

# This Week in The Journal

## ● Cellular/Molecular

### *Making Dendritic Spikes*

Sonia Gasparini, Michele Migliore, and Jeffrey C. Magee  
(see pages 11046–11056)

Not so long ago, dendrites were considered passive conduits, not active, spike-generating compartments. We now know that in some neurons, action potentials can originate in dendrites and propagate to the soma, and these spikes can also backpropagate from the soma into dendrites. This week, Gasparini et al. examine the conditions required for distal spike generation using dendritic and somatic recording in CA1 pyramidal neurons. They used computer simulation to explore the factors regulating dendritic spikes. In dendrites, the action potential threshold was  $\sim 10$  mV more depolarized than at the soma, a property determined by the dendritic sodium and potassium channel density. To evoke dendritic spikes, synaptic inputs had to be clustered in time and space, within 100  $\mu\text{m}$  and a few milliseconds. However, when they occurred, dendritic spikes triggered short-latency spike output in the axons. Thus with clustered incoming activity, dendritic spikes can provide more bang for the buck.

## ▲ Development/Plasticity/Repair

### *Bax-Less Living in the Dentate Gyrus*

Woong Sun, Adam Winseck, Sharon Vinsant, Ok-hee Park, Hyun Kim, and Ronald W. Oppenheim  
(see pages 11205–11213)

Making it in the adult world can be tough. Although new neurons are continuously produced in the dentate gyrus of the hippocampus throughout life, programmed cell death kills off 60–80%. The Bcl-2 family proapoptotic protein Bax has been implicated in this cell death. This week, Sun et al. examine the impact of Bax on neuronal paring in the adult. Unlike wild-type mice, adult mice lacking Bax showed no evidence of apoptosis in the subgranular zone of the dentate gyrus, whereas the size of the dentate gyrus increased. Mark-

ers of mature granule cells such as neuronal-specific nuclear protein (NeuN) confirmed an increase in mature neurons. Bromodeoxyuridine labeling showed that cell proliferation remained intact, consistent with an effect of Bax on cell death, not production of new cells. Granule cell migration was also disrupted in mice lacking Bax, perhaps a confirmation that more neurons are not necessarily better.

## ■ Behavioral/Systems/Cognitive

### *Wake–Sleep Cycling in the Rat*

Damien Gervasoni, Shih-Chieh Lin, Sidarta Ribeiro, Ernesto S. Soares, Janaina Pantoja, and Miguel A. L. Nicolelis  
(see pages 11137–11147)

Just as behavior differs greatly between waking and sleep states, so too does electrical brain activity, from the fast oscillating pattern of wakefulness to the slower waves of sleep states and then back to the fast activity associated with rapid eye movement (REM) sleep. This week, Gervasoni et al. examine the dynamics of the wake–sleep cycle. They classified rat behavior into five states and created state maps by recording local field potentials (LFPs) in several forebrain areas: cortex, hippocampus, thalamus, and striatum. The LFPs were tightly correlated with the behavioral states and several substates. Remarkably, the transitions between states occurred simultaneously throughout remote areas of the forebrain, indicating a high degree of functional integration across time and space. The authors postu-

late that the transient oscillatory synchronization of synaptic inputs that drive the state transitions may facilitate information exchange between different brain areas.

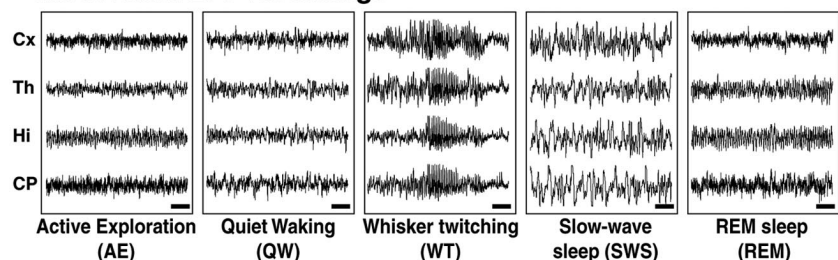
## ◆ Neurobiology of Disease

### *Insulin, IDE, and $\beta$ -Amyloid*

Lixia Zhao, Bruce Teter, Takashi Morihara, Giselle P. Lim, Surendra S. Ambegaokar, Oliver J. Ubeda, Sally A. Frautschy, and Greg M. Cole  
(see pages 11120–11126)

Insulin-degrading enzyme (IDE), despite its name, can also degrade  $\beta$ -amyloid ( $A\beta$ ) peptide that accumulates in Alzheimer's disease (AD). There are some tantalizing clues linking IDE and AD. For example, IDE is decreased in AD brain tissue, and genetic linkage studies implicate chromosome 10q in AD, the area where the IDE gene resides. This evidence has prompted some to suggest that IDE upregulation could be a therapeutic strategy in AD. This week Zhao et al. examine several aspects of this idea. With insulin treatment, cultured hippocampal neurons upregulated IDE, apparently through phosphatidylinositol-3 (PI3) kinase activation. In the hippocampus and cortex of human AD brains, the authors found reduced levels of both IDE and the P85 subunit of PI3. Finally, they examined an animal model of AD, the Tg2576 Swedish amyloid precursor protein mutant mouse. After a high-fat diet to exacerbate pathogenesis, the mutant mice expressed lower levels of IDE and increased  $A\beta$  monomers.

