

# This Week in The Journal

## ● Cellular/Molecular

### *Regulation of Intracellular Chloride Concentration and GABA<sub>A</sub> Signaling*

Audrey C. Brumback and Kevin J. Staley

(see pages 1301–1312)

Activating GABA<sub>A</sub> receptors opens an intrinsic chloride channel, which normally results in chloride influx and hyperpolarization. In some contexts, however (e.g., development, neuropathic pain, and epilepsy), the intracellular chloride concentration ( $Cl_i$ ) is elevated; thus, GABA<sub>A</sub> receptor activation leads to chloride efflux and depolarization. New experiments by Brumback and Staley reveal that the elevated  $Cl_i$  during development in rats is maintained by the ion transporter NKCC1. They showed that the direction of  $Cl^-$  transport by NKCC1 depends solely on the electrochemical driving force of  $Na^+$ ,  $K^+$ , and  $Cl^-$ . Because of this,  $Cl_i$  can be regulated by altering the intracellular  $Na^+$  concentration ( $Na_i$ ). The authors demonstrate this by triggering trains of action potentials in hippocampal neurons. The resulting  $Na^+$ / $K^+$ -pump-dependent changes in  $Na_i$  led to  $Cl_i$  elevation by NKCC1. Thus, the synaptic signal provided by GABA may depend on whether a neuron has been recently active.

## ▲ Development/Plasticity/Repair

### *Acetylcholine-Induced, Activity-Independent Long-Term Potentiation*

David Fernández de Sevilla, Angel Núñez, Michel Borde, Roberto Malinow, and Washington Buño

(see pages 1469–1478)

Activation of muscarinic acetylcholine receptors (mAChRs) can induce synaptic enhancement in the absence of correlated activity, as demonstrated this week by Fernández de Sevilla et al. Puffing ACh onto the apical dendrites of rat hippocampal pyramidal cells in slice cultures resulted in a long-lasting increase in the EPSC. Experiments using calcium imaging, receptor blockers, and

uncaging of inositol trisphosphate ( $IP_3$ ) revealed that this form of long-term potentiation (LTP) requires mAChR activation,  $IP_3$  production, activation of  $IP_3$  receptors, and subsequent release of calcium from internal stores. The authors therefore named this form of plasticity  $LTP_{IP_3}$ . Tracking of fluorescently tagged glutamate receptors (GluR1 and GluR2) suggested that  $LTP_{IP_3}$  may result from increased insertion of AMPA receptors in dendritic spines.  $LTP_{IP_3}$  did not require NMDA receptor activation, occurred even when action potentials were blocked with tetrodotoxin, and, when paired with traditional LTP-inducing protocols, doubled the amount of potentiation.

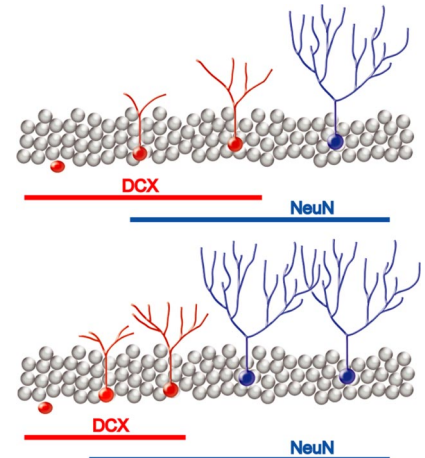
## ■ Behavioral/Systems/Cognitive

### *Fear Extinction in Developing Rats*

Jee Hyun Kim and Rick Richardson

(see pages 1282–1290)

Conditioned responses (CRs) to fear can be extinguished by repeatedly presenting the conditioned stimulus (CS) without the unconditioned stimulus (US). In adult rats, extinction is thought to be produced primarily by inhibiting the CR, rather than by eliminating the conditioned memory, because the CR often reappears without reconditioning. Developing rats, however, don't show spontaneous recovery of the CR after extinction, suggesting that a different mechanism is involved. This week, Kim and Richardson provide more evidence that this is the case. They inactivated the amygdala in young rats during extinction training, reconditioning, or reextinction and found that initial extinction was amygdala dependent at postnatal day 17 (P17) and P24. After subsequent reconditioning at P26, however, reextinction was amygdala dependent only in those rats initially extinguished at P17. Interestingly, the switch from amygdala dependence to independence parallels temporally the maturation of hippocampus and medial prefrontal cortex—structures thought to be involved in extinction in adults.



Schematic drawing illustrates accelerated development in fluoxetine-treated neurons. The immature doublecortin (DCX)-expressing period is shorter, NeuN expression starts sooner, and dendrites develop more quickly in fluoxetine-treated neurons (bottom) than in controls (top). See the article by Wang et al. for details.

## ◆ Neurobiology of Disease

### *Accelerated Neuronal Maturation with Antidepressant Treatment*

Jing-Wen Wang, Denis J. David, James E. Monckton, Fortunato Battaglia, and René Hen

(see pages 1373–1383)

Selective serotonin reuptake inhibitors (SSRIs) speed maturation of neurons as well as increase neurogenesis, according to a report by Wang et al. in this issue. Like others, these authors found that chronic treatment with the SSRI fluoxetine (Prozac) increased the number of newborn neurons in adult rats, as indicated by immunostaining for bromodeoxyuridine (BrdU) and the neuronal-specific nuclear protein (NeuN). In addition, fluoxetine treatment decreased the proportion of BrdU- and NeuN-positive neurons that expressed doublecortin, a marker of young neurons, suggesting that more newborn neurons had fully matured. Furthermore, those neurons that did express doublecortin had more extensive dendritic arbors than neurons in controls. Killing new neurons with x-irradiation prevented the reduced latency to eat food in a novel environment and blocked the enhancement of a form of LTP that is normally observed in rats chronically treated with fluoxetine, indicating that neurogenesis is required for some physiological and behavioral effects of fluoxetine.

