our studies thus indicate that Mints are important regulators of presynaptic neurotransmitter release that are essential for mouse survival.

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DEVELOPMENT/PLASTICITY/REPAIR

Ephrin-As and Patterned Retinal Activity Act Together in the Development of Topographic Maps in the Primary Visual System

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The development of topographic maps in the primary visual system is thought to rely on a combination of EphA/ephrin-A interactions and patterned neural activity. Here, we characterize the retinogeniculate and retinocollicular maps of mice mutant for ephrins-A2, -A3, and -A5 (the three ephrin-As expressed in the mouse visual system), mice mutant for the $\beta 2$ subunit of the nicotinic acetylcholine receptor (that lack early patterned retinal activity), and mice mutant for both ephrin-As and $\beta 2$. We also

provide the first comprehensive anatomical description of the topographic connections between the retina and the dorsal lateral geniculate nucleus. We find that, although ephrin-A2/A3/A5 triple knock-out mice have severe mapping defects in both projections, they do not completely lack topography. Mice lacking $\beta 2$ -dependent retinal activity have nearly normal topography but fail to refine axonal arbors. Mice mutant for both ephrin-As and $\beta 2$ have synergistic mapping defects that result in a near absence of map in the retinocollicular projection; however, the retinogeniculate projection is not as severely disrupted as the retinocollicular projection is in these mutants. These results show that ephrin-As and patterned retinal activity act together to establish topographic maps, and demonstrate that midbrain and forebrain connections have a differential requirement for ephrin-As and patterned retinal activity in topographic map development.

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Differential Reelin-Induced Enhancement of NMDA and AMPA Receptor Activity in the Adult Hippocampus

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The developmental lamination of the hippocampus and other cortical structures requires a signaling cascade initiated by reelin and its receptors, apoER2 (apolipoprotein E receptor 2) and VLDLR (very-low-density lipoprotein receptor). However, the functional significance of continued reelin expression in the postnatal brain remains poorly understood. Here, we show that reelin application to adult mice hippocampal slices leads to enhanced glutamatergic transmission mediated by NMDA receptors (NMDARs) and AMPA receptors (AMPA) through distinct mechanisms. Application of recombinant reelin enhanced NMDAR-mediated currents through postsynaptic mechanisms, as revealed by the variance-mean analysis of synaptic NMDAR currents, assessment of spontaneous miniature events, and the levels of NMDAR subunits at synaptic surface. In comparison, nonstationary fluctuation analysis of miniature AMPAR currents and quantification of synaptic surface proteins revealed that reelin-induced enhancement of AMPAR responses was mediated by increased AMPAR numbers. Reelin enhancement of synaptic NMDAR currents was abolished when receptor-associated protein (RAP) or the Src inhibitor 4-amino-5-(4-methylphenyl)-7-(*t*-butyl)pyrazolo[3,4-*d*]-pyrimidine (PPI) was bath applied and was abrogated by including PPI in the recording electrodes. In comparison, including RAP or an inactive PPI analog PP3 in the recording electrode was without effect. Interestingly, the increased AMPAR response after reelin application was not blocked by PPI but was blocked by the phosphoinositide-3' kinase (PI3K) inhibitors wortmannin and LY294002 [2-(4-morpholinyl)-8-phenyl-1(4*H*)-benzopyran-4-one hydrochloride]. Furthermore, reelin-induced, PI3K-dependent AMPAR surface insertion was also observed in cultured hippocampal neurons. Together, these results reveal a differential functional coupling of reelin signaling with NMDAR and AMPAR function and define a novel mechanism for controlling synaptic strength and plasticity in the adult hippocampus.

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GABA Regulates Dendritic Growth by Stabilizing Lamellipodia in Newly Generated Interneurons of the Olfactory Bulb

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The initial formation and growth of dendrites is a critical step leading to the integration of newly generated neurons into postnatal functional networks. However, the cellular mechanisms and extracellular signals regulating this process remain mostly unknown. By directly observing newborn neurons derived from the subventricular zone in culture as well as in olfactory bulb slices, we show that ambient GABA acting through GABA_A receptors is essential for the temporal stability of lamellipodial protrusions in dendritic growth cones but did not interfere with filopodia dynamics. Furthermore, we provide direct evidence that ambient GABA is required for the proper initiation and elongation of dendrites by promoting the rapid stabilization of new dendritic segments after their extension. The effects of GABA on the initial formation of dendrites depend on depolarization and Ca²⁺ influx and are associated with a higher stability of microtubules. Together, our results indicate that ambient GABA is a key regulator of dendritic initiation in postnatally generated olfactory interneurons and offer a mechanism by which this neurotransmitter drives early dendritic growth.

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A Neurovascular Niche for Neurogenesis after Stroke

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Stroke causes cell death but also birth and migration of new neurons within sites of ischemic damage. The cellular environment that induces neuronal regeneration and migration after stroke has not been defined. We have used a model of long-distance migration of newly born neurons from the subventricular zone to cortex after stroke to define the cellular cues that induce neuronal regeneration after CNS injury. Mitotic, genetic, and viral labeling and chemokine/growth factor gain- and loss-of-function studies show that stroke induces neurogenesis from a GFAP-expressing progenitor cell in the subventricular zone and migration of newly born neurons into a unique neurovascular niche in peri-infarct cortex. Within this neurovascular niche, newly born, immature neurons closely associate with the remodeling vasculature. Neurogenesis and angiogenesis are causally linked through vascular production of stromal-derived factor 1 (SDF1) and angiopoietin 1 (Ang1). Furthermore, SDF1 and Ang1 promote

post-stroke neuroblast migration and behavioral recovery. These experiments define a novel brain environment for neuronal regeneration after stroke and identify molecular mechanisms that are shared between angiogenesis and neurogenesis during functional recovery from brain injury.

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BEHAVIORAL/SYSTEMS/COGNITIVE

Facilitation of Saccadic Eye Movements by Postsaccadic Electrical Stimulation in the Primate Caudate

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Sensorimotor experience followed by positive feedback leads to motor learning. Although the striatum, an input channel of the basal ganglia, has been implicated to play a key role in motor learning, little is known about how reward information modulates the neuronal processes in the striatum that causes behavioral changes. Here, we report that direct manipulation of the neuronal signal in the primate caudate yields behavioral changes comparable with those induced by natural reward. Electrical stimulation in the oculomotor region of the caudate immediately after saccades to a fixed direction led to selective facilitation of saccades in that direction. The facilitation remained even after stimulation was stopped, indicating a plastic change. These effects were observed when stimulation was applied after, not before, saccades. We propose that the caudate plays a causal role in behavioral changes by integrating selective sensorimotor and reward information in a temporally specific manner.

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Elevated Sleep Spindle Density after Learning or after Retrieval in Rats

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Non-rapid eye movement sleep has been strongly implicated in consolidation of both declarative and procedural memory in humans. Elevated sleep-spindle density in slow-wave sleep after learning has been shown recently in humans. It has been proposed that sleep spindles, 12–15 Hz oscillations superimposed on slow waves (<1 Hz), in concert with high-frequency hippocampal sharp waves/ripples, promote neural plasticity underlying remote memory formation. The present study reports the first indication of learning-associated increase in spindle density in the rat, providing an animal model to study the role of brain oscillations in memory consolidation during sleep. An odor–reward association task, analogous in many respects to human paired-associate learning, is rapidly learned and leads to robust memory in rats. Rats learned the task over 10 massed trials within a single session, and EEG was monitored for 3 h after learning. Learning-induced increase in spindle density is reliably reproduced in rats in two different learning situations, differing primarily in the behavioral component of the task. This increase in spindle density is also present after reactivation of remote memory and in situations when memory update is required; it is not observed after noncontingent exposure to reward and training context. The latter results substantially extend findings in humans. The magnitude of increase (~25%) and the time window of maximal effect (~1 h after sleep onset) were remarkably similar to human data, making this a valid rodent model to study network interactions through the use of simultaneous unit recordings and local field potentials during postlearning sleep.

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A Physiologically Plausible Model of Action Selection and Oscillatory Activity in the Basal Ganglia

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The basal ganglia (BG) have long been implicated in both motor function and dysfunction. It has been proposed that the BG form a centralized action selection circuit, resolving conflict between multiple neural systems competing for access to the final common motor pathway. We present a new spiking neuron model of the BG circuitry to test this proposal, incorporating all major features and many physiologically plausible details. We include the following: effects of dopamine in the subthalamic nucleus (STN) and globus pallidus (GP), transmission delays between neurons, and specific distributions of synaptic inputs over dendrites. All main parameters were derived from experimental studies. We find that the BG circuitry supports motor program selection and switching, which deteriorates under dopamine-depleted and dopamine-excessive conditions in a manner consistent with some pathologies associated with those dopamine states. We also validated the model against data describing oscillatory properties of BG. We find that the same model displayed detailed features of both γ -band (30–80 Hz) and slow (~1 Hz) oscillatory phenomena reported by Brown et al. (2002) and Magill et al. (2001), respectively. Only the parameters required to mimic experimental conditions (e.g., anesthetic) or manipulations (e.g., lesions) were changed. From the results, we derive the following novel predictions about the STN–GP feedback loop: (1) the loop is functionally decoupled by tonic dopamine under normal conditions and recoupled by dopamine depletion; (2) the loop does not show pacemaking activity under normal conditions *in vivo* (but does after combined dopamine depletion and cortical lesion); (3) the loop has a resonant frequency in the γ -band.

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Regional Differentiation of the Medial Prefrontal Cortex in Regulating Adaptive Responses to Acute Emotional Stress

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The medial prefrontal cortex (mPFC) is an important neural substrate for integrating cognitive-affective information and regulating the hypothalamo-pituitary-adrenal (HPA) axis response to emotional stress. mPFC modulation of stress responses is effected in part via the paraventricular hypothalamic nucleus (PVH), which houses both autonomic (sympathoadrenal) and neuroendocrine (HPA) effector mechanisms. Although the weight of evidence suggests that mPFC influences on stress-related PVH outputs are inhibitory, discordant findings have been reported, and such work has tended to treat this cortical region as a unitary structure. Here we compared the effects of lesions of the dorsal versus ventral aspects of mPFC, centered in the prelimbic and infralimbic fields, respectively, on acute restraint stress-induced activation of PVH cell groups mediating autonomic and neuroendocrine responses. Lesions to the dorsal mPFC enhanced restraint-induced Fos and corticotropin-releasing factor (CRF) mRNA expression in the neurosecretory region of PVH. Ablation of the ventral mPFC decreased stress-induced Fos protein and CRF mRNA expression in this compartment but increased Fos induction in PVH regions involved in central autonomic control. Repetition of the experiments in rats bearing retrograde tracer deposits to label PVH-autonomic projections confirmed that ventral mPFC lesions selectively increased stress-induced Fos expression in identified preautonomic neurons. Finally, hormonal indices of HPA activation in response to acute stress were augmented after dorsal mPFC lesions and attenuated after ventral mPFC lesions. These results suggest that dorsal and ventral aspects of the mPFC differentially regulate neuroendocrine and autonomic PVH outputs in response to emotional stress.

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Translational Control via the Mammalian Target of Rapamycin Pathway Is Critical for the Formation and Stability of Long-Term Fear Memory in Amygdala Neurons

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The mammalian target of rapamycin kinase (mTOR) regulates protein synthesis in neurons at the translational level through phosphorylation of several intracellular targets. Recent work in invertebrates indicates that mTOR-dependent translational control may be critical for the induction and maintenance of activity-dependent synaptic plasticity underlying memory formation. Here, we report that training rats in a simple fear conditioning procedure evokes a time-dependent increase in the phosphorylation of p70s6 kinase, a major direct downstream target of mTOR. When the activation of mTOR was prevented by posttraining injection of rapamycin into the amygdala, formation of the memory and the increase in p70s6 kinase phosphorylation was attenuated. Furthermore, when rapamycin was applied to the amygdala after the recall of a previously stored fear memory, subsequent retention was disrupted, indicating that local translational control at active synapses is required for the stability as well as the formation of long-term memory in this system.

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The Molecular Gatekeeper Dexas1 Sculpts the Photic Responsiveness of the Mammalian Circadian Clock

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The mammalian master clock, located in the suprachiasmatic nucleus (SCN), is exquisitely sensitive to photic timing cues, but the key molecular events that sculpt both the phasing and magnitude of responsiveness are not understood. Here, we show that the Ras-like G-protein Dexas1 is a critical factor in these processes. *Dexas1*-deficient mice (*dexas1*^{-/-}) exhibit a restructured nighttime phase response curve and a loss of gating to photic resetting during the day. Dexas1 affects the photic sensitivity by repressing or activating time-of-day-specific signaling pathways that regulate extracellular signal-regulated kinase (ERK)/mitogen-activated protein kinase (MAPK). During the late night, Dexas1 limits the capacity of pituitary adenylate cyclase (PAC) activating peptide (PACAP)/PAC1 to affect ERK/MAPK, and in the early night, light-induced phase delays, which are mediated predominantly by NMDA receptors, are reduced as reported previously. Daytime photic phase advances are mediated by a novel signaling pathway that does not affect the SCN core but rather stimulates ERK/MAPK in the SCN shell and triggers downregulation of clock protein expression.

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Discrimination Training Alters Object Representations in Human Extrastriate Cortex

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Visual object recognition relies critically on learning. However, little is known about the effect of object learning in human visual cortex, and in particular how the spatial distribution of training effects relates to the distribution of object and face selectivity across the cortex before training. We scanned human subjects with high-resolution functional magnetic resonance imaging (fMRI) while they viewed novel object classes, both before and after extensive training to discriminate between exemplars within one of these object classes. Training increased the strength of the response in visual cortex to trained objects compared with untrained objects. However, training did not simply induce a uniform increase in the response to trained objects: the magnitude of this training effect varied substantially across subregions of extrastriate cortex, with some showing a twofold increase in response to trained objects and others (including the right fusiform face area) showing no significant effect of training. Furthermore, the spatial distribution of training effects could not be predicted from the spatial distribution of either pretrained responses or face selectivity. Instead, training changed the spatial distribution of activity across the cortex. These findings support a dynamic view of the ventral visual pathway in which the cortical representation of an object category is continuously modulated by experience.

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Differential Target-Dependent Actions of Coexpressed Inhibitory Dynorphin and Excitatory Hypocretin/ Orexin Neuropeptides

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The hypocretin/orexin arousal system plays a key role in maintaining an alert wake state. The hypocretin peptide is colocalized with an opioid peptide, dynorphin. As dynorphin may be coreleased with hypocretin, we asked what action simultaneous stimulation with the excitatory neuropeptide hypocretin and the inhibitory peptide dynorphin might exert on cells postsynaptic to hypocretin axons, including hypocretin neurons. Hypocretin neurons received direct synaptic contact from other hypocretin neurons but showed little direct response to hypocretin. Here, we show that mouse hypocretin neurons are acutely sensitive to dynorphin. Dynorphin inhibits the hypocretin system by direct postsynaptic actions (hyperpolarization, decreased spike frequency, increased GIRK (G-protein-gated inwardly rectifying K⁺ channel) current, and attenuated calcium current, and indirectly by reducing excitatory synaptic tone. Interestingly, a selective antagonist of κ -opioid receptors enhanced activity of the hypocretin system, suggesting ongoing depression by endogenous hypothalamic opioids. Electrical stimulation of hypothalamic microslices that contained hypocretin cells and their axons evoked dynorphin release. Costimulation with dynorphin and hypocretin had three different effects on neurons postsynaptic to hypocretin axons: direct response to only one or the other of the two peptides [hypocretin cells respond to dynorphin, arcuate neuropeptide Y (NPY) cells respond to hypocretin], differential desensitization causing shift from inhibitory current to excitatory current with repeated coexposure (melanin-concentrating hormone neurons), synergistic direct excitation by hypocretin and presynaptic attenuation of inhibition by dynorphin (arcuate NPY neurons). These results suggest that hypocretin neurons may be able to exercise a high degree of modulatory control over postsynaptic targets using multiple neuropeptides with target-dependent actions.

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Activation of Opioid Receptor Like-1 Receptor in the Spinal Cord Produces Sex-Specific Antinociception in the Rat: Estrogen Attenuates Antinociception in the Female, whereas Testosterone Is Required for the Expression of Antinociception in the Male

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Sex-related differences in the perception and modulation of pain have been reported. The present study is the first to investigate systematically whether activation of opioid receptor-like 1 receptor (ORL₁) by orphanin FQ (OFQ) produces sex-specific modulation of spinal nociception and whether estrogen or testosterone contributes to these differences using the rat as an experimental animal. Two behavioral models, the NMDA and heat-induced nociceptive tests, were used to examine sex-specific modulation of spinal nociception. Intrathecal microinjection of OFQ in male, ovariectomized (OVX), and diestrous rats produced a significant antinociceptive effect on both tests. However, OFQ failed to produce antinociception in proestrous rats, the phase of the estrous cycle with the highest levels of circulating estradiol, and produced a dose-dependent effect in OVX females treated with 1 ng to 100 μ g of estradiol. The antinociceptive effects of OFQ were dose dependent in male and OVX animals and were reversibly antagonized by UFP-101 ([Nphe¹, Arg¹⁴, Lys¹⁵]N/OFQ(1–13)-NH₂), an ORL₁ receptor-selective antagonist. Interestingly, OFQ was ineffective in gonadectomized (GDX) males, whereas testosterone replacement restored the antinociceptive effect of OFQ in GDX males. We conclude that OFQ produces sex-specific modulation of spinal nociception; estrogen attenuates antinociception in the female in parallel with normal cycling of estrogen levels, and testosterone is required for the expression of antinociception in the male; thus, the sensitivity of the male to the antinociceptive effects of OFQ is not simply attributable to the intrinsically low estrogen levels in these animals.

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Positive Emotions Preferentially Engage an Auditory–Motor “Mirror” System

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Social interaction relies on the ability to react to communication signals. Although cortical sensory–motor “mirror” networks are thought to play a key role in visual aspects of primate communication, evidence for a similar generic role for auditory–motor interaction in primate nonverbal communication is lacking. We demonstrate that a network of human premotor cortical regions activated during facial movement is also involved in auditory processing of affective nonverbal vocalizations. Within this auditory–motor mirror network, distinct functional subsystems respond preferentially to emotional valence and arousal properties of heard vocalizations. Positive emotional valence enhanced activation in a left posterior inferior frontal region involved in representation of prototypic actions, whereas increasing arousal enhanced activation in presupplementary motor area cortex involved in higher-order motor control. Our findings demonstrate that listening to nonverbal vocalizations can automatically engage preparation of responsive orofacial gestures, an effect that is greatest for positive-valence and high-arousal emotions. The automatic engagement of responsive orofacial gestures by emotional vocalizations suggests that auditory–motor interactions provide a fundamental mechanism for mirroring the emotional states of others during primate social behavior. Motor facilitation by positive vocal emotions suggests a basic neural mechanism for establishing cohesive bonds within primate social groups.

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NEUROBIOLOGY OF DISEASE

Bim and Noxa Are Candidates to Mediate the Deleterious Effect of the NF- κ B Subunit RelA in Cerebral Ischemia

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The transcription factor nuclear factor κ B (NF- κ B) is well known for its antiapoptotic action. However, in some disorders, such as cerebral ischemia, a proapoptotic function of NF- κ B has been demonstrated. To analyze which subunit of NF- κ B is functional in cerebral ischemia, we induced focal cerebral ischemia in mice with a germline deletion of the *p52* or *c-Rel* gene or with a conditional deletion of RelA in the brain. Only RelA deficiency reduced infarct size. Interestingly, expression of the proapoptotic BH3 (Bcl-2 homology domain 3)-only genes *Bim* and *Noxa* in cerebral ischemia depended on RelA and the upstream kinase IKK (I κ B kinase). RelA stimulated *Bim* and *Noxa* gene transcription in primary cortical neurons and bound to the promoter of both genes. Thus, the deleterious function in cerebral ischemia is specific for the NF- κ B subunit RelA and may be mediated through *Bim* and *Noxa*.

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Suppression of Microglial Inflammatory Activity by Myelin Phagocytosis: Role of p47-PHOX-Mediated Generation of Reactive Oxygen Species

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Multiple sclerosis (MS) is pathologically characterized by inflammatory demyelination and neuronal injury. Although phagocytosis of myelin debris by microglia and macrophages in acute MS lesions is well documented, its pathophysiological significance is unclear. Using real-time quantitative PCR, flow cytometry, ELISA, and reactive oxygen species (ROS) measurement assays, we demonstrated that phagocytosis of myelin modulates activation of microglial cells prestimulated by interferon- γ (IFN- γ) or a combination of IFN- γ and lipopolysaccharide with a biphasic temporal pattern, i.e., enhanced production of proinflammatory mediators during the first phase (≤ 6 h), followed by suppression during the second (6–24 h) phase. In this second phase, myelin phagocytosis leads to an enhanced release of prostaglandin E2 and ROS in microglia, whereas the production of anti-inflammatory cytokines (particularly interleukin-10) remains unchanged. Suppression of inflammatory microglial activation by myelin phagocytosis was reversed by treatment with superoxide dismutase and catalase, by inhibition of the NADPH-oxidase complex, or by specific knockdown of the NADPH-oxidase-required adaptor p47-phagocyte oxidase (PHOX). Furthermore, we observed that myelin phagocytosis destabilized tumor necrosis factor- α and

interferon-induced protein-10 mRNA through an adenine–uridine-rich elements-involved mechanism, which was reversed by blocking the function of NADPH–oxidase complex. We conclude that phagocytosis of myelin suppresses microglial inflammatory activities via enhancement of p47-PHOX-mediated ROS generation. These results suggest that intervention in ROS generation could represent a novel therapeutic strategy to reduce neuroinflammation in MS.

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Amyloid- β -Induced Pathological Behaviors Are Suppressed by *Ginkgo biloba* Extract EGb 761 and Ginkgolides in Transgenic *Caenorhabditis elegans*

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Amyloid- β ($A\beta$) toxicity has been postulated to initiate synaptic loss and subsequent neuronal degeneration seen in Alzheimer's disease (AD). We previously demonstrated that the standardized *Ginkgo biloba* extract EGb 761, commonly used to enhance memory and by AD patients for dementia, inhibits $A\beta$ -induced apoptosis in neuroblastoma cells. In this study, we use EGb 761 and its single constituents to associate $A\beta$ species with $A\beta$ -induced pathological behaviors in a model organism, *Caenorhabditis elegans*. We report that EGb 761 and one of its components, ginkgolide A, alleviates $A\beta$ -induced pathological behaviors, including paralysis, and reduces chemotaxis behavior and 5-HT hypersensitivity in a transgenic *C. elegans*. We also show that EGb 761 inhibits $A\beta$ oligomerization and $A\beta$ deposits in the worms. Moreover, reducing oxidative stress is not the mechanism by which EGb 761 and ginkgolide A suppress $A\beta$ -induced paralysis because the antioxidant L-ascorbic acid reduced intracellular levels of hydrogen peroxide to the same extent as EGb 761, but was not nearly as effective in suppressing paralysis in the transgenic *C. elegans*. These findings suggest that (1) EGb 761 suppresses $A\beta$ -related pathological behaviors, (2) the protection against $A\beta$ toxicity by EGb 761 is mediated primarily by modulating $A\beta$ oligomeric species, and (3) ginkgolide A has therapeutic potential for prevention and treatment of AD.

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