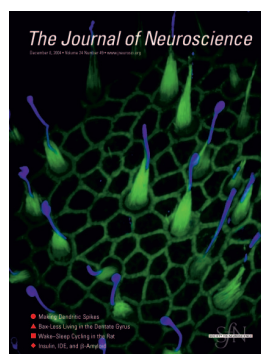
### Intracranial LFP recordings



Intracranial LFPs during five brain behavioral states. Cx, Somatosensory cortex; Hi, hippocampus; CP, caudate–putamen; Th, thalamus. See the article by Gervasoni et al. for details.

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**Cover picture:** Confocal image of the sensory epithelium excised from the developing mouse utricle, a vestibular organ, at embryonic day 18 (E18).

The tissue was stained with phalloidin (green) to illuminate the actin-rich hair bundles and the actin belt that rings the apical surface of each cell. Anti-tubulin (blue) labels the kinocilium. Significant functional maturation of vestibular hair cells occurs the week before birth (approximately E20). For details, see the article by Géléoc et al. in this issue (pages 11148–11159).

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## Behavioral Stress Modifies Hippocampal Synaptic Plasticity through Corticosterone-Induced Sustained Extracellular Signal-Regulated Kinase/Mitogen-Activated Protein Kinase Activation

Chih-Hao Yang,<sup>1,2</sup> Chiung-Chun Huang,<sup>1</sup> and Kuei-Sen Hsu<sup>1,2</sup>

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The induction of hippocampal long-term synaptic plasticity is exquisitely sensitive to behavioral stress, but the underlying mechanisms are still unclear. We report here that hippocampal slices prepared from adult rats that had experienced unpredictable and inescapable restraint tail-shock stress showed marked impairments of long-term potentiation (LTP) in the CA1 region. The same stress promoted the induction of long-term depression (LTD). These effects were prevented when the animals were given the glucocorticoid receptor antagonist 11 $\beta$ ,17 $\beta$ -11[4-(dimethylamino)phenyl]-17-hydroxy-17-(1-propynyl)-estra-4-9-dien-3-one before the stress. Immunoblotting analyses revealed that stress induced a profound and prolonged extracellular signal-regulated kinase/mitogen-activated protein kinase (ERK1/2 MAPK) hyperphosphorylation through small GTPase Ras, Raf-1, and MAPK kinase 1/2 (MEK1/2). Furthermore, the stress effects were obviated by the intrahippocampal injection of specific inhibitors of MEK1/2 (U0126), protein kinase C (bisindolylmaleimide I), tyrosine kinase (K252a), and BDNF antisense oligonucleotides. These results suggest that the effects of stress on LTP and LTD originate from the corticosterone-induced sustained activation of ERK1/2-coupled signaling cascades.

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## A “Synaptoplasmic Cistern” Mediates Rapid Inhibition of Cochlear Hair Cells

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Cochlear hair cells are inhibited by cholinergic efferent neurons. The acetylcholine (ACh) receptor of the hair cell is a ligand-gated cation channel through which calcium enters to activate potassium channels and hyperpolarize the cell. It has been proposed that calcium-induced calcium release (CICR) from a near-membrane postsynaptic store supplements this process. Here, we demonstrate expression of type I ryanodine receptors in outer hair cells in the apical turn of the rat cochlea. Consistent with this finding, ryanodine and other store-active compounds alter the amplitude of transient currents produced by synaptic release of ACh, as well as the response of the hair cell to exogenous ACh. Like the sarcoplasmic reticulum of muscle, the “synaptoplasmic” cistern of the hair cell efficiently couples synaptic input to CICR.

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## Articles

### CELLULAR/MOLECULAR

## Interactions of Postsynaptic Density-95 and the NMDA Receptor 2 Subunit Control Calpain-Mediated Cleavage of the NMDA Receptor

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The calcium-dependent protease calpain cleaves the NMDA receptor 2 (NR2) subunit of the NMDA receptor both *in vitro* and *in vivo* and thus potentially modulates NMDA receptor function and turnover. We examined the ability of postsynaptic density-95 (PSD-95) protein to alter the calpain-mediated cleavage of NR2A and NR2B. Coexpression of PSD-95 with NMDA receptors in human embryonic kidney 293 cells blocked cleavage of NR2A and NR2B by NMDA receptor-activated calpain. NR2A cleavage by calpain occurred in the cell surface and intracellular fractions and required the presence of NR1 subunits. The blocking effect of PSD-95 did not result from decreased calpain activity, lowered intracellular calcium responses, or the blockade of internalization. Instead, this effect was eliminated by deletion of the C-terminal ESDV motif of NR2A or by overexpression of a palmitoylation-deficient PSD-95 mutant lacking the ability to cluster and to interact with NMDA receptors *in situ*, suggesting a role for association between the C terminus of NR2A and clustered PSD-95. Synapse-associated protein 102, a membrane-associated guanylate kinase interacting with NR2A but lacking palmitoylation motifs and the ability to cluster, did not protect NR2A from cleavage by calpain. Pharmacological inhibition of palmitoylation disrupted the interaction of PSD-95 with NMDA receptors in cortical neurons and allowed NR2A to be cleaved by calpain, whereas NR2A could not be cleaved in untreated neurons. These results indicate that PSD-95 clustering and direct association of NR2A and PSD-95 mediate the blocking effect of PSD-95 on calpain cleavage. PSD-95 could regulate the susceptibility of NMDA receptors to calpain-mediated cleavage during synaptic transmission and excitotoxicity.

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# On the Initiation and Propagation of Dendritic Spikes in CA1 Pyramidal Neurons

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Under certain conditions, regenerative voltage spikes can be initiated locally in the dendrites of CA1 pyramidal neurons. These are interesting events that could potentially provide neurons with additional computational abilities. Using whole-cell dendritic recordings from the distal apical trunk and proximal tuft regions and realistic computer modeling, we have determined that highly synchronized and moderately clustered inputs are required for dendritic spike initiation: ~50 synaptic inputs spread over 100  $\mu\text{m}$  of the apical trunk/tuft need to be activated within 3 msec. Dendritic spikes are characterized by a more depolarized voltage threshold than at the soma [ $-48 \pm 1 \text{ mV}$  ( $n = 30$ ) vs  $-56 \pm 1 \text{ mV}$  ( $n = 7$ ), respectively] and are mainly generated and shaped by dendritic  $\text{Na}^+$  and  $\text{K}^+$  currents. The relative contribution of AMPA and NMDA currents is also important in determining the actual spatiotemporal requirements for dendritic spike initiation. Once initiated, dendritic spikes can easily reach the soma, but their propagation is only moderately strong, so that it can be modulated by physiologically relevant factors such as changes in the  $V_m$  and the ionic composition of the extracellular solution. With effective spike propagation, an extremely short-latency neuronal output is produced for greatly reduced input levels. Therefore, dendritic spikes function as efficient detectors of specific input patterns, ensuring that the neuronal response to high levels of input synchrony is a precisely timed action potential output.

The Journal of Neuroscience, December 8, 2004 • 24(49):11046–11056

# Independent Presynaptic and Postsynaptic Mechanisms Regulate Endocannabinoid Signaling at Multiple Synapses in the Ventral Tegmental Area

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Dopamine (DA) neurons in the ventral tegmental area have been implicated in psychiatric disorders and drug abuse. Understanding the mechanisms through which their activity is regulated via the modulation of afferent input is imperative to understanding their roles in these conditions. Here we demonstrate that endocannabinoids liberated from DA neurons activate cannabinoid CB1 receptors located on glutamatergic axons and on GABAergic terminals targeting GABA<sub>B</sub> receptors located on these cells. Endocannabinoid release was initiated by inhibiting either presynaptic type-III metabotropic glutamate receptors or postsynaptic calcium-activated potassium channels, two conditions that also promote enhanced DA neuron excitability and bursting. Thus, activity-dependent release of endocannabinoids may act as a regulatory feedback mechanism to inhibit synaptic inputs in response to DA neuron bursting, thereby regulating firing patterns that may fine-tune DA release from afferent terminals.

The Journal of Neuroscience, December 8, 2004 • 24(49):11070–11078

# Spontaneous Opening of T-Type $\text{Ca}^{2+}$ Channels Contributes to the Irregular Firing of Dopamine Neurons in Neonatal Rats

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During early postnatal development, midbrain dopamine (DA) neurons display anomalous firing patterns and amphetamine response. Spontaneous miniature hyperpolarizations (SMHs) are observed in DA neurons during the same period but not in adults. These hyperpolarizations have been shown to be dependent on the release of  $\text{Ca}^{2+}$  from internal stores and the subsequent activation of  $\text{Ca}^{2+}$ -sensitive  $\text{K}^+$  channels. However, the triggering mechanism and the functional significance of SMHs remain poorly understood. To address these issues, using brain slices, we recorded spontaneous miniature outward currents (SMOCs) in DA neurons of neonatal rats. Two types of SMOCs were identified based on the peak amplitude. Both types were suppressed by intracellular dialysis of ruthenium red, a ryanodine receptor (RyR) antagonist, yet none of the known  $\text{Ca}^{2+}$ -releasing messengers were involved. T-type  $\text{Ca}^{2+}$  channel blockers ( $\text{Ni}^{2+}$  and mibefradil) inhibited large-amplitude SMOCs without affecting the small-amplitude ones. The voltage dependence of SMOCs displayed a peak of approximately  $-50 \text{ mV}$ , consistent with the involvement of low-threshold T-type  $\text{Ca}^{2+}$  channels. Blockade of SMOCs with cyclopiazonic acid or ryanodine converted the irregular firing of DA neurons in neonatal rats into an adult-like pacemaker pattern. This effect was reversed by the injection of artificial currents mimicking SMOCs. Finally, amphetamine inhibited SMOCs and transformed the irregular firing pattern into a more regular one. These data demonstrate that  $\text{Ca}^{2+}$  influx through T-type  $\text{Ca}^{2+}$  channels, followed by  $\text{Ca}^{2+}$ -induced  $\text{Ca}^{2+}$  release via RyRs, contributes to the generation of SMOCs. We propose that SMOCs–SMHs may underlie the anomalous firing and amphetamine response of DA neurons during the postnatal developmental period.