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**Cover legend:** Confocal images of white matter axons and glial cells labeled with the multi-diolistic technique. Beads of various mixtures of DiO, Dil, and DiD were delivered to the preparation using a gene gun. Images were acquired by Selva Baltan and Jamie Grutzendler and pseudocolored by NancyAnn Oberheim. For more information, see the article by Baltan et al. in this issue (pages 1479–1489).

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**Corrections:** In the article "Generation of Distinct Types of Periglomerular Olfactory Bulb Interneurons during Development and in Adult Mice: Implication for Intrinsic Properties of the Subventricular Zone Progenitor Population" by Silvia De Marchis, Serena Bovetti, Barbara Carletti, Yi-Chun Hsieh, Donatella Garzotto, Paolo Peretto, Aldo Fasolo, Adam C. Puche, and Ferdinando Rossi, which appeared on pages 657–664 of the January 17, 2007 issue, panels *D* and *F* in Figure 4 were erroneously labeled; they should have been labeled TH and CB, respectively. Also, in legend of the figure, the statement "*D–N*, Representative images of EGFP-positive cells double stained for CB (*D, G, L*), CR (*E, H, M*), and TH (*F, I, N*)" should read "*D–N*, Representative images of EGFP-positive cells double stained for TH (*D, G, L*), CR (*E, H, M*), and CB (*F, I, N*)."

In the article "Regulation of Spine Development by Semaphorin3A through Cyclin-Dependent Kinase 5 Phosphorylation of Collapsin Response Mediator Protein 1" by Naoya Yamashita, Asa Morita, Yutaka Uchida, Fumio Nakamura, Hiroshi Usui, Toshio Ohshima, Masahiko Taniguchi, Jérôme Honnorat, Nicole Thomasset, Kohtaro Takei, Takuya Takahashi, Pappachan Kolattukudy, and Yoshio Goshima, which appeared on pages 12546–12554 of the November 14, 2007 issue, Figure 1 contained photomicrographs that were previously published in Figure 7 of the article "Regulation of Dendritic Branching and Spine Maturation by Semaphorin3A-Fyn Signaling" by Asa Morita, Naoya Yamashita, Yukio Sasaki, Yutaka Uchida, Oumi Nakajima, Fumio Nakamura, Takeshi Yagi, Masahiko Taniguchi, Hiroshi Usui, Ritsuko Katoh-Semba, Kohtaro Takei, and Yoshio Goshima, which appeared on pages 2971–2980 of the March 15, 2006 issue. The authors acknowledge that portions of this figure were duplicated without copyright permission or proper acknowledgement required by the Guidelines for Responsible Conduct Regarding Scientific Communication of the Society for Neuroscience.

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## Beyond Feeling: Chronic Pain Hurts the Brain, Disrupting the Default-Mode Network Dynamics

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Chronic pain patients suffer from more than just pain; depression and anxiety, sleep disturbances, and decision-making abnormalities (Apkarian et al., 2004a) also significantly diminish their quality of life. Recent studies have demonstrated that chronic pain harms cortical areas unrelated to pain (Apkarian et al., 2004b; Acerra and Moseley, 2005), but whether these structural impairments and behavioral deficits are connected by a single mechanism is as of yet unknown. Here we propose that long-term pain alters the functional connectivity of cortical regions known to be active at rest, i.e., the components of the “default mode network” (DMN). This DMN (Raichle et al., 2001; Greicius et al., 2003; Vincent et al., 2007) is marked by balanced positive and negative correlations between activity in component brain regions. In several disorders, however this balance is disrupted (Fox and Raichle, 2007). Using well validated functional magnetic resonance imaging (fMRI) paradigms to study the DMN (Fox et al., 2005), we investigated whether the impairments of chronic pain patients could be rooted in disturbed DMN dynamics. Studying with fMRI a group of chronic back pain (CBP) patients and healthy controls while executing a simple visual attention task, we discovered that CBP patients, despite performing the task equally well as controls, displayed reduced deactivation in several key DMN regions. These findings demonstrate that chronic pain has a widespread impact on overall brain function, and suggest that disruptions of the DMN may underlie the cognitive and behavioral impairments accompanying chronic pain.

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## Chronic Exposure to Nerve Growth Factor Increases Acetylcholine and Glutamate Release from Cholinergic Neurons of the Rat Medial Septum and Diagonal Band of Broca via Mechanisms Mediated by p75<sup>NTR</sup>

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Basal forebrain neurons play an important role in memory and attention. In addition to cholinergic and GABAergic neurons, glutamatergic neurons and neurons that can corelease acetylcholine and glutamate have recently been described in the basal forebrain. Although it is well known that nerve growth factor (NGF) promotes synaptic function of cholinergic basal forebrain neurons, how NGF affects the newly identified basal forebrain neurons remains undetermined. Here, we examined the effects of NGF on synaptic transmission of medial septum and diagonal band of Broca (MS-DBB) neurons expressing different neurotransmitter phenotypes. We used MS-DBB neurons from 10- to 13-d-old rats, cultured on astrocytic microislands to promote the development of autaptic connections. Evoked and spontaneous postsynaptic currents were recorded, and neurotransmitters released were characterized pharmacologically. We found that chronic exposure to NGF significantly increased acetylcholine and glutamate release from cholinergic MS-DBB neurons, whereas glutamate and GABA transmission from noncholinergic MS-DBB neurons were not affected by NGF. Interestingly, the NGF-induced increase in neurotransmission was mediated by p75<sup>NTR</sup>. These results demonstrate a previously unidentified role of NGF and its receptor p75<sup>NTR</sup>; their interactions are crucial for cholinergic and glutamatergic transmission in the septohippocampal pathway.

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## Which GABA<sub>A</sub> Receptor Subunits Are Necessary for Tonic Inhibition in the Hippocampus?

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GABA<sub>A</sub> receptors (GABA<sub>A</sub>Rs) assembled of different subunits mediate tonic and phasic inhibition in hippocampal neurons. CA1/CA3 pyramidal cells (PCs) predominantly express  $\alpha 5$  subunits whereas dentate gyrus granule cells (DGGCs) and molecular layer (ML) interneurons predominantly express  $\delta$  subunits. Both  $\alpha 5$ - and  $\delta$ -containing GABA<sub>A</sub>Rs mediate tonic inhibition. We have shown previously that mice lacking  $\alpha 5$  subunits (*Gabra5*<sup>-/-</sup>) have a residual tonic current in CA1/CA3 PCs because of an upregulation of  $\delta$  subunits, but the basis of the residual tonic current in DGGCs and ML interneurons of mice lacking the  $\delta$  subunit (*Gabrd*<sup>-/-</sup>) is still unknown. We now show that wild-type DGGCs have a small tonic current mediated by  $\alpha 5$  subunit-containing GABA<sub>A</sub>Rs responsible for ~29% of the total tonic current. To better identify the GABA<sub>A</sub>Rs mediating tonic inhibition in hippocampal neurons, we generated mice lacking both  $\alpha 5$  and  $\delta$  subunits (*Gabra5/Gabrd*<sup>-/-</sup>). Recordings from CA1/CA3 PCs, DGGCs, and ML interneurons in these mice show an absence of tonic currents without compensatory changes in spontaneous IPSCs (sIPSCs), sEPSCs, and membrane resistance. The absence of tonic inhibition results in spontaneous gamma oscillations recordable *in vitro* in the CA3 pyramidal layer of these mice, which can be mimicked in wild-type mice by blocking  $\alpha 5$  subunit-containing GABA<sub>A</sub>Rs with 50 nM L-655,708. In conclusion, depending on the cell type, the  $\alpha 5$  and  $\delta$  subunits are the principal GABA<sub>A</sub>R subunits responsible for mediating the lion's share of tonic inhibition in hippocampal neurons.

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# Developing Postmitotic Mammalian Neurons *In Vivo* Lacking Apaf-1 Undergo Programmed Cell Death by a Caspase-Independent, Nonapoptotic Pathway Involving Autophagy

Ronald W. Oppenheim,<sup>1</sup> Klas Blomgren,<sup>2</sup> Douglas W. Ethell,<sup>3</sup> Masato Koike,<sup>4</sup> Masaaki Komatsu,<sup>5</sup> David Prevette,<sup>1</sup> Kevin A. Roth,<sup>6</sup> Yasuo Uchiyama,<sup>4</sup> Sharon Vinsant,<sup>1</sup> and Changlian Zhu<sup>2</sup>

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Previous studies have shown that caspases and Apaf-1 are required for the normal programmed cell death (PCD) *in vivo* of immature postmitotic neurons and mitotically active neuronal precursor cells. In contrast, caspase activity is not necessary for the normal PCD of more mature postmitotic neurons that are establishing synaptic connections. Although normally these cells use caspases for PCD, in the absence of caspase activity these neurons undergo a distinct nonapoptotic type of degeneration. We examined the survival of these more mature postmitotic neuronal populations in mice in which Apaf-1 has been genetically deleted and find that they exhibit quantitatively normal PCD of developing postmitotic neurons. We next characterized the morphological mode of PCD in these mice and show that the neurons degenerate by a caspase-independent, nonapoptotic pathway that involves autophagy. However, autophagy does not appear to be involved in the normal PCD of postmitotic neurons in which caspases and Apaf-1 are present and functional because quantitatively normal neuronal PCD occurred in the absence of a key gene required for autophagy (ATG7). Finally, we examined the possible role of another caspase-independent type of neuronal PCD involving the apoptosis-inducing factor (AIF). Mice deficient in AIF also exhibit quantitatively normal PCD of postmitotic neurons after caspase inhibition. Together, these data indicate that, when key components of the type I apoptotic pathway (i.e., caspases and Apaf-1) are perturbed *in vivo*, developing postmitotic neurons nonetheless undergo quantitatively normal PCD by a caspase-independent pathway involving autophagy and not requiring AIF.

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## Articles

### CELLULAR/MOLECULAR

## Kv3.3 Channels at the Purkinje Cell Soma Are Necessary for Generation of the Classical Complex Spike Waveform

Edward Zagha,<sup>1,3,4</sup> Eric J. Lang,<sup>1</sup> and Bernardo Rudy<sup>1,2,3</sup>

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Voltage-gated potassium channel subunit Kv3.3 is prominently expressed in cerebellar Purkinje cells and is known to be important for cerebellar function, as human and mouse movement disorders result from mutations in Kv3.3. To understand these behavioral deficits, it is necessary to know the role of Kv3.3 channels on the physiological responses of Purkinje cells. We studied the function of Kv3.3 channels in regulating the synaptically evoked Purkinje cell complex spike, the massive postsynaptic response to the activation of climbing fiber afferents, believed to be fundamental to cerebellar physiology. Acute slice recordings revealed that Kv3.3 channels are required for generation of the repetitive spikelets of the complex spike. We found that spikelet expression is regulated by somatic, and not by dendritic, Kv3 activity, which is consistent with dual somatic–dendritic recordings that demonstrate spikelet generation at axosomatic membranes. Simulations of Purkinje cell Na<sup>+</sup> currents show that the unique electrical properties of Kv3 and resurgent Na<sup>+</sup> channels are coordinated to limit accumulation of Na<sup>+</sup> channel inactivation and enable rapid, repetitive firing. We additionally show that Kv3.3 knock-out mice produce altered complex spikes *in vitro* and *in vivo*, which is likely a cellular substrate of the cerebellar phenotypes observed in these mice. This characterization presents new tools to study complex spike function, cerebellar signaling, and Kv3.3-dependent human and mouse phenotypes.

The Journal of Neuroscience, February 6, 2008 • 28(6):1291–1300

## Thermodynamic Regulation of NKCC1-Mediated Cl<sup>-</sup> Cotransport Underlies Plasticity of GABA<sub>A</sub> Signaling in Neonatal Neurons

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In the adult brain, chloride (Cl<sup>-</sup>) influx through GABA<sub>A</sub> receptors is an important mechanism of synaptic inhibition. However, under a variety of circumstances, including acquired epilepsy, neuropathic pain, after trains of action potentials or trauma, and during normal early brain development, GABA<sub>A</sub> receptor activation excites neurons by gating Cl<sup>-</sup> efflux because the intracellular Cl<sup>-</sup> concentration (Cl<sub>i</sub>) is elevated. These findings require an inducible, active mechanism of chloride accumulation. We used gramicidin-perforated patch recordings to characterize Cl<sup>-</sup> transport via NKCC1, the principal neuronal Cl<sup>-</sup> accumulator, in neonatal CA1 pyramidal neurons. NKCC1

activity was required to maintain elevated  $Cl_i$  such that GABA<sub>A</sub> receptor activation was depolarizing. Kinetic analysis of NKCC1 revealed reversible transmembrane  $Cl^-$  transport characterized by a large maximum velocity ( $v_{max}$ ) and high affinity ( $K_m$ ), so that NKCC1 transport was limited only by the net electrochemical driving force for  $Na^+$ ,  $K^+$ , and  $Cl^-$ . At the steady-state  $Cl_i$ , NKCC1 was at thermodynamic equilibrium, and there was no evidence of net  $Cl^-$  transport. Trains of action potentials that have been previously shown to induce persistent changes in neuronal  $E_{Cl}$  (reversal potential for  $Cl^-$ ) did not alter  $v_{max}$  or  $K_m$  of NKCC1. Rather, action potentials shifted the thermodynamic set point, the steady-state  $Cl_i$  at which there was no net NKCC1-mediated  $Cl^-$  transport. The persistent increase in  $Cl_i$  required intact  $\alpha 2/\alpha 3 Na^+ - K^+ - ATPase$  activity, indicating that trains of action potentials reset the thermodynamic equilibrium for NKCC1 transport by lowering  $Na_i$ . Activity-induced changes in  $Na^+ - K^+ - ATPase$  activity comprise a novel mechanism for persistent alterations in synaptic signaling mediated by GABA.

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## Light Adaptation in Salamander L-Cone Photoreceptors

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The responses of individual salamander L-cones to light steps of moderate intensity (bleaching 0.3–3% of the total photopigment) and duration (between 5 and 90 s) were recorded using suction electrodes. Light initially suppressed the circulating current, which partially recovered or “sagged” over several seconds. The sensitivity of the cone to dim flashes decreased rapidly after light onset and approached a minimum within 500 ms. Background light did not affect the rising phase of the dim flash response, a measure of the initial gain of phototransduction. When the light was extinguished, the circulating current transiently exceeded or “overshot” its level in darkness. During the overshoot, the sensitivity of the cone required several seconds to recover. The sag and overshoot remained in voltage-clamped cones. Comparison with theory suggests that three mechanisms cause the sag, overshoot, and slow recovery of sensitivity after the light step: a gradual increase in the rate of inactivation of the phototransduction cascade during the light step, residual activity of the transduction cascade after the step is extinguished, and an increase in guanylate cyclase activity during the light step that persists after the light is extinguished.

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## The Clustering of GABA<sub>A</sub> Receptor Subtypes at Inhibitory Synapses is Facilitated via the Direct Binding of Receptor $\alpha 2$ Subunits to Gephyrin

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Classical benzodiazepine sensitive GABA<sub>A</sub> receptor subtypes, the major mediators of fast synaptic inhibition in the brain are heteropentamers that can be assembled from  $\alpha 1-3/5$ ,  $\beta 1-3$ , and  $\gamma 2$  subunits, but how neurons orchestrate their selective accumulation at synapses remains obscure. We have identified a 10 amino acid hydrophobic motif within the intracellular domain of the  $\alpha 2$  subunit that regulates the accumulation of GABA<sub>A</sub> receptors at inhibitory synaptic sites on both axon initial segments and dendrites in a mechanism dependent on the inhibitory scaffold protein gephyrin. This motif was sufficient to target CD4 (cluster of differentiation molecule 4) molecules to inhibitory synapses, and was also critical in regulating the direct binding of  $\alpha 2$  subunits to gephyrin *in vitro*. Our results thus reveal that the specific accumulation of GABA<sub>A</sub> receptor subtypes containing  $\alpha 2$  subunits at inhibitory synapses is dependent on their ability to bind gephyrin.

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## Quantitative Cortical Mapping of Fractional Anisotropy in Developing Rat Brains

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Cortical development is associated with a series of events that involve axon and dendrite growth and synaptic formation. Although these developmental processes have been investigated in detail with histology, three-dimensional and quantitative imaging methods for rodent brains may be useful for genetic and pharmacological studies in which cortical developmental abnormalities are suspected. It has been shown that diffusion tensor imaging (DTI) can delineate the columnar organization of the fetal and early neonatal cortex based on a high degree of diffusion anisotropy along the columnar structures. This anisotropy is known to decrease during brain development. In this study, we applied DTI to developing rat brains at five developmental stages, postnatal days 0, 3, 7, 11 and 19, and used diffusion anisotropy as an index to characterize the structural change. Statistical analysis reveals four distinctive cortical areas that demonstrate a characteristic time course of anisotropy loss. This method may provide a means to delineate specific cortical areas and a quantitative method to detect abnormalities in cortical development in rodent pathological models.

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# Role of Protein Phosphatase 2A in Regulating the Visual Signaling in *Drosophila*

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*Drosophila* visual signaling, a G-protein-coupled phospholipase C $\beta$  (PLC $\beta$ )-mediated mechanism, is regulated by eye-protein kinase C (PKC) that promotes light adaptation and fast deactivation, most likely via phosphorylation of inactivation no afterpotential D (INAD) and TRP (transient receptor potential). To reveal the critical phosphatases that dephosphorylate INAD, we used several biochemical analyses and identified protein phosphatase 2A (PP2A) as a candidate. Importantly, the catalytic subunit of PP2A, microtubule star (MTS), is copurified with INAD, and an elevated phosphorylation of INAD by eye-PKC was observed in three *mts* heterozygotes. To explore whether PP2A (MTS) regulates dephosphorylation of INAD by counteracting eye-PKC [INAC (inactivation no afterpotential C)] *in vivo*, we performed ERG recordings. We discovered that *inaC*<sup>P209</sup> was semidominant, because *inaC*<sup>P209</sup> heterozygotes displayed abnormal light adaptation and slow deactivation. Interestingly, the deactivation defect of *inaC*<sup>P209</sup> heterozygotes was rescued by the *mts*<sup>XE2258</sup> heterozygous background. In contrast, *mts*<sup>XE2258</sup> failed to modify the severe deactivation of *norpA*<sup>P16</sup>, indicating that MTS does not modulate NORPA (no receptor potential A) (PLC $\beta$ ). Together, our results strongly indicate that dephosphorylation of INAD is catalyzed by PP2A, and a reduction of PP2A can compensate for a partial loss of function in eye-PKC, restoring the fast deactivation kinetics *in vivo*. We thus propose that the fast deactivation of the visual response is modulated in part by the phosphorylation of INAD.

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# Differential Regulation of Synaptic Plasticity and Cerebellar Motor Learning by the C-Terminal PDZ-Binding Motif of GluR $\delta$ 2

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The  $\delta$ 2 glutamate receptor (GluR $\delta$ 2) is predominantly expressed in Purkinje cells and plays crucial roles in cerebellar functions: *GluR $\delta$ 2*<sup>-/-</sup> mice display ataxia and impaired motor learning. In addition, long-term depression (LTD) at parallel fiber (PF)–Purkinje cell synapses is abrogated, and synapse formation with PFs and climbing fibers (CFs) is severely disturbed in *GluR $\delta$ 2*<sup>-/-</sup> Purkinje cells. Recently, we demonstrated that abrogated LTD was restored in *GluR $\delta$ 2*<sup>-/-</sup> Purkinje cells by the virus-mediated expression of the wild-type *GluR $\delta$ 2* transgene (*Tg*<sub>wild</sub>) but not by that of mutant *GluR $\delta$ 2* lacking the C-terminal seven residues to which several PDZ proteins bind (*Tg* <sub>$\Delta$ CT7</sub>). These results indicated that the C terminus of GluR $\delta$ 2 conveys the signal(s) necessary for LTD. In contrast, other phenotypes of *GluR $\delta$ 2*<sup>-/-</sup> cerebellum, especially morphological abnormalities at PF and CF synapses, could not be rescued by virus-mediated transient expression. Thus, whether these phenotypes are mediated by the same signaling pathway remains unclear. To address these issues and to further delineate the function of GluR $\delta$ 2 *in vivo*, we generated transgenic mice that expressed *Tg* <sub>$\Delta$ CT7</sub> on a *GluR $\delta$ 2*<sup>-/-</sup> background. Interestingly, although *Tg* <sub>$\Delta$ CT7</sub> restored abnormal PF and CF synapse formation almost completely, it could not rescue abrogated LTD in *GluR $\delta$ 2*<sup>-/-</sup> Purkinje cells. Furthermore, although the gross motor discoordination of *GluR $\delta$ 2*<sup>-/-</sup> mice was restored, the cerebellar motor learning underlying delayed eyeblink conditioning remained impaired. These results indicate that LTD induction and motor learning are regulated by signaling via the C-terminal end of GluR $\delta$ 2, whereas other functions may be differentially regulated by other regions of GluR $\delta$ 2.

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# Acid Sensing Ion Channels in Dorsal Spinal Cord Neurons

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Acid-sensing ion channels (ASICs) are broadly expressed in the CNS, including the spinal cord. However, very little is known about the properties of ASICs in spinal cord neurons compared with brain. We show here that ASIC1a and ASIC2a are the most abundant ASICs in mouse adult spinal cord and are coexpressed by most neurons throughout all the laminae. ASIC currents in cultured embryonic day 14 mouse dorsal spinal neurons mainly flow through homomeric ASIC1a (34% of neurons) and heteromeric ASIC1a plus 2a channels at a ratio of 2:1 (83% of neurons). ASIC2b only has a minor contribution to these currents. The two channel subtypes show different active pH ranges and different inactivation and reactivation kinetics supporting complementary functional properties. One striking property of native dorsal spinal neuron currents and recombinant currents is the pH dependence of the reactivation process. A light sustained acidosis induces a threefold slow-down of the homomeric ASIC1a (from pH 7.4 to pH 7.3) and heteromeric ASIC1a plus 2a (from pH 7.4 to pH 7.2) current reactivation ( $T_{0.5}$  increasing from 5.77 to 16.84 s and from 0.98 to 3.2 s, respectively), whereas a larger acidosis to pH 6.6 induces a 32-fold slow-down of the ASIC1a plus 2a current reactivation ( $T_{0.5}$  values increasing to 31.30 s). The pH dependence of ASIC channel reactivation is likely to modulate neuronal excitability associated with repetitive firing in response to extracellular pH oscillations, which can be induced, for example, by intense synaptic activity of central neurons.

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## Cholinergic-Mediated IP<sub>3</sub>-Receptor Activation Induces Long-Lasting Synaptic Enhancement in CA1 Pyramidal Neurons

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Cholinergic–glutamatergic interactions influence forms of synaptic plasticity that are thought to mediate memory and learning. We tested *in vitro* the induction of long-lasting synaptic enhancement at Schaffer collaterals by acetylcholine (ACh) at the apical dendrite of CA1 pyramidal neurons and *in vivo* by stimulation of cholinergic afferents. *In vitro* ACh induced a Ca<sup>2+</sup> wave and synaptic enhancement mediated by insertion of AMPA receptors in spines. Activation of muscarinic ACh receptors (mAChRs) and Ca<sup>2+</sup> release from inositol 1,4,5-trisphosphate (IP<sub>3</sub>)-sensitive stores were required for this synaptic enhancement that was insensitive to blockade of NMDA receptors and also triggered by IP<sub>3</sub> uncaging. Activation of cholinergic afferents *in vivo* induced an analogous atropine-sensitive synaptic enhancement. We describe a novel form of synaptic enhancement (LTP<sub>IP<sub>3</sub></sub>) that is induced *in vitro* and *in vivo* by activation of mAChRs. We conclude that Ca<sup>2+</sup> released from postsynaptic endoplasmic reticulum stores is the critical event in the induction of this unique form of long-lasting synaptic enhancement.

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## Multidigit Movement Synergies of the Human Hand in an Unconstrained Haptic Exploration Task

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Although the human hand has a complex structure with many individual degrees of freedom, joint movements are correlated. Studies involving simple tasks (grasping) or skilled tasks (typing or finger spelling) have shown that a small number of combined joint motions (i.e., synergies) can account for most of the variance in observed hand postures. However, those paradigms evoked a limited set of hand postures and as such the reported correlation patterns of joint motions may be task-specific. Here, we used an unconstrained haptic exploration task to evoke a set of hand postures that is representative of most naturalistic postures during object manipulation. Principal component analysis on this set revealed that the first seven principal components capture >90% of the observed variance in hand postures. Further, we identified nine eigenvectors (or synergies) that are remarkably similar across multiple subjects and across manipulations of different sets of objects within a subject. We then determined that these synergies are used broadly by showing that they account for the changes in hand postures during other tasks. These include hand motions such as reach and grasp of objects that vary in width, curvature and angle, and skilled motions such as precision pinch. Our results demonstrate that the synergies reported here generalize across tasks, and suggest that they represent basic building blocks underlying natural human hand motions.

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## The Effect of Temporary Amygdala Inactivation on Extinction and Reextinction of Fear in the Developing Rat: Unlearning as a Potential Mechanism for Extinction Early in Development

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It is well accepted that fear extinction does not cause erasure of the original conditioned stimulus (CS)–unconditioned stimulus association in the adult rat because the extinguished fear often returns (e.g., renewal and reinstatement). Furthermore, extinction is NMDA and GABA dependent, showing that extinction involves new inhibitory learning. We have recently observed each of these extinction-related phenomena in 24-d-old but not in 17-d-old rats. These results suggest that different neural processes mediate extinction early in development. However, the neural processes underlying extinction in the developing rat are unknown. Therefore, the present study investigated amygdala involvement in extinction and reextinction during development. In experiment 1, temporary inactivation of the amygdala (using bupivacaine, a sodium channel modulator) during extinction training impaired extinction of conditioned fear in 17- and 24-d-old rats. In experiment 2, 17- and 24-d-old rats were conditioned, extinguished, and then reconditioned to the same CS. After reconditioning, the CS was reextinguished; at this time, some rats at each age had their amygdala temporarily inactivated. Reextinction was amygdala independent in 24-d-old rats, as previously shown in adult rats. However, reextinction was still amygdala dependent in 17-d-old rats. In Experiment 3, the age at conditioning, reconditioning, reextinction, and test was held constant, but the age of initial extinction varied across groups; reextinction was found to be amygdala independent if initial extinction occurred at 24 d of age but amygdala dependent if it occurred at 17 d of age. Consistent with previous findings, these results show that there are fundamental differences in the neural mechanisms of fear extinction across development.

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# Mapping White Matter Integrity and Neurobehavioral Correlates in Children with Fetal Alcohol Spectrum Disorders

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Brain structural abnormalities and neurocognitive dysfunction have been observed in individuals with fetal alcohol spectrum disorders (FASDs). Little is known about how white matter integrity is related to these functional and morphological deficits. We used a combination of diffusion tensor and T1-weighted magnetic resonance imaging to evaluate white matter integrity in individuals with FASDs and related these findings to neurocognitive deficits. Seventeen children and adolescents with FASDs were compared with 19 typically developing age- and gender-matched controls. Lower fractional anisotropy (FA) was observed in individuals with FASDs relative to controls in the right lateral temporal lobe and bilaterally in the lateral aspects of the splenium of the corpus callosum. White matter density was also lower in some, but not all regions in which FA was lower. FA abnormalities were confirmed to be in areas of white matter in *post hoc* region of interest analyses, further supporting that less myelin or disorganized fiber tracts are associated with heavy prenatal alcohol exposure. Significant correlations between performance on a test of visuomotor integration and FA in bilateral splenium, but not temporal regions were observed within the FASD group. Correlations between the visuomotor task and FA within the splenium were not significant within the control group, and were not significant for measures of reading ability. This suggests that this region of white matter is particularly susceptible to damage from prenatal alcohol exposure and that disruption of splenial fibers in this group is associated with poorer visuomotor integration.

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# Cysteine-Rich Protein 2, a Novel Downstream Effector of cGMP/cGMP-Dependent Protein Kinase I-Mediated Persistent Inflammatory Pain

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The cGMP/cGMP-dependent protein kinase I (cGKI) signaling pathway plays an important role in spinal nociceptive processing. However, downstream targets of cGKI in this context have not been identified to date. Using a yeast two-hybrid screen, we isolated cysteine-rich protein 2 (CRP2) as a novel cGKI interactor in the spinal cord. CRP2 is expressed in laminae I and II of the mouse spinal cord and is colocalized with cGKI, calcitonin gene-related peptide, and isolectin B4. Moreover, the majority of CRP2 mRNA-positive dorsal root ganglion (DRG) neurons express cGKI and peripherin. CRP2 is phosphorylated in a cGMP-dependent manner, and its expression increases in the spinal cord and in DRGs after noxious stimulation of a hindpaw. To elucidate the functional role of CRP2 in nociception, we analyzed mice with a targeted deletion of CRP2. CRP2-deficient (CRP2<sup>-/-</sup>) mice demonstrate normal behavioral responses to acute nociception and after axonal injury of the sciatic nerve, but increased nociceptive behavior in models of inflammatory hyperalgesia compared with wild-type mice. Intrathecal administration of cGMP analogs increases the nociceptive behavior in wild-type but not in CRP2<sup>-/-</sup> mice, indicating that the presence of CRP2 is important for cGMP-mediated nociception. These data suggest that CRP2 is a new downstream effector of cGKI-mediated spinal nociceptive processing and point to an inhibitory role of CRP2 in the generation of inflammatory pain.

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# The Effect of Middle Temporal Spike Phase on Sensory Encoding and Correlates with Behavior during a Motion- Detection Task

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Previous studies have shown that sensory neurons that are the most informative of the stimulus tend to be the best correlated with the subject's perceptual decision. We wanted to know whether this relationship might also apply to short time segments of a neuron's response. We asked whether spikes that conveyed more information about a motion stimulus were also more tightly linked to the perceptual behavior. We examined single-neuron activity in middle temporal (MT) area while monkeys performed a motion-detection task. Because of a slow stimulus update (every 27 ms), activity in many MT neurons was entrained and phase-locked to the stimulus. These stimulus-entrained neuronal oscillations allowed us to separate spikes based on phase. We observed a large amount of variability in how spikes at different phases of the oscillation encoded the stimulus, as revealed by the spike-triggered average of the motion. Spikes during certain phases of the cycle were much more informative about the presence of coherent motion than others. Importantly, we found that the phases that were the most informative about the motion stimulus were also more correlated with the behavioral performance and reaction time of the animal. Our results suggest that the relationship between a neuron's spikes, the stimulus, and behavior can vary on a time scale of tens of milliseconds.

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# Neural Control of Motion-to-Force Transitions with the Fingertip

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The neural control of tasks such as rapid acquisition of precision pinch remains unknown. Therefore, we investigated the neural control of finger musculature when the index fingertip abruptly transitions from motion to static force production. Nine subjects produced a downward tapping motion followed by vertical fingertip force against a rigid surface. We simultaneously recorded three-dimensional fingertip force, plus the complete muscle coordination pattern using intramuscular electromyograms from all seven index finger muscles. We found that the muscle coordination pattern clearly switched from that for motion to that for isometric force ~65 ms before contact ( $p = 0.0004$ ). Mathematical modeling and analysis revealed that the underlying neural control also switched between mutually incompatible strategies in a time-critical manner. Importantly, this abrupt switch in underlying neural control polluted fingertip force vector direction beyond what is explained by muscle activation-contraction dynamics and neuromuscular noise ( $p \leq 0.003$ ). We further ruled out an impedance control strategy in a separate test showing no systematic change in initial force magnitude for catch trials where the tapping surface was surreptitiously lowered and raised ( $p = 0.93$ ). We conclude that the nervous system predictively switches between mutually incompatible neural control strategies to bridge the abrupt transition in mechanical constraints between motion and static force. Moreover because the nervous system cannot switch between control strategies instantaneously or exactly, there arise physical limits to the accuracy of force production on contact. The need for such a neurally demanding and time-critical strategy for routine motion-to-force transitions with the fingertip may explain the existence of specialized neural circuits for the human hand.

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# Endocannabinoid Signaling Mediates Cocaine-Induced Inhibitory Synaptic Plasticity in Midbrain Dopamine Neurons

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Drugs that increase GABA levels in the brain reduce cocaine seeking in rodents and humans, suggesting that GABAergic inhibition regulates cocaine-seeking behavior. We previously reported that repeated cocaine exposure *in vivo* facilitates long-term potentiation by reducing the strength of GABAergic inhibition in dopamine neurons of the ventral tegmental area (VTA). Selective blockade of cocaine-induced reduction of GABAergic inhibition in the VTA might diminish cocaine-induced aberrant synaptic plasticity and addictive behavior. Here, we investigated the mechanism for cocaine-induced reduction of GABAergic inhibition. We show that a pathophysiologically relevant concentration of cocaine enables a normally ineffective stimulus to induce long-term depression (LTD) of IPSCs (I-LTD) in VTA dopamine neurons of midbrain slices. Activation of D<sub>2</sub> dopamine receptors and group I metabotropic glutamate receptors and subsequent recruitment of endocannabinoid signaling are required for I-LTD induction. We further demonstrate that *in vivo* pretreatment with antagonists to these receptors blocks cocaine-induced reduction of GABAergic inhibition and that repeated cocaine exposure *in vivo* occludes the subsequent induction of I-LTD *ex vivo*. Together, these results suggest that repeated cocaine exposure reduces the strength of GABAergic inhibition in dopamine neurons by inducing I-LTD-like modification *in vivo*.

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# Cue-Elicited Reward-Seeking Requires Extracellular Signal-Regulated Kinase Activation in the Nucleus Accumbens

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The motivation to seek out rewards can come under the control of stimuli associated with reward delivery. The ability of cues to motivate reward-seeking behavior depends on the nucleus accumbens (NAcc). The molecular mechanisms in the NAcc that underlie the ability of a cue to motivate reward-seeking are not well understood. We examined whether extracellular signal-regulated kinase (ERK), an important intracellular signaling pathway in learning and memory, has a role in these motivational processes. We first examined p42 ERK (ERK2) activation in the NAcc after rats were trained to associate an auditory stimulus with food delivery and found that, as a consequence of training, presentation of the auditory cue itself was sufficient to increase ERK2 activation in the NAcc. To examine whether inhibition of ERK in the NAcc prevents cue-induced reward-seeking, we infused an inhibitor of ERK, U0126, into the NAcc before assessing rats' instrumental responding in the presence versus absence of the conditioned cue. We found that, whereas vehicle-infused rats showed increased instrumental responding during cue presentation, rats infused with U0126 showed a profound impairment in cue-induced instrumental responding. In contrast, intra-NAcc U0126 infusion had no effect on rats' food-reinforced instrumental responding or their ability to execute conditioned approach behavior. Our results demonstrate learning-related changes in ERK signaling in the NAcc, and that disruption of ERK activation in this structure interferes with the incentive-motivational effects of conditioned stimuli. The molecular mechanisms described here may have implications for cue-elicited drug craving after repeated exposure to drugs of abuse.

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# A Synaptic Basis for Auditory–Vocal Integration in the Songbird

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Songbirds learn to sing by memorizing a tutor song that they then vocally mimic using auditory feedback. This developmental sequence suggests that brain areas that encode auditory memories communicate with brain areas for learned vocal control. In the songbird, the secondary auditory telencephalic region caudal mesopallium (CM) contains neurons that encode aspects of auditory experience. We investigated whether CM is an important source of auditory input to two sensorimotor structures implicated in singing, the telencephalic song nucleus interface (Nif) and HVC. We used reversible inactivation methods to show that activity in CM is necessary for much of the auditory-evoked activity that can be detected in Nif and HVC of anesthetized adult male zebra finches. Furthermore, extracellular and intracellular recordings along with spike-triggered averaging methods indicate that auditory selectivity for the bird's own song is enhanced between CM and Nif. We used lentiviral-mediated tracing methods to confirm that CM neurons directly innervate Nif. To our surprise, these tracing studies also revealed a direct projection from CM to HVC. We combined irreversible lesions of Nif with reversible inactivation of CM to establish that CM supplies a direct source of auditory drive to HVC. Finally, using chronic recording methods, we found that CM neurons are active in response to song playback and during singing, indicating their potential importance to song perception and processing of auditory feedback. These results establish the functional synaptic linkage between sites of auditory and vocal learning and may identify an important substrate for learned vocal communication.

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# Stimulus-Specific Adaptations in the Gaze Control System of the Barn Owl

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Abrupt orientation to novel stimuli is a critical, memory-dependent task performed by the brain. In the present study, we examined two gaze control centers of the barn owl: the optic tectum (OT) and the arcopallium gaze fields (AGFs). Responses of neurons to long sequences of dichotic sound bursts comprised of two sounds differing in the probability of appearance were analyzed. We report that auditory neurons in the OT and in the AGFs tend to respond stronger to rarely presented sounds (novel sounds) than to the same sounds when presented frequently. This history-dependent phenomenon, known as stimulus-specific adaptation (SSA), was demonstrated for rare sound frequencies, binaural localization cues [interaural time difference (ITD) and level difference (ILD)] and sound amplitudes. The manifestation of SSA in such a variety of independent acoustic features, in the midbrain and in the forebrain, supports the notion that SSA is involved in sensory memory and novelty detection. To track the origin of SSA, we analyzed responses of neurons in the external nucleus of the inferior colliculus (ICX; the source of auditory input to the OT) to similar sequences of sound bursts. Neurons in the ICX responded stronger to rare sound frequencies, but did not respond differently to rare ITDs, ILDs, or sound amplitudes. We hypothesize that part of the SSA reported here is computed in high-level networks, giving rise to novelty signals that modulate tectal responses in a context-dependent manner.

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## NEUROBIOLOGY OF DISEASE

# Chronic Fluoxetine Stimulates Maