

This Week in The Journal

● Cellular/Molecular

Neuron, Astrocyte, and Oligodendrocyte Transcriptomes

John D. Cahoy, Ben Emery, Amit Kaushal, Lynette C. Foo, Jennifer L. Zamanian, Karen S. Christopherson, Yi Xing, Jane L. Lubischer, Paul A. Krieg, Sergey A. Krupenko, Wesley J. Thompson, and Ben A. Barres

(see pages 264–278)

In this issue, Cahoy et al. describe a comprehensive database of quantitative gene expression data from neurons, astrocytes, and oligodendrocytes isolated from mouse forebrain at different developmental stages. To do this, the authors first developed a novel purification strategy, involving immunopanning and fluorescence-activated cell sorting, to obtain nearly pure samples of each cell type, including mature astrocytes. They then profiled the expression of >20,000 genes using GeneChip arrays. Besides providing a wealth of quantitative gene enrichment data for the neuroscience community, their analysis revealed that the transcriptomes of astrocytes and oligodendrocytes are as different from each other as they are from neurons and that cultured astrocytes are substantially different from mature astrocytes (the former may be more akin to reactive astrocytes). In addition, a new, highly specific, broadly expressed astrocyte marker, *aldhL1*, was identified. Finally, analysis of molecular pathway genes suggested that phagocytosis may be a major function of astrocytes.

▲ Development/Plasticity/Repair

Extended Period of Retinocollicular Synaptic Plasticity in $\beta 2^{-/-}$ Mice

Ruchir D. Shah and Michael C. Crair

(see pages 292–303)

Spontaneous retinal waves, mediated by cholinergic synapses during the first postnatal week, are thought to drive refinement of the retinotopic map in the superior colliculus. This week, Shah and Crair use mice lacking the nicotinic acetylcholine receptor $\beta 2$ subunit ($\beta 2^{-/-}$) to investigate mechanisms of synaptic plasticity in retinocollicular synapses. In normal mice, the AMPA/NMDA receptor ratio and the amplitude of AMPA currents increased from postnatal day 3 (P3) to P7, while the proportion of silent synapses decreased. These changes also occurred in $\beta 2^{-/-}$ mice but only after the second postnatal week, when glutamate-mediated retinal waves occur. The delay in maturation of retinocollicular synapses in $\beta 2^{-/-}$ mice was paralleled by an extended period in which long-term potentiation (LTP) could be elicited: a stimulation paradigm that mimicked retinal wave bursts elicited LTP in a majority of synapses in P3–P4 control mice, but not P6–P7 controls, whereas this stimulation produced LTP in $\beta 2^{-/-}$ mice at both ages.

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■ Behavioral/Systems/Cognitive

Antimanic Drugs and AMPA Receptors

Jing Du, Thomas K. Creson, Long-Jun Wu, Ming Ren, Neil A. Gray, Cynthia Falke, Yanling Wei, Yun Wang, Rayah Blumenthal, Rodrigo Machado-Vieira, Peixiong Yuan, Guang Chen, Min Zhuo, and Husseini K. Manji

(see pages 68–79)

Although lithium and valproate have long been used to treat manic disorder, the mode of action of these structurally dissimilar treatments is not fully understood. Du et al. now suggest that these drugs reduce trafficking of AMPA receptor GluR1/GluR2 tetramers to synapses. Chronic treatment of rats with therapeutic doses of lithium or valproate reduced levels of GluR2 in hippocampal synaptosomes, as had been shown previously for GluR1. The overall expression level of GluR2 in hippocampus was unchanged, however, suggesting that the reduction in membrane expression may result from decreased receptor trafficking. This hypothesis was supported by chronically treating rats with a fusion peptide that specifically blocks PKA phosphorylation of GluR1 at Ser 845, a site necessary for insertion of GluR1/2 tetramers into membranes. The treatment mimicked the effects of lithium and valproate on GluR1 and GluR2 localization, and it also de-

creased amphetamine-induced hyperactivity, a common model of mania.

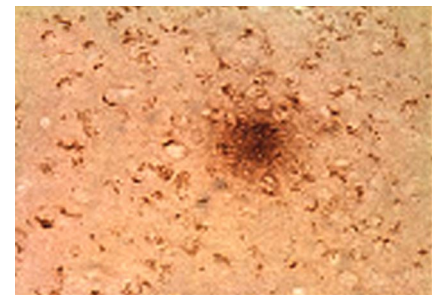
◆ Neurobiology of Disease

Environmental Trigger for Alzheimer's Disease

Jinfang Wu, Md. Riyaz Basha, Brian Brock, David P. Cox, Fernando Cardozo-Pelaez, Christopher A. McPherson, Jean Harry, Deborah C. Rice, Bryan Maloney, Demao Chen, Debomoy K. Lahiri, and Nasser H. Zawia

(see pages 3–9)

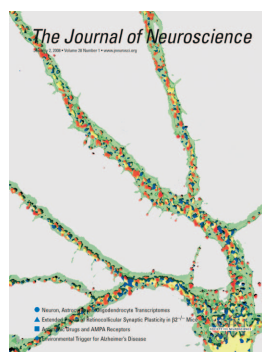
Early exposure to environmental toxins can lead to diseases much later in life. This week, Wu et al. report that primates exposed to lead as infants showed Alzheimer's disease (AD)-like pathology years later. From birth to 400 d of age, monkeys were exposed to lead levels that produced no obvious sign of toxicity. Although by young adulthood blood lead levels in exposed monkeys were indistinguishable from those of controls, when examined at approximately 23 years of age, the brains of lead-exposed monkeys exhibited many hallmarks of AD, including $A\beta$ plaques and neurofibrillary tangles, as well as increased expression of $A\beta$ precursor protein (APP) and Sp1, a transcription factor that regulates APP expression. DNA methyltransferase I activity was reduced in lead-exposed monkeys, whereas oxidative damage to DNA was increased. These results indicate that lead exposure early in life can predispose animals to later neurodegenerative disease, possibly through alterations in DNA methylation and oxidation.



AD-like pathology in forebrain section of 23-year-old monkeys exposed to lead as infants. Anti- $A\beta$ antibody staining reveals granular and intracellular staining and plaques. See the article by Wu et al. for details.

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Cover legend: Immunofluorescence and 3-D reconstruction of a cultured hippocampal neuron labeled for phosphorylated FMRP (red), total FMRP (blue), and F-actin using phalloidin (green). Colocalization of FMRP and phosphorylated FMRP is shown in yellow. FMRP is transiently dephosphorylated by PP2A in response to mGluR activation to allow translation of FMRP-bound mRNAs. For more information, see the article by Narayanan et al. in the December 26, 2007 issue (pages 14349-14357).

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BRIEF COMMUNICATIONS

NF-Protocadherin and TAF1 Regulate Retinal Axon Initiation and Elongation *In Vivo*

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NF-protocadherin (NFPC)-mediated cell–cell adhesion plays a critical role in vertebrate neural tube formation. NFPC is also expressed during the period of axon tract formation, but little is known about its function in axonogenesis. Here we have tested the role of NFPC and its cytosolic cofactor template-activating factor 1 (TAF1) in the emergence of the *Xenopus* retinotectal projection. NFPC is expressed in the developing retina and optic pathway and is abundant in growing retinal axons. Inhibition of NFPC function in developing retinal ganglion cells (RGCs) severely reduces axon initiation and elongation and suppresses dendrite genesis. Furthermore, an identical phenotype occurs when TAF1 function is blocked. These data provide evidence that NFPC regulates axon initiation and elongation and indicate a conserved role for TAF1, a transcriptional regulator, as a downstream cytosolic effector of NFPC in RGCs.

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The Magnocellular Mediodorsal Thalamus is Necessary for Memory Acquisition, But Not Retrieval

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Damage to the magnocellular mediodorsal thalamic nucleus (MDmc) in the human brain is associated with both retrograde and anterograde amnesia. In the present study we made selective neurotoxic MDmc lesions in rhesus monkeys and compared the effects of these lesions on memory acquisition and retrieval. Monkeys learned 300 unique scene discriminations preoperatively and retention was assessed in a one-trial preoperative retrieval test. Bilateral neurotoxic lesions of the MDmc, produced by $10 \times 1 \mu\text{l}$ injections of a mixture of ibotenate and NMDA did not affect performance in the postoperative one-trial retrieval test. In contrast, new postoperative learning of a further 100 novel scene discriminations was substantially impaired. Thus, MDmc is required for new learning of scene discriminations but not for their retention and retrieval. This finding is the first evidence that MDmc plays a specific role in memory acquisition.

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Articles

CELLULAR/MOLECULAR

Phosphorylation of SNAP-25 at Ser187 Mediates Enhancement of Exocytosis by a Phorbol Ester in INS-1 Cells

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Activation of diacylglycerol (DAG) signaling pathways with phorbol esters dramatically enhances Ca^{2+} -triggered exocytosis from both endocrine cells and neurons, however the relevant targets of DAG are controversial. A possible effector mechanism for this signaling pathway is phosphorylation of SNAP-25 (25 kDa synaptosome-associated protein) at Ser187 by PKC. Here, we investigated the role of Ser187 in the enhancement of exocytosis by the phorbol ester PMA (phorbol 12-myristate 13-acetate). We used patch-clamp measurements of membrane capacitance together with photorelease of caged- Ca^{2+} and membrane depolarization to study exocytosis. Expression of the nonphosphorylatable S187C SNAP-25 mutant did not attenuate the enhancement of exocytosis by PMA in either bovine chromaffin cells or the INS-1 insulin-secreting cell line. To test the effects of Ser187 mutations under conditions in which the endogenous SNAP-25 is disabled, we expressed botulinum toxin serotype E to cleave SNAP-25 in INS-1 cells. Coexpression of a toxin-resistant mutant (TR), but not wild-type SNAP-25, was able to rescue PMA-modulated exocytosis. Coexpression of the toxin with the TR-S187C SNAP-25 mutant was able to completely block the enhancement of exocytosis by PMA in response to photoelevation of $[\text{Ca}^{2+}]_i$ to low μM levels or to a depolarizing train. The phospho-mimetic S187E mutation enhanced the small, fast burst of exocytosis evoked by photoelevation of Ca^{2+} , but, like PMA, had smaller effects on exocytosis evoked by a depolarizing train. This work supports the hypothesis that phosphorylation of Ser187 of SNAP-25 by PKC is a key step in the enhancement of exocytosis by DAG.

The Journal of Neuroscience, January 2, 2008 • 28(1):21–30

Mutations in a *Drosophila* $\alpha_2\delta$ Voltage-Gated Calcium Channel Subunit Reveal a Crucial Synaptic Function

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Voltage-dependent calcium channels regulate many aspects of neuronal biology, including synaptic transmission. In addition to their α_1 subunit, which encodes the essential voltage gate and selective pore, calcium channels also contain auxiliary $\alpha_2\delta$, β , and γ subunits. Despite progress in understanding the biophysical properties of calcium channels, the *in vivo* functions of these auxiliary subunits remain unclear. We have isolated mutations in the gene encoding an $\alpha_2\delta$ calcium channel subunit (*d $\alpha_2\delta$ -3*) using a forward genetic screen in *Drosophila*. Null mutations in this gene are embryonic lethal and can be rescued by expression in the nervous system, demonstrating that the essential function of this subunit is neuronal. The photoreceptor phenotype of *d $\alpha_2\delta$ -3* mutants resembles that of the calcium channel α_1 mutant *cacophony* (*cac*), suggesting shared functions. We have examined in detail genotypes that survive to the third-instar stage. Electrophysiological recordings demonstrate that synaptic transmission is severely impaired in these mutants. Thus the $\alpha_2\delta$ calcium channel subunit is critical for calcium-dependent synaptic function. As such, this *Drosophila* isoform is the likely partner to the presynaptic calcium channel α_1 subunit encoded by the *cac* locus. Consistent with this hypothesis, *cacGFP* fluorescence at the neuromuscular junction is reduced in *d $\alpha_2\delta$ -3* mutants. This is the first characterization of an $\alpha_2\delta$ -3 mutant in any organism and indicates a necessary role for $\alpha_2\delta$ -3 in presynaptic vesicle release and calcium channel expression at active zones.

The Journal of Neuroscience, January 2, 2008 • 28(1):31–38

Lrig1 Is an Endogenous Inhibitor of Ret Receptor Tyrosine Kinase Activation, Downstream Signaling, and Biological Responses to GDNF

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Glial cell line-derived neurotrophic factor (GDNF)/Ret signaling has potent trophic effects on ventral midbrain dopaminergic, motor, sensory, and sympathetic neurons. The molecular mechanisms that restrict Ret receptor tyrosine kinase activation are not well understood. Here, we show that Lrig1, a transmembrane protein containing leucine-rich repeats and Ig-like domains in its extracellular region, acts in a negative feedback loop to regulate the activity of Ret receptor tyrosine kinase. In particular, we demonstrate that Lrig1 is capable of physically interacting with Ret and that Lrig1/Ret association inhibits GDNF binding, recruitment of Ret to lipid rafts, receptor autophosphorylation, and mitogen-activated protein kinase (MAPK) activation in response to GDNF. In neuronal cells, Lrig1 overexpression also inhibits GDNF/Ret-induced neurite outgrowth in a cell-autonomous manner. Downregulation of Lrig1 using small interference RNA knock-down experiments potentiates both neuronal differentiation and MAPK activation in response to GDNF. Together, these results provide an insight into Lrig1 function and establish a new physiological mechanism to restrict signaling and biological responses induced by GDNF and Ret in neuronal cells.

The Journal of Neuroscience, January 2, 2008 • 28(1):39–49

Taurine Is a Potent Activator of Extrasynaptic GABA_A Receptors in the Thalamus

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Taurine is one of the most abundant free amino acids in the brain. In a number of studies, taurine has been reported to activate glycine receptors (Gly-Rs) at moderate concentrations ($\geq 100 \mu\text{M}$), and to be a weak agonist at GABA_A receptors (GABA_A-Rs), which are usually activated at high concentrations ($\geq 1 \text{ mM}$). In this study, we show that taurine reduced the excitability of thalamocortical relay neurons and activated both extrasynaptic GABA_A-Rs and Gly-Rs in neurons in the mouse ventrobasal (VB) thalamus. Low concentrations of taurine (10 – $100 \mu\text{M}$) decreased neuronal input resistance and firing frequency, and elicited a steady outward current under voltage clamp, but had no effects on fast inhibitory synaptic currents. Currents elicited by $50 \mu\text{M}$ taurine were abolished by gabazine, insensitive to midazolam, and partially blocked by $20 \mu\text{M}$ Zn^{2+} , consistent with the pharmacological properties of extrasynaptic GABA_A-Rs ($\alpha 4\beta 2\delta$ subtype) involved in tonic inhibition in the thalamus. Tonic inhibition was enhanced by an inhibitor of taurine transport, suggesting that taurine can act as an endogenous activator of these receptors. Taurine-evoked currents were absent in relay neurons from GABA_A-R $\alpha 4$ subunit knock-out mice. The amplitude of the taurine current was larger in neurons from adult mice than juvenile mice. Taurine was a more potent agonist at recombinant $\alpha 4\beta 2\delta$ GABA_A-Rs than at $\alpha 1\beta 2\gamma 2$ GABA_A-Rs. We conclude that physiological concentrations of taurine can inhibit VB neurons via activation of extrasynaptic GABA_A-Rs and that taurine may function as an endogenous regulator of excitability and network activity in the thalamus.

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Order-Dependent Coincidence Detection in Cerebellar Purkinje Neurons at the Inositol Trisphosphate Receptor

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Associative long-term depression (LTD) at cerebellar parallel fiber–Purkinje cell synapses is sensitive to the temporal order in which the parallel fiber is coactivated with the climbing fiber input, but how order sensitivity is achieved is unknown. Here we show that the cerebellar inositol-1,4,5-trisphosphate (IP₃) receptor, whose activation is required for LTD induction, is sensitive *in situ* to the order of presentation of its coagonists, IP₃ and cytoplasmic calcium. By focally photolyzing a novel caged IP₃ compound in dendritic spines, we find that pairing IP₃ with climbing fiber-mediated calcium entry leads to a large calcium release transient if the climbing fiber is activated up to 100 ms before or up to 500 ms after IP₃ uncaging. This asymmetric timing window for coactivation follows the kinetics of calcium removal and IP₃ unbinding from the receptor and is not limited by IP₃ metabolism. IP₃ receptor binding thus acts as an eligibility trace that can drive temporal order-dependent calcium release and LTD induction in Purkinje cells and event order-dependent sensory plasticity in the whole animal.

The Journal of Neuroscience, January 2, 2008 • 28(1):133–142

A Transcriptome Database for Astrocytes, Neurons, and Oligodendrocytes: A New Resource for Understanding Brain Development and Function

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Understanding the cell–cell interactions that control CNS development and function has long been limited by the lack of methods to cleanly separate neural cell types. Here we describe methods for the prospective isolation and purification of astrocytes, neurons, and oligodendrocytes from developing and mature mouse forebrain. We used FACS (fluorescent-activated cell sorting) to isolate astrocytes from transgenic mice that express enhanced green fluorescent protein (EGFP) under the control of an S100 β promoter. Using Affymetrix GeneChip Arrays, we then created a transcriptome database of the expression levels of >20,000 genes by gene profiling these three main CNS neural cell types at various postnatal ages between postnatal day 1 (P1) and P30. This database provides a detailed global characterization and comparison of the genes expressed by acutely isolated astrocytes, neurons, and oligodendrocytes. We found that Aldh1L1 is a highly specific antigenic marker for astrocytes with a substantially broader pattern of astrocyte expression than the traditional astrocyte marker GFAP. Astrocytes were enriched in specific metabolic and lipid synthetic pathways, as well as the draper/Megf10 and MerTK/integrin $\alpha_v\beta_3$ phagocytic pathways suggesting that astrocytes are professional phagocytes. Our findings call into question the concept of a “glial” cell class as the gene profiles of astrocytes and oligodendrocytes are as dissimilar to each other as they are to neurons. This transcriptome database of acutely isolated purified astrocytes, neurons, and oligodendrocytes provides a resource to the neuroscience community by providing improved cell-type-specific markers and for better understanding of neural development, function, and disease.

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Heterogeneity in Synaptic Vesicle Release at Neuromuscular Synapses of Mice Expressing SynaptopHluorin

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Mammalian neuromuscular junctions are useful model synapses to study the relationship between synaptic structure and function, although these have rarely been studied together at the same synapses. To do this, we generated transgenic lines of mice in which the *thy1.2* promoter drives expression of synaptopHluorin (spH) as a means of optically measuring synaptic vesicle distribution and release. spH is colocalized with other synaptic vesicle proteins in presynaptic terminals and does not alter normal synaptic function. Nerve stimulation leads to readily detectable and reproducible fluorescence changes in motor axon terminals that vary with stimulus frequency and, when compared with electrophysiological recordings, are reliable indicators of neurotransmitter release. Measurements of fluorescence intensity changes reveal a surprising amount of heterogeneity in synaptic vesicle release throughout individual presynaptic motor axon terminals. Some discrete terminal regions consistently displayed a greater rate and extent of release than others, regardless of stimulation frequency. The amount of release at a particular site is highly correlated to the relative abundance of synaptic vesicles there, indicating that a relatively constant fraction of the total vesicular pool, ~30%, is released in response to activity. These studies reveal previously unknown relationships between synaptic structure and function at mammalian neuromuscular junctions and demonstrate the usefulness of spH expressing mice as a tool for studying neuromuscular synapses in adults, as well as during development and diseases that affect neuromuscular synaptic function.

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Mechanisms of Compartmentalized Expression of Mrg Class G-Protein-Coupled Sensory Receptors

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Mrg class G-protein-coupled receptors (GPCRs) are expressed exclusively in sensory neurons in the trigeminal and dorsal root ganglia. Pharmacological activation of Mrg proteins is capable of modulating sensory neuron activities and elicits nociceptive effects. In this study, we illustrate a control mechanism that allows the Runx1 runt domain transcription factor to generate compartmentalized expression of these sensory GPCRs. Expression of *MrgA*, *MrgB*, and *MrgC* subclasses is confined to an “A/B/C” neuronal compartment that expresses Runx1 transiently (or does not express Runx1), whereas *MrgD* expression is restricted to a “D” compartment with persistent Runx1 expression. *Runx1* is initially required for the expression of all *Mrg* genes. However, during late development Runx1 becomes a repressor for *MrgA/B/C* genes. As a result, *MrgA/B/C* expression persists only in the Runx1[−] “A/B/C” compartment. In $\Delta 446$ mice, in which Runx1 lacks the C-terminal repression domain, expression of *MrgA/B/C* genes is dramatically expanded into the Runx1⁺ “D” compartment. *MrgD* expression, however, is resistant to Runx1-mediated repression in the “D” compartment. Therefore, the creation of Runx1⁺ and Runx1[−] compartments, in conjunction with different responses of *Mrg* genes to Runx1-mediated repression, results in the compartmentalized expression of *MrgA/B/C* versus *MrgD* genes. Within the *MrgA/B/C* compartment, *MrgB4*-expressing neurons innervate exclusively the hairy skin. Here we found that Smad4, a downstream component of bone morphological protein-mediated signaling, is required selectively for the expression of *MrgB4*. Our study suggests a new line of evidence that specification of sensory subtypes is established progressively during perinatal and postnatal development.

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Differential Outgrowth of Axons and their Branches Is Regulated by Localized Calcium Transients

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During development axon outgrowth and branching are independently regulated such that axons can stall or retract while their interstitial branches extend toward targets. Previous studies have shown that guidance cues and intracellular signaling components can promote branching of cortical axons without affecting axon outgrowth. However, the mechanisms that regulate differential outgrowth of axons and their branches are not well understood. Based on our previous work showing the importance of localized repetitive calcium transients in netrin-1-induced cortical axon branching, we sought to investigate the role of calcium signaling in regulating differential outgrowth of axons and their branches. Using fluorescence calcium imaging of dissociated developing cortical neurons, we show that localized spontaneous calcium transients of different frequencies occur in restricted regions of axons and their branches. Higher frequencies occur in more rapidly extending processes whereas lower frequencies occur in processes that stall or retract. Direct induction of localized calcium transients with photolysis of caged calcium induced rapid outgrowth of axonal processes. Surprisingly outgrowth of one axonal process was almost invariably accompanied by simultaneous retraction of another process belonging to the same axon, suggesting a competitive mechanism for differential process outgrowth. Conversely, reducing frequencies of calcium transients with nifedipine and TTX reduced the incidence of differential process outgrowth. Together these results suggest a novel activity-dependent mechanism whereby intrinsic localized calcium transients regulate the competitive growth of axons and their branches. These mechanisms may also be important for the development of cortical connectivity *in vivo*.

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L1 Interaction with Ankyrin Regulates Mediolateral Topography in the Retinocollicular Projection

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Dynamic modulation of adhesion provided by anchorage of axonal receptors with the cytoskeleton contributes to attractant or repellent responses that guide axons to topographic targets in the brain. The neural cell adhesion molecule L1 engages the spectrin-actin cytoskeleton through reversible linkage of its cytoplasmic domain to ankyrin. To investigate a role for L1 association with the cytoskeleton in topographic guidance of retinal axons to the superior colliculus, a novel mouse strain was generated by genetic knock-in that expresses an L1 point mutation (Tyr1229His) abolishing ankyrin binding. Axon tracing revealed a striking mistargeting of mutant ganglion cell axons from the ventral retina, which express high levels of ephrinB receptors, to abnormally lateral sites in the contralateral superior colliculus, where they formed multiple ectopic arborizations. These axons were compromised in extending interstitial branches in the medial direction, a normal response to the high medial to low lateral SC gradient of ephrinB1. Furthermore, ventral but not dorsal L1(Y1229H) retinal cells were impaired for ephrinB1-stimulated adhesion through $\beta 1$ integrins in culture. The

retinocollicular phenotype of the L1(Tyr1229His) mutant provides the first evidence that L1 regulates topographic mapping of retinal axons through adhesion mediated by linkage to the actin cytoskeleton and functional interaction with the ephrinB/EphB targeting system.
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Testosterone-Induced Matrix Metalloproteinase Activation Is a Checkpoint for Neuronal Addition to the Adult Songbird Brain

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Testosterone-induced neuronal addition to the adult songbird vocal control center, HVC, requires the androgenic induction of vascular endothelial growth factor (VEGF), followed by VEGF-stimulated angiogenesis. The expanded vasculature acts as a source of BDNF, which supports the immigration of new neurons from the overlying ventricular zone. In tumorigenesis, a similar process of adult angiogenesis is regulated by matrix metalloproteinase (MMP) activity, in particular that of the gelatinases. We therefore investigated the role of the gelatinases in neuronal addition to the HVC of adult female canaries. *In situ* zymography of the caudal forebrain revealed that testosterone-induced perivascular gelatinase activity that was most prominent in HVC. High-resolution gels revealed distinct MMP activities that comigrated with MMP2 and MMP9, and PCR cloning yielded MMP2 and MMP9 orthologues of 1465 and 1044 bp, respectively. Quantitative PCR revealed that HVC MMP2 mRNA levels doubled within 8 d of testosterone, whereas MMP9 transcript levels were stable. Moreover, isolated adult canary forebrain endothelial cells secreted MMP2, and VEGF substantially increased endothelial MMP2 gelatinase activity. To assess the importance of androgen-regulated, VEGF-induced MMP2 to adult angiogenesis and neurogenesis, we treated testosterone-implanted females with the gelatinase inhibitor SB-3CT. *In situ* zymography confirmed that SB-3CT suppressed gelatinase activity in HVC, and histological analysis revealed that SB-3CT-treated birds exhibited a decreased endothelial mitotic index and substantially diminished neuronal recruitment to HVC. These data suggest that the androgenic induction of endothelial MMP2 is a critical regulator of neuronal addition to the adult HVC, and as such comprises an important regulatory step in adult neurogenesis.

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Pigment Dispersing Factor-Dependent and -Independent Circadian Locomotor Behavioral Rhythms

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Circadian pacemaker circuits consist of ensembles of neurons, each expressing molecular oscillations, but how circuit-wide coordination of multiple oscillators regulates rhythmic physiological and behavioral outputs remains an open question. To investigate the relationship between the pattern of oscillator phase throughout the circadian pacemaker circuit and locomotor activity rhythms in *Drosophila*, we perturbed the electrical activity and pigment dispersing factor (PDF) levels of the lateral ventral neurons (LN_v) and assayed their combinatorial effect on molecular oscillations in different parts of the circuit and on locomotor activity behavior. Altered electrical activity of PDF-expressing LN_v causes initial behavioral arrhythmicity followed by gradual long-term emergence of two concurrent short- and long-period circadian behavioral activity bouts in ~60% of flies. Initial desynchrony of circuit-wide molecular oscillations is followed by the emergence of a novel pattern of period (PER) synchrony whereby two subgroups of dorsal neurons (DN1 and DN2) exhibit PER oscillation peaks coinciding with two activity bouts, whereas other neuronal subgroups exhibit a single PER peak coinciding with one of the two activity bouts. The emergence of this novel pattern of circuit-wide oscillator synchrony is not accompanied by concurrent change in the electrical activity of the LN_v. In PDF-null flies, altered electrical activity of LN_v drives a short-period circadian activity bout only, indicating that PDF-independent factors underlie the short-period circadian activity component and that the long-period circadian component is PDF-dependent. Thus, polyrhythmic behavioral patterns in electrically manipulated flies are regulated by circuit-wide coordination of molecular oscillations and electrical activity of LN_v via PDF-dependent and -independent factors.

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Establishment of a Scaffold for Orientation Maps in Primary Visual Cortex of Higher Mammals

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In higher mammals, environmentally driven patterns of neural activity do not play a role in the establishment of orientation specificity and maps. It has been proposed that specific long-range interactions provide the scaffold for developing orientation maps. Our model aims at explaining how such a scaffold could develop in the first place. Broad spontaneous activity waves and locally evoked spatially periodic response patterns are used. The model is discussed in relation to biological evidence, and experiments to test the model are proposed. We show that reliable orientation specificity cannot be a result of haphazard cortical wiring, as has been proposed.

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Retinocollicular Synapse Maturation and Plasticity Are Regulated by Correlated Retinal Waves

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During development, spontaneous retinal waves are thought to provide an instructive signal for retinotopic map formation in the superior colliculus. In mice lacking the $\beta 2$ subunit of nicotinic ACh receptors ($\beta 2^{-/-}$), correlated retinal waves are absent during the first postnatal week, but return during the second postnatal week. In control retinocollicular synapses, *in vitro* analysis reveals that AMPA/NMDA ratios and AMPA quantal amplitudes increase during the first postnatal week while the prevalence of silent synapses decreases. In age-matched $\beta 2^{-/-}$ mice, however, these parameters remain unchanged through the first postnatal week in the absence of retinal waves, but quickly mature to control levels by the end of the second week, suggesting that the delayed onset of correlated waves is able to drive synapse maturation. To examine whether such a mechanistic relationship exists, we applied a “burst-based” plasticity protocol that mimics coincident activity during retinal waves. We find that this pattern of activation is indeed capable of inducing synaptic strengthening [long-term potentiation (LTP)] on average across genotypes early in the first postnatal week [postnatal day 3 (P3) to P4] and, interestingly, that the capacity for LTP at the end of the first week (P6–P7) is significantly greater in immature $\beta 2^{-/-}$ synapses than in mature control synapses. Together, our results suggest that retinal waves drive retinocollicular synapse maturation through a learning rule that is physiologically relevant to natural wave statistics and that these synaptic changes may serve an instructive role during retinotopic map refinement.

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p75 Neurotrophin Receptor Mediates Neuronal Cell Death by Activating GIRK Channels through Phosphatidylinositol 4,5-Bisphosphate

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The pan neurotrophin receptor p75^{NTR} signals programmed cell death both during nervous system development and after neural trauma and disease in the adult. However, the molecular pathways by which death is mediated remain poorly understood. Here, we show that this cell death is initiated by activation of G-protein-coupled inwardly rectifying potassium (GIRK/Kir3) channels and a consequent potassium efflux. Death signals stimulated by neurotrophin-mediated cleavage of p75^{NTR} activate GIRK channels through the generation and binding of phosphatidylinositol 4,5-bisphosphate [PtdIns(4,5)P₂/PIP₂] to GIRK channels. Both GIRK channel activity and p75^{NTR}-mediated neuronal death are inhibited by sequestration of PtdIns(4,5)P₂ and application of GIRK channel inhibitors, whereas pertussis toxin treatment has no effect. Thus, p75^{NTR} activates GIRK channels without the need for G_{i/o}-proteins. Our results demonstrate a novel mode of activation of GIRK channels, representing an early step in the p75^{NTR}-mediated cell death pathway and suggesting a function for these channels during nervous system development.

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BEHAVIORAL/SYSTEMS/COGNITIVE

Cerebellar Dysfunction Explains the Extinction-Like Abolition of Conditioned Eyeblinks After NBQX Injections in the Inferior Olive

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Classical conditioning of the eyeblink response is a form of motor learning that is controlled by the intermediate cerebellum and related brainstem structures. The inferior olive (IO) is commonly thought to provide the cerebellum with a “teaching” unconditioned stimulus (US) signal required for cerebellar learning. Testing this concept has been difficult because the IO, in addition to its putative learning function, also controls tonic activity in the cerebellum. Previously, it was reported that inactivation of AMPA/kainate receptors in the IO produces extinction of conditioned responses (CRs), suggesting that it blocks the transmission of US signals without perturbing the functional state of the cerebellum. However, the electrophysiological support for this critical finding was lacking, mostly because of methodological difficulties in maintaining stable recordings from the same set of single units throughout long drug injection sessions in awake rabbits. To address this critical issue, we used our microwire-based multiple single-unit recording method. The IO in trained rabbits was injected with the AMPA/kainate receptor blocker, 1,2,3,4-tetrahydro-6-nitro-2,3-dioxo-benzof[*j*]quinoxaline-7-sulfonamide (NBQX), and its effects on CR expression and neuronal activity in the cerebellar interposed nuclei (IN) were examined. We found that NBQX abolished CR expression and that delayed drug effects were independent of the presentation of the conditioned stimulus and were therefore not related to extinction. In parallel to these behavioral effects, the spontaneous neuronal activity and CR-related neuronal responses in the IN were suppressed, suggesting cerebellar dysfunction. These findings indicate that testing the role of IO in learning requires methods that do not alter the functional state of the cerebellum.

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The Role of Hippocampal GluR1 and GluR2 Receptors in Manic-Like Behavior

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The cellular basis underlying the complex clinical symptomatology of bipolar disorder and the mechanisms underlying the actions of its effective treatments have not yet been fully elucidated. This study investigated the role of hippocampal synaptic AMPA receptors. We found that chronic administration of the antimanic agents lithium and valproate (VPA) reduced synaptic AMPA receptor GluR1/2 in hippocampal neurons *in vitro* and *in vivo*. Electrophysiological studies confirmed that the AMPA/NMDA ratio was reduced in CA1 regions of hippocampal slices from lithium-treated animals. Reduction in GluR1 phosphorylation at its cAMP-dependent protein kinase A site by the synthetic peptide TAT-S845, which mimics the effects of lithium or VPA, was sufficient to attenuate surface and synaptic GluR1/2 levels in hippocampal neurons *in vitro* and *in vivo*. Intrahippocampal infusion studies with the AMPA-specific inhibitor GYKI 52466 [4-(8-methyl-9H-1,3-dioxolo[4,5-h][2,3]benzodiazepin-5-yl)-benzenamine hydrochloride], a GluR1-specific TAT-S845 peptide, showed that GluR1/2 was essential for the development of manic/hedonic-like behaviors such as amphetamine-induced hyperactivity. These studies provide novel insights into the role of hippocampal GluR1/2 receptors in mediating facets of the manic syndrome and offer avenues for the development of novel therapeutics for these disorders.

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Glycinergic “Inhibition” Mediates Selective Excitatory Responses to Combinations of Sounds

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In the mustached bat's inferior colliculus (IC), combination-sensitive neurons display time-sensitive facilitatory interactions between inputs tuned to distinct spectral elements in sonar or social vocalizations. Here we compare roles of ionotropic receptors to glutamate (iGluRs), glycine (GlyRs), and GABA (GABA_ARs) in facilitatory combination-sensitive interactions. Facilitatory responses to 36 single IC neurons were recorded before, during, and after local application of antagonists to these receptors. The NMDA receptor antagonist CPP [(±)-3-(2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid], alone ($n = 14$) or combined with AMPA receptor antagonist NBQX ($n = 22$), significantly reduced or eliminated responses to best frequency (BF) sounds across a broad range of sound levels, but did not eliminate combination-sensitive facilitation. In a subset of neurons, GABA_AR blockers bicuculline or gabazine were applied in addition to iGluR blockers. GABA_AR blockers did not “uncover” residual iGluR-mediated excitation, and only rarely eliminated facilitation. In nearly all neurons for which the GlyR antagonist strychnine was applied in addition to iGluR blockade (22 of 23 neurons, with or without GABA_AR blockade), facilitatory interactions were eliminated. Thus, neither glutamate nor GABA neurotransmission are required for facilitatory combination-sensitive interactions in IC. Instead, facilitation may depend entirely on glycinergic inputs that are presumed to be inhibitory. We propose that glycinergic inputs tuned to two distinct spectral elements in vocal signals each activate postinhibitory rebound excitation. When rebound excitations from two spectral elements coincide, the neuron discharges. Excitation from glutamatergic inputs, tuned to the BF of the neuron, is superimposed onto this facilitatory interaction, presumably mediating responses to a broader range of acoustic signals.

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Systems Neuroplasticity in the Aging Brain: Recruiting Additional Neural Resources for Successful Motor Performance in Elderly Persons

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Functional imaging studies have shown that seniors exhibit more elaborate brain activation than younger controls while performing motor tasks. Here, we investigated whether this age-related overactivation reflects compensation or dedifferentiation mechanisms. “Compensation” refers to additional activation that counteracts age-related decline of brain function and supports successful performance, whereas “dedifferentiation” reflects age-related difficulties in recruiting specialized neural mechanisms and is not relevant to task performance. To test these predictions, performance on a complex interlimb coordination task was correlated with brain activation. Findings revealed that coordination resulted in activation of classical motor coordination regions, but also higher-level sensorimotor regions, and frontal regions in the elderly. Interestingly, a positive correlation between activation level in these latter regions and motor performance was observed in the elderly. This performance enhancing additional recruitment is consistent with the compensation hypothesis and characterizes neuroplasticity at the systems level in the aging brain.

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Medial Temporal Lobe Activity Predicts Successful Relational Memory Binding

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Previous neuropsychological findings have implicated medial temporal lobe (MTL) structures in retaining object-location relations over the course of short delays, but MTL effects have not always been reported in neuroimaging investigations with similar short-term memory requirements. Here, we used event-related functional magnetic

resonance imaging to test the hypothesis that the hippocampus and related MTL structures support accurate retention of relational memory representations, even across short delays. On every trial, four objects were presented, each in one of nine possible locations of a three-dimensional grid. Participants were to mentally rotate the grid and then maintain the rotated representation in anticipation of a test stimulus: a rendering of the grid, rotated 90° from the original viewpoint. The test stimulus was either a “match” display, in which object-location relations were intact, or a “mismatch” display, in which one object occupied a new, previously unfilled location (mismatch position), or two objects had swapped locations (mismatch swap). Encoding phase activation in anterior and posterior regions of the left hippocampus, and in bilateral perirhinal cortex, predicted subsequent accuracy on the short-term memory decision, as did bilateral posterior hippocampal activity after the test stimulus. Notably, activation in these posterior hippocampal regions was also sensitive to the degree to which object-location bindings were preserved in the test stimulus; activation was greatest for match displays, followed by mismatch-position displays, and finally mismatch-swap displays. These results indicate that the hippocampus and related MTL structures contribute to successful encoding and retrieval of relational information in visual short-term memory.

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Speed, Spatial, and Temporal Tuning of Rod and Cone Vision in Mouse

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Rods and cones subserve mouse vision over a 100 million-fold range of light intensity (-6 to $2 \log \text{cd m}^{-2}$). Rod pathways tune vision to the temporal frequency of stimuli (peak, 0.75 Hz) and cone pathways to their speed (peak, $\sim 12^\circ/\text{s}$). Both pathways tune vision to the spatial components of stimuli (0.064–0.128 cycles/°). The specific photoreceptor contributions were determined by two-alternative, forced-choice measures of contrast thresholds for optomotor responses of C57BL/6J mice with normal vision, *Gnat2^{cpfl3}* mice without functional cones, and *Gnat1^{-/-}* mice without functional rods. *Gnat2^{cpfl3}* mice (threshold, $-6.0 \log \text{cd m}^{-2}$) cannot see rotating gratings above $-2.0 \log \text{cd m}^{-2}$ (photopic vision), and *Gnat1^{-/-}* mice (threshold, $-4.0 \log \text{cd m}^{-2}$) are blind below $-4.0 \log \text{cd m}^{-2}$ (scotopic vision). Both genotypes can see in the transitional mesopic range (-4.0 to $-2.0 \log \text{cd m}^{-2}$). Mouse rod and cone sensitivities are similar to those of human. This parametric study characterizes the functional properties of the mouse visual system, revealing the rod and cone contributions to contrast sensitivity and to the temporal processing of visual stimuli.

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Inhibition of Serotonin But Not Norepinephrine Transport during Development Produces Delayed, Persistent Perturbations of Emotional Behaviors in Mice

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Serotonin (5-HT) acts as a neurotransmitter, but also modulates brain maturation during early development. The demonstrated influence of genetic variants on brain function, personality traits, and susceptibility to neuropsychiatric disorders suggests a critical importance of developmental mechanisms. However, little is known about how and when developmentally perturbed 5-HT signaling affects circuitry and resulting behavior. The 5-HT transporter (5-HTT) is a key regulator of extracellular 5-HT levels and we used pharmacologic strategies to manipulate 5-HTT function during development and determine behavioral consequences. Transient exposure to the 5-HTT inhibitors fluoxetine, clomipramine, and citalopram from postnatal day 4 (P4) to P21 produced abnormal emotional behaviors in adult mice. Similar treatment with the norepinephrine transporter (NET) inhibitor, desipramine, did not adversely affect adult behavior, suggesting that 5-HT and norepinephrine (NE) do not share the same effects on brain development. Shifting our period of treatment/testing to P90/P185 failed to mimic the effect of earlier exposure, demonstrating that 5-HT effects on adult behavior are developmentally specific. We have hypothesized that early-life perturbations of 5-HT signaling affect corticolimbic circuits that do not reach maturity until the peri-adolescent period. In support of this idea, we found that abnormal behaviors resulting from postnatal fluoxetine exposure have a post-pubescent onset and persist long after reaching adult age. A better understanding of the underlying 5-HT sensitive circuits and how they are perturbed should lead to new insights into how various genetic polymorphisms confer their risk to carriers. Furthermore, these studies should help determine whether *in utero* exposure to 5-HTT blocking drugs poses a risk for behavioral abnormalities in later life.

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Vasopressin Increases Locomotion through a V1a Receptor in Orexin/Hypocretin Neurons: Implications for Water Homeostasis

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Water homeostasis is a critical challenge to survival for land mammals. Mice display increased locomotor activity when dehydrated, a behavior that improves the likelihood of locating new sources of water and simultaneously places additional demands on compromised hydration levels. The neurophysiology underlying this well known behavior has not been previously elucidated. We report that the anti-diuretic hormone arginine-vasopressin (AVP) is involved in this response. AVP and oxytocin directly induced depolarization and an inward current in orexin/hypocretin neurons. AVP-induced activation of orexin neurons was inhibited by a V1a receptor (V1aR)-selective antagonist and was not observed in V1aR knock-out mice, suggesting an involvement of V1aR. Subsequently activation of phospholipase C β triggers an increase in

intracellular calcium by both calcium influx through nonselective cation channels and calcium release from calcium stores in orexin neurons. Intracerebroventricular injection of AVP or water deprivation increased locomotor activity in wild-type mice, but not in transgenic mice lacking orexin neurons. V1aR knock-out mice were less active than wild-type mice. These results suggest that the activation of orexin neurons by AVP or oxytocin has an important role in the regulation of spontaneous locomotor activity in mice. This system appears to play a key role in water deprivation-induced hyperlocomotor activity, a response to dehydration that increases the chance of locating water in nature.

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Universal Memory Mechanism for Familiarity Recognition and Identification

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Macaque monkeys were tested on a delayed-match-to-multiple-sample task, with either a limited set of well trained images (in randomized sequence) or with never-before-seen images. They performed much better with novel images. False positives were mostly limited to catch-trial image repetitions from the preceding trial. This result implies extremely effective one-shot learning, resembling Standing’s finding that people detect familiarity for 10,000 once-seen pictures (with 80% accuracy) (Standing, 1973). Familiarity memory may differ essentially from identification, which embeds and generates contextual information. When encountering another person, we can say immediately whether his or her face is familiar. However, it may be difficult for us to identify the same person. To accompany the psychophysical findings, we present a generic neural network model reproducing these behaviors, based on the same conservative Hebbian synaptic plasticity that generates delay activity identification memory. Familiarity becomes the first step toward establishing identification. Adding an inter-trial reset mechanism limits false positives for previous-trial images. The model, unlike previous proposals, relates repetition–recognition with enhanced neural activity, as recently observed experimentally in 92% of differential cells in prefrontal cortex, an area directly involved in familiarity recognition. There may be an essential functional difference between enhanced responses to novel versus to familiar images: The maximal signal from temporal cortex is for novel stimuli, facilitating additional sensory processing of newly acquired stimuli. The maximal signal for familiar stimuli arising in prefrontal cortex facilitates the formation of selective delay activity, as well as additional consolidation of the memory of the image in an upstream cortical module.

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Retinoid Hyposignaling Contributes to Aging-Related Decline in Hippocampal Function in Short-Term/Working Memory Organization and Long-Term Declarative Memory Encoding in Mice

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An increasing body of evidence indicates that the vitamin A metabolite retinoic acid (RA) plays a role in adult brain plasticity by activating gene transcription through nuclear receptors. Our previous studies in mice have shown that a moderate downregulation of retinoid-mediated transcription contributed to aging-related deficits in hippocampal long-term potentiation and long-term declarative memory (LTDM). Here, knock-out, pharmacological, and nutritional approaches were used in a series of radial-arm maze experiments with mice to further assess the hypothesis that retinoid-mediated nuclear events are causally involved in preferential degradation of hippocampal function in aging. Molecular and behavioral findings confirmed our hypothesis. First, a lifelong vitamin A supplementation, like short-term RA administration, was shown to counteract the aging-related hippocampal (but not striatal) hypoexpression of a plasticity-related retinoid target-gene, GAP43 (reverse transcription-PCR analyses, experiment 1), as well as short-term/working memory (STWM) deterioration seen particularly in organization demanding trials (STWM task, experiment 2). Second, using a two-stage paradigm of LTDM, we demonstrated that the vitamin A supplementation normalized memory encoding-induced recruitment of (hippocampoprefrontal) declarative memory circuits, without affecting (striatal) procedural memory system activity in aged mice (Fos neuroimaging, experiment 3A) and alleviated their LTDM impairment (experiment 3B). Finally, we showed that (knock-out, experiment 4) RA receptor β and retinoid X receptor γ , known to be involved in STWM (Wietrzych et al., 2005), are also required for LTDM. Hence, aging-related retinoid signaling hypoexpression disrupts hippocampal cellular properties critically required for STWM organization and LTDM formation, and nutritional vitamin A supplementation represents a preventive strategy. These findings are discussed within current neurobiological perspectives questioning the historical consensus on STWM and LTDM system partition.

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DLGS97/SAP97 Is Developmentally Upregulated and Is Required for Complex Adult Behaviors and Synapse Morphology and Function

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The synaptic membrane-associated guanylate kinase (MAGUK) scaffolding protein family is thought to play key roles in synapse assembly and synaptic plasticity. Evidence supporting these roles *in vivo* is scarce, as a consequence of gene redundancy in mammals. The genome of *Drosophila* contains only one MAGUK gene, *discs large (dlg)*, from which two major proteins originate: DLGA [PSD95 (postsynaptic density 95)-like] and DLGS97 [SAP97 (synapse-associated protein)-like]. These differ only by the inclusion in DLGS97 of an L27 domain, important for the formation of supramolecular assemblies. Known *dlg* mutations affect both forms and are lethal at larval stages attributable to tumoral overgrowth of epithelia. We generated independent null mutations for each, *dlgA* and *dlgS97*. These allowed unveiling of a shift in expression during the development of the nervous system: predominant expression of DLGA in the embryo, balanced expression of both during larval stages, and almost exclusive DLGS97 expression in the adult brain. Loss of embryonic DLGS97 does not alter the development of the nervous system. At larval stages, DLGA and DLGS97 fulfill both unique and partially redundant functions in the neuromuscular junction. Contrary to *dlg* and *dlgA* mutants, *dlgS97* mutants are viable to adulthood, but they exhibit marked alterations in complex behaviors such as phototaxis, circadian activity, and courtship, whereas simpler behaviors like locomotion and odor and light perception are spared. We propose that the increased repertoire of associations of a synaptic scaffold protein given by an additional domain of protein–protein interaction underlies its ability to integrate molecular networks required for complex functions in adult synapses.

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NEUROBIOLOGY OF DISEASE

Alzheimer's Disease (AD)-Like Pathology in Aged Monkeys after Infantile Exposure to Environmental Metal Lead (Pb): Evidence for a Developmental Origin and Environmental Link for AD

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The sporadic nature of Alzheimer's disease (AD) argues for an environmental link that may drive AD pathogenesis; however, the triggering factors and the period of their action are unknown. Recent studies in rodents have shown that exposure to lead (Pb) during brain development predetermined the expression and regulation of the amyloid precursor protein (APP) and its amyloidogenic β -amyloid ($A\beta$) product in old age. Here, we report that the expression of AD-related genes [APP, BACE1 (β -site APP cleaving enzyme 1)] as well as their transcriptional regulator (Sp1) were elevated in aged (23-year-old) monkeys exposed to Pb as infants. Furthermore, developmental exposure to Pb altered the levels, characteristics, and intracellular distribution of $A\beta$ staining and amyloid plaques in the frontal association cortex. These latent effects were accompanied by a decrease in DNA methyltransferase activity and higher levels of oxidative damage to DNA, indicating that epigenetic imprinting in early life influenced the expression of AD-related genes and promoted DNA damage and pathogenesis. These data suggest that AD pathogenesis is influenced by early life exposures and argue for both an environmental trigger and a developmental origin of AD.

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Ubiquitin–Proteasome-Mediated Synaptic Reorganization: A Novel Mechanism Underlying Rapid Ischemic Tolerance

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Ischemic tolerance is an endogenous neuroprotective mechanism in brain and other organs, whereby prior exposure to brief ischemia produces resilience to subsequent normally injurious ischemia. Although many molecular mechanisms mediate delayed (gene-mediated) ischemic tolerance, the mechanisms underlying rapid (protein synthesis-independent) ischemic tolerance are relatively unknown. Here we describe a novel mechanism for the induction of rapid ischemic tolerance mediated by the ubiquitin–proteasome system. Rapid ischemic tolerance is blocked by multiple proteasome inhibitors [carbobenzoxy-L-leucyl-L-leucyl-L-leucinal (MG132), MG115 (carbobenzoxy-L-leucyl-L-leucyl-L-norvalinal), and clasto-lactacystin- β -lactone]. A proteomics strategy was used to identify ubiquitinated proteins after preconditioning ischemia. We focused our studies on two actin-binding proteins of the postsynaptic density that were ubiquitinated after rapid preconditioning: myristoylated, alanine-rich C-kinase substrate (MARCKS) and fascin. Immunoblots confirm the degradation of MARCKS and fascin after preconditioning ischemia. The loss of actin-binding proteins promoted actin reorganization in the postsynaptic density and transient retraction of dendritic spines. This rapid and reversible synaptic remodeling reduced NMDA-mediated electrophysiological responses and renders the cells refractory to NMDA receptor-mediated toxicity. The dendritic spine retraction and NMDA neuroprotection after preconditioning ischemia are blocked by actin stabilization with jasplakinolide, as well as proteasome inhibition with MG132. Together these data suggest that rapid tolerance results from changes to the postsynaptic density mediated by the ubiquitin–proteasome system, rendering neurons resistant to excitotoxicity.

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Expression of a Mutant Form of the Ferritin Light Chain Gene Induces Neurodegeneration and Iron Overload in Transgenic Mice

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Increased iron levels and iron-mediated oxidative stress play an important role in the pathogenesis of many neurodegenerative diseases. The finding that mutations in the ferritin light polypeptide (*FTL*) gene cause a neurodegenerative disease known as neuroferritinopathy or hereditary ferritinopathy (HF) provided a direct connection between abnormal brain iron storage and neurodegeneration. HF is characterized by a severe movement disorder and by the presence of nuclear and cytoplasmic ferritin inclusion bodies in glia and neurons throughout the CNS and in tissues of multiple organ systems. Here we report that the expression in transgenic mice of a human *FTL* cDNA carrying a thymidine and cytidine insertion at position 498 (*FTL498–499InsTC*) leads to the formation of nuclear and cytoplasmic ferritin inclusion bodies. As in HF, ferritin inclusions are seen in glia and neurons throughout the CNS as well as in cells of other organ systems. Our studies show histological, immunohistochemical, and biochemical similarities between ferritin inclusion bodies found in transgenic mice and in individuals with HF. Expression of the transgene in mice leads to a significant decrease in motor performance and a shorter life span, formation of ferritin inclusion bodies, misregulation of iron metabolism, accumulation of ubiquitinated proteins, and incorporation of elements of the proteasome into inclusions. This new transgenic mouse represents a relevant model of HF in which to study the pathways that lead to neurodegeneration in HF, to evaluate the role of iron mismanagement in neurodegenerative disorders, and to evaluate potential therapies for HF and related neurodegenerative diseases.

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Protein Phosphatase 1-Dependent Bidirectional Synaptic Plasticity Controls Ischemic Recovery in the Adult Brain

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Protein kinases and phosphatases can alter the impact of excitotoxicity resulting from ischemia by concurrently modulating apoptotic/survival pathways. Here, we show that protein phosphatase 1 (PP1), known to constrain neuronal signaling and synaptic strength (Mansuy et al., 1998; Morishita et al., 2001), critically regulates neuroprotective pathways in the adult brain. When PP1 is inhibited pharmacologically or genetically, recovery from oxygen/glucose deprivation (OGD) *in vitro*, or ischemia *in vivo* is impaired. Furthermore, *in vitro*, inducing LTP shortly before OGD similarly impairs recovery, an effect that correlates with strong PP1 inhibition. Conversely, inducing LTD before OGD elicits full recovery by preserving PP1 activity, an effect that is abolished by PP1 inhibition. The mechanisms of action of PP1 appear to be coupled with several components of apoptotic pathways, in particular ERK1/2 (extracellular signal-regulated kinase 1/2) whose activation is increased by PP1 inhibition both *in vitro* and

in vivo. Together, these results reveal that the mechanisms of recovery in the adult brain critically involve PP1, and highlight a novel physiological function for long-term potentiation and long-term depression in the control of brain damage and repair.

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Pulse Inhibition of Histone Deacetylases Induces Complete Resistance to Oxidative Death in Cortical Neurons without Toxicity and Reveals a Role for Cytoplasmic p21^{waf1/cip1} in Cell Cycle-Independent Neuroprotection

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Histone deacetylase (HDAC) inhibitors are currently in human clinical trials as antitumor drugs because of their ability to induce cell dysfunction and death in cancer cells. The toxic effects of HDAC inhibitors are also apparent in cortical neurons *in vitro*, despite the ability of these agents to induce significant protection in the cells they do not kill. Here we demonstrate that pulse exposure of cortical neurons (2 h) in an *in vitro* model of oxidative stress results in durable neuroprotection without toxicity. Protection was associated with transcriptional upregulation of the cell cycle inhibitor, p21^{waf1/cip1}, both in this model and in an *in vivo* model of permanent ischemia. Transgenic overexpression of p21^{waf1/cip1} in neurons can mimic the protective effect of HDAC inhibitors against oxidative stress-induced toxicity, including death induced by glutathione depletion or peroxide addition. The protective effect of p21^{waf1/cip1} in the context of oxidative stress appears to be unrelated to its ability to act in the nucleus to inhibit cell cycle progression. However, although p21^{waf1/cip1} is sufficient for neuroprotection, it is not necessary for HDAC inhibitor neuroprotection, because these agents can completely protect neurons cultured from p21^{waf1/cip1}-null mice. Together these findings demonstrate (1) that pulse inhibition of HDACs in cortical neurons can induce neuroprotection without apparent toxicity; (2) that p21^{waf1/cip1} is sufficient but not necessary to mimic the protective effects of HDAC inhibition; and (3) that oxidative stress in this model induces neuronal cell death via cell cycle-independent pathways that can be inhibited by a cytosolic, noncanonical action of p21^{waf1/cip1}.

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