The Journal of Neuroscience, December 8, 2004 • 24(49):11079–11087

# Boosting of Action Potential Backpropagation by Neocortical Network Activity *In Vivo*

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Action potentials backpropagate into the dendritic trees of pyramidal neurons, reporting output activity to the sites of synaptic input and provoking long-lasting changes in synaptic strength. It is unclear how this retrograde signal is modified by neural network activity. Using whole-cell recordings from somata, apical trunks, and dendritic tuft branches of layer 2/3 pyramidal neurons *in vivo*, we show that network-driven subthreshold membrane depolarizations (“up states”) occur simultaneously throughout

the apical dendritic tree. This spontaneous synaptic activity enhances action potential-evoked calcium influx into the distal apical dendrite by promoting action potential backpropagation. Hence, somatic feedback to the dendrites becomes stronger with increasing network activity.

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## Developmental Acquisition of Voltage-Dependent Conductances and Sensory Signaling in Hair Cells of the Embryonic Mouse Inner Ear

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How and when sensory hair cells acquire the remarkable ability to detect and transmit mechanical information carried by sound and head movements has not been illuminated. Previously, we defined the onset of mechanotransduction in embryonic hair cells of mouse vestibular organs to be at approximately embryonic day 16 (E16). Here we examine the functional maturation of hair cells in intact sensory epithelia excised from the inner ears of embryonic mice. Hair cells were studied at stages between E14 and postnatal day 2 using the whole-cell, tight-seal recording technique. We tracked the developmental acquisition of four voltage-dependent conductances. We found a delayed rectifier potassium conductance that appeared as early as E14 and grew in amplitude over the subsequent prenatal week. Interestingly, we also found a low-voltage-activated potassium conductance present at E18, ~1 week earlier than reported previously. An inward rectifier conductance appeared at approximately E15 and doubled in size over the next few days. We also noted transient expression of a voltage-gated sodium conductance that peaked between E16 and E18 and then declined to near zero at birth. We propose that hair cells undergo a stereotyped developmental pattern of ion channel acquisition and that the precise pattern may underlie other developmental processes such as synaptogenesis and functional differentiation into type I and type II hair cells. In addition, we find that the developmental acquisition of basolateral conductances shapes the hair cell receptor potential and therefore comprises an important step in the signal cascade from mechanotransduction to neurotransmission.

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### DEVELOPMENT/PLASTICITY/REPAIR

## Fluorescent Proteins Expressed in Mouse Transgenic Lines Mark Subsets of Glia, Neurons, Macrophages, and Dendritic Cells for Vital Examination

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To enable vital observation of glia at the neuromuscular junction, transgenic mice were generated that express proteins of the green fluorescent protein family under control of transcriptional regulatory sequences of the human S100B gene. Terminal Schwann cells were imaged repetitively in living animals of one of the transgenic lines to show that, except for extension and retraction of short processes, the glial coverings of the adult neuromuscular synapse are stable. In other lines, subsets of Schwann cells were labeled. The distribution of label suggests that Schwann cells at individual synapses are clonally related, a finding with implications for how these cells might be sorted during postnatal development. Other labeling patterns, some present in unique lines, included astrocytes, microglia, and subsets of cerebellar Bergmann glia, spinal motor neurons, macrophages, and dendritic cells. We show that lines with labeled macrophages can be used to follow the accumulation of these cells at sites of injury.

The Journal of Neuroscience, December 8, 2004 • 24(49):11010–11016

## Tumor Necrosis Factor Receptor 1 and Its Signaling Intermediates Are Recruited to Lipid Rafts in the Traumatized Brain

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The tumor necrosis factor (TNF) ligand–receptor system plays an essential role in apoptosis that contributes to secondary damage after traumatic brain injury (TBI). TNF also stimulates inflammation by activation of gene transcription through the I $\kappa$ B kinase (IKK)/NF- $\kappa$ B and JNK (c-Jun N-terminal protein kinase)/AP-1 signaling cascades. The mechanism by which TNF signals between cell death and survival and the role of receptor localization in the activation of downstream signaling events are not fully understood. Here, TNF receptor 1 (TNFR1) signaling complexes in lipid rafts were investigated in the cerebral cortex of adult male Sprague Dawley rats subjected to moderate (1.8–2.2 atmospheres) fluid-percussion TBI and naive controls. In the normal rat cortex, a portion of TNFR1 was present in lipid raft microdomains, where it associated with the adaptor proteins TRADD (TNF receptor-associated death domain), TNF receptor-associated factor-2 (TRAF-2), the Ser/Thr kinase RIP (receptor-interacting protein), TRAF1, and cIAP-1 (cellular inhibitor of apoptosis protein-1), forming a survival signaling complex. Moderate TBI resulted in rapid recruitment of TNFR1, but not TNFR2 or Fas, to lipid rafts and induced alterations in the composition of signaling intermediates. TNFR1 and TRAF1 were polyubiquitinated in lipid rafts after TBI. Subsequently, the signaling complex contained activated caspase-8, thus initiating apoptosis. In addition, TBI caused a transient activation of NF- $\kappa$ B, but receptor

signaling interacting proteins IKK $\alpha$  and IKK $\beta$  were not detected in raft-containing fractions. Thus, redistribution of TNFR1 in lipid rafts and nonraft regions of the plasma membrane may regulate the diversity of signaling responses initiated by these receptors in the normal brain and after TBI.  
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## Retina-Driven Dephosphorylation of the NR2A Subunit Correlates with Faster NMDA Receptor Kinetics at Developing Retinocollicular Synapses

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We describe a homeostatic mechanism that limits NMDA receptor currents in response to early light activation of a developing visual pathway. During the second postnatal week of rodent retinocollicular development, the Ca<sup>2+</sup>-activated phosphatase calcineurin (CaN) mediates a rapid, activity-induced shortening in the decay time of NMDA receptor (NMDAR) currents. We show that protein kinase A acts in opposition to CaN to maintain NMDAR currents with long decay times. The CaN-mediated change is coincident with the initial expression of the NMDAR subunit NR2A. Using NR2A knock-out mice and dialyzing neurons with a constitutively active CaN, we demonstrate that NR2A subunits are necessary for the effect of CaN on NMDAR current kinetics. In wild-type mice, Ser900 of NR2A, previously implicated in CaN-mediated glycine-independent desensitization, becomes chronically dephosphorylated by postnatal day 11 as NMDAR current decay times become faster. Pharmacologically disrupting early photoreceptor-driven activity in the retina eliminates the dephosphorylation of NR2A and prevents the shortening in NMDAR current decay time. These data suggest that the developmental onset of retinal activity increases CaN-mediated dephosphorylation of NR2A subunits newly incorporated into synaptic NMDARs of the superior colliculus, thereby providing a mechanism for the early and rapid reduction of NMDAR current decay time in visual neurons.

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## Defective Postnatal Neurogenesis and Disorganization of the Rostral Migratory Stream in Absence of the *Vax1* Homeobox Gene

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The subventricular zone (SVZ) is one of the sources of adult neural stem cells (ANSCs) in the mouse brain. Precursor cells proliferate in the SVZ and migrate through the rostral migratory stream (RMS) to the olfactory bulb (OB), where they differentiate into granule and periglomerular cells. Few transcription factors are known to be responsible for regulating NSC proliferation, migration, and differentiation processes; even fewer have been found to be responsible for the organization of the SVZ and RMS. For this reason, we studied the ventral anterior homeobox (*Vax1*) gene in NSC proliferation and in SVZ organization. We found that *Vax1* is strongly expressed in the SVZ and in the RMS and that, in the absence of *Vax1*, embryonic precursor cells proliferate 100 times more than wild-type controls, *in vitro*. The SVZ of *Vax1*<sup>-/-</sup> brains is hyperplastic and mostly disorganized, and the RMS is missing, causing a failure of precursor cell migration to the OBs, which as a result are severely hypoplastic. Moreover, we found that *Vax1* is essential for the correct differentiation of ependyma and astrocytes.

Together, these data indicate that *Vax1* is a potent regulator of SVZ organization and NSC proliferation, with important consequences on postnatal neurogenesis.  
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## Programmed Cell Death of Adult-Generated Hippocampal Neurons Is Mediated by the Proapoptotic Gene Bax

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In the dentate gyrus (DG) of the adult mouse hippocampus, a substantial number of new cells are generated daily, but only a subset of these survive and differentiate into mature neurons, whereas the majority undergo programmed cell death (PCD). However, neither the intracellular machinery required for adult stem cell-derived neuronal death nor the biological implications of the significant loss of these newly generated cells have been examined. Several markers for apoptosis failed to reveal cell death in Bax-deficient mice, and this, together with a progressive increase in neuron number in the DG of the Bax knock-out, indicates that Bax is critical for the PCD of adult-generated hippocampal neurons. Whereas the proliferation of neural progenitor cells was not altered in the Bax-knock-out, there was an accumulation of doublecortin, calretinin<sup>+</sup>, and neuronal-specific nuclear protein<sup>+</sup> postmitotic neurons, suggesting that Bax-mediated PCD of adult-generated neurons takes place during an early phase of differentiation. The absence of PCD in the adult also influenced the migration and maturation of adult-generated DG neurons. These results suggest that PCD in the adult brain plays a significant role in the regulation of multiple aspects of adult neurogenesis.

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## Executive Dysfunction in Cocaine Addiction: Evidence for Discordant Frontal, Cingulate, and Cerebellar Activity

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Using a GO–NOGO response inhibition task in which working memory (WM) demands can be varied, we demonstrate that the compromised abilities of cocaine users to exert control over strong prepotent urges are associated with reduced activity in anterior cingulate and right prefrontal cortices, two regions thought to be critical for implementing cognitive control. Furthermore, unlike drug-naïve controls, and opposite to the anterior cingulate pattern, cocaine users showed an over-reliance on the left cerebellum, a compensatory pattern previously seen in alcohol addiction. The results indicate that cocaine users find it difficult to inhibit their own actions, particularly when WM demands, which have been shown previously to increase during cue-induced craving for the drug, are increased. The results reveal a neuroanatomical basis for this dysexecutive component to addiction, supporting the suggested importance cognitive functions may play in prolonging abuse or predisposing users toward relapse. *The Journal of Neuroscience*, December 8, 2004 • 24(49):11017–11022

## Perirhinal and Postrhinal Contributions to Remote Memory for Context

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The perirhinal (PER) and postrhinal (POR) cortices, two components of the medial temporal lobe memory system, are reciprocally connected with the hippocampus both directly and via the entorhinal cortex. Damage to PER or POR before or shortly after training on a contextual fear conditioning task causes deficits in the subsequent expression of contextual fear, implicating these regions in the acquisition or expression of contextual memory. Here, we examined the contribution of PER and POR to the processing of remotely learned contextual information. Male Long–Evans rats were trained in an unsignaled contextual fear conditioning paradigm. After training, rats received bilateral neurotoxic lesions to PER or POR or sham control surgeries at three different training-to-lesion intervals: 1, 28, or 100 d after training. Two weeks after surgery, lesioned and control rats were returned to the training context to assess contextual fear as measured by freezing. Rats with PER or POR damage froze significantly less in the training context than control rats but were not different from each other. The severity of the deficit did not differ across training-to-lesion intervals for any group. This pattern of deficits differs from that of posttraining hippocampal lesions, for which longer training-to-lesion intervals produce significantly more fear-conditioned contextual freezing than shorter training-to-lesion intervals. In the absence of such a retrograde gradient in the present study, our interpretation is that PER and POR have an ongoing role in the storage or retrieval of representations for context. Alternatively, these regions may be involved in a more extended consolidation process that becomes apparent beyond 100 d after learning.

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## Persistence of Parahippocampal Representation in the Absence of Stimulus Input Enhances Long-Term Encoding: A Functional Magnetic Resonance Imaging Study of Subsequent Memory after a Delayed Match-to-Sample Task

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Recent theoretical models based on cellular processes in parahippocampal structures show that persistent neuronal spiking in the absence of stimulus input is important for encoding. The goal of this study was to examine in humans how sustained activity in the parahippocampal gyrus may underlie long-term encoding as well as active maintenance of novel information. The relationship between long-term encoding and active maintenance of novel information during brief memory delays was studied using functional magnetic resonance imaging (fMRI) in humans performing a delayed matching-to-sample (DMS) task and a post-scan subsequent recognition memory task of items encountered during DMS task performance. Multiple regression analyses revealed fMRI activity in parahippocampal structures associated with the active maintenance of trial-unique visual information during a brief memory delay. In addition to a role in active maintenance, we found that the subsequent memory for the sample stimuli as measured by the post-scan subsequent recognition memory task correlated with activity in the parahippocampal gyrus during the delay period. The results provide direct evidence that encoding mechanisms are engaged during brief memory delays when novel information is actively maintained. The relationship between active maintenance during the delay period and long-term subsequent memory is consistent with current theoretical models and experimental data that suggest that long-term encoding is enhanced by sustained parahippocampal activity.

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# Spatial and Temporal Organization of Ensemble Representations for Different Odor Classes in the Moth Antennal Lobe

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In the insect antennal lobe, odor discrimination depends on the ability of the brain to read neural activity patterns across arrays of uniquely identifiable olfactory glomeruli. Less is understood about the complex temporal dynamics and interglomerular interactions that underlie these spatial patterns. Using neural-ensemble recording, we show that the evoked firing patterns within and between groups of glomeruli are odor dependent and organized in both space and time. Simultaneous recordings from up to 15 units per ensemble were obtained from four zones of glomerular neuropil in response to four classes of odorants: pheromones, monoterpenoids, aromatics, and aliphatics. Each odor class evoked a different pattern of excitation and inhibition across recording zones. The excitatory response field for each class was spatially defined, but inhibitory activity was spread across the antennal lobe, reflecting a center-surround organization. Some chemically related odorants were not easily distinguished by their spatial patterns, but each odorant evoked transient synchronous firing across a uniquely different subset of ensemble units. Examination of 535 cell pairs revealed a strong relationship between their recording positions, temporal correlations, and similarity of odor response profiles. These findings provide the first definitive support for a nested architecture in the insect olfactory system that uses both spatial and temporal coordination of firing to encode chemosensory signals. The spatial extent of the representation is defined by a stereotyped focus of glomerular activity for each odorant class, whereas the transient temporal correlations embedded within the ensemble provide a second coding dimension that can facilitate discrimination between chemically similar volatiles.

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# Global Forebrain Dynamics Predict Rat Behavioral States and Their Transitions

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The wake–sleep cycle, a spontaneous succession of global brain states that correspond to major overt behaviors, occurs in all higher vertebrates. The transitions between these states, at once rapid and drastic, remain poorly understood. Here, intracranial local field potentials (LFPs) recorded in the cortex, hippocampus, striatum, and thalamus were used to characterize the neurophysiological correlates of the rat wake–sleep cycle. By way of a new method for the objective classification and quantitative investigation of all major brain states, we demonstrate that global brain state transitions occur simultaneously across multiple forebrain areas as specific spectral trajectories with characteristic path, duration, and coherence bandwidth. During state transitions, striking changes in neural synchronization are effected by the prominent narrow-band LFP oscillations that mark state boundaries. Our results demonstrate that distant forebrain areas tightly coordinate the processing of neural information during and between global brain states, indicating a very high degree of functional integration across the entire wake–sleep cycle. We propose that transient oscillatory synchronization of synaptic inputs, which underlie the rapid switching of global brain states, may facilitate the exchange of information within and across brain areas at the boundaries of very distinct neural processing regimens.

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# Convergence and Segregation of the Multiple Rod Pathways in Mammalian Retina

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Using a multidisciplinary approach, we demonstrate that three different pathways are responsible for the transmission of rod signals across the mouse retina. Each pathway serves a primarily nonoverlapping range of stimulus intensities, with ganglion cells receiving either segregated or convergent inputs. For both on-center (ON) and off-center (OFF) ganglion cells, the primary rod pathway carries signals with the lowest threshold, whereas the secondary rod pathway is less sensitive by  $\sim 1$  log unit. In addition, OFF signaling uses a tertiary rod pathway that is  $\sim 1$  log unit less sensitive than the secondary. Although some ganglion cells received rod inputs exclusively from one of the pathways, others showed convergent inputs. Using pharmacological and genetic approaches, we defined classes of ON and OFF ganglion cells for which the scotopic inputs derive only from the primary pathway or from both primary and secondary pathways. In addition, we observed a class of OFF ganglion cell receiving mixed input from primary and tertiary pathways. Interestingly, OFF ganglion cells receiving convergent inputs from all three rod pathways or from the secondary and tertiary pathways together were never observed. Overall, our data show a complex arrangement of convergence and segregation of rod inputs to ganglion cells in the mammalian retina.

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# Receptive Field Properties of the Macaque Second Somatosensory Cortex: Evidence for Multiple Functional Representations

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The detailed functional organization of the macaque second somatosensory cortex (SII) is not well understood. Here we report the results of a study of the functional organization of the SII hand region that combines microelectrode mapping using hand-held stimuli with single-unit recordings using a motorized, computer-controlled tactile oriented bar. The data indicate that the SII hand region extends ~10 mm in the anteroposterior (AP) dimension, primarily within the upper bank of the lateral sulcus. Furthermore, we find evidence that this region consists of multiple functional fields, with a central field containing neurons that are driven well by cutaneous stimuli, flanked by an anterior field and a posterior field that each contain neurons that are driven well by proprioceptive stimuli and less well by cutaneous stimuli. The anterior field extends ~4–5 mm AP, the central field extends ~3–4 mm, and the posterior field extends ~3 mm. Data from the motorized stimulator indicate that neurons in the central field are more responsive to oriented bars, more frequently exhibit orientation tuning, and have larger receptive fields than neurons in the anterior and posterior fields. We speculate that the three putative fields play different functional roles in tactile perception; the anterior and posterior fields process information that involves both proprioceptive and cutaneous input such as sensorimotor integration or stereognosis, whereas the central field processes primarily cutaneous information.

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## NEUROBIOLOGY OF DISEASE

# Sodium Influx Pathways during and after Anoxia in Rat Hippocampal Neurons

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Mechanisms that contribute to Na<sup>+</sup> influx during and immediately after 5 min anoxia were investigated in cultured rat hippocampal neurons loaded with the Na<sup>+</sup>-sensitive fluorophore sodium-binding benzofuran isophthalate. During anoxia, an influx of Na<sup>+</sup> in the face of reduced Na<sup>+</sup>,K<sup>+</sup>-ATPase activity caused a rise in [Na<sup>+</sup>]<sub>i</sub>. After the return to normoxia, Na<sup>+</sup>,K<sup>+</sup>-ATPase activity mediated the recovery of [Na<sup>+</sup>]<sub>i</sub>; despite continued Na<sup>+</sup> entry. Sodium influx during and after anoxia occurred through multiple pathways and increased the longer neurons were maintained in culture. Under the experimental conditions used, Na<sup>+</sup> entry during anoxia did not reflect the activation of ionotropic glutamate receptors, TTX- or lidocaine-sensitive Na<sup>+</sup> channels, plasmalemmal Na<sup>+</sup>/Ca<sup>2+</sup> exchange, Na<sup>+</sup>/H<sup>+</sup> exchange, or HCO<sub>3</sub><sup>-</sup>-dependent mechanisms; rather, contributions were received from a Gd<sup>3+</sup>-sensitive pathway activated by reactive oxygen species and Na<sup>+</sup>/K<sup>+</sup>/2Cl<sup>-</sup> cotransport in neurons maintained for 6–10 and 11–14 d *in vitro* (DIV), respectively. Sodium entry immediately after anoxia was not attributable to the activation of ionotropic glutamate receptors, voltage-activated Na<sup>+</sup> channels, or Na<sup>+</sup>/K<sup>+</sup>/2Cl<sup>-</sup> cotransport; rather, it occurred via Na<sup>+</sup>/Ca<sup>2+</sup> exchange, Na<sup>+</sup>/H<sup>+</sup> exchange, and a Gd<sup>3+</sup>-sensitive pathway similar to that observed during anoxia; 11–14 DIV neurons received an additional contribution from an HCO<sub>3</sub><sup>-</sup>-dependent mechanism(s). The results provide insight into the intrinsic mechanisms that contribute to disturbed internal Na<sup>+</sup> homeostasis during and immediately after anoxia in rat hippocampal neurons and, in this way, may play a role in the pathogenesis of anoxic or ischemic cell injury.

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# Insulin-Degrading Enzyme as a Downstream Target of Insulin Receptor Signaling Cascade: Implications for Alzheimer's Disease Intervention

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Insulin-degrading enzyme (IDE) is one of the proteins that has been demonstrated to play a key role in degrading  $\beta$ -amyloid (A $\beta$ ) monomer *in vitro* and *in vivo*, raising the possibility of upregulating IDE as an approach to reduce A $\beta$ . Little is known, however, about the cellular and molecular regulation of IDE protein. Because one of the main functions of IDE is to degrade insulin, we hypothesized that there is a negative feedback mechanism whereby stimulation of insulin receptor-mediated signaling upregulates IDE to prevent chronic activation of the pathway. We show that treatment of primary hippocampal neurons with insulin increased IDE protein levels by ~25%. Insulin treatment also led to phosphatidylinositol-3 (PI3) kinase activation evidenced by Akt phosphorylation, which was blocked by PI3 kinase inhibitors, wortmannin and LY 294002. Inhibition of PI3 kinase abolished the IDE upregulation by insulin, indicating a cause–effect relationship between insulin signaling and IDE upregulation. Further support for this link was provided by the findings that deficient insulin signaling (decreased PI3 kinase subunit P85) was correlated with reduced IDE in Alzheimer's disease (AD) brains and in Tg2576 Swedish amyloid precursor protein transgenic mice fed a safflower oil-enriched (“Bad”) diet used to accelerate pathogenesis. Consistent with IDE function in the degradation of A $\beta$  monomer, the IDE decrease in the Bad diet-fed Tg2576 mice was associated with increased A $\beta$  monomer levels. These *in vitro* and *in vivo* analyses validate the use of enhanced CNS insulin signaling as a potential strategy for AD intervention to correct the IDE defects occurring in AD.

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# Role of $\alpha$ -Synuclein in Presynaptic Dopamine Recruitment

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Real-time monitoring of stimulated dopamine release in mice with different  $\alpha$ -synuclein expression was used to study the role of  $\alpha$ -synuclein in presynaptic dopamine recruitment. Repeated electrical stimulations of ascending dopaminergic pathways decreased the capacity of the readily releasable pool (RRP) and temporarily increased its refilling rate, significantly slowing the rate of dopamine decline in mice with normally expressed  $\alpha$ -synuclein. Mice with  $\alpha$ -synuclein null mutation demonstrated a permanent increase of the refilling rate. This increase maintained stable dopamine release during stimulation (which induced dopamine decline in other animals) and served as an adaptation to altered dopamine compartmentalization. Mice without  $\alpha$ -synuclein and with overexpression of human A30P mutated  $\alpha$ -synuclein had a lower capacity of the dopamine storage pool than other animals. Reducing capacity of the storage pool in transgenic A30P mice led to paradoxical effects of L-dopa, which elevated dopamine release in response to single stimulation but decreased the refilling rate of the RRP.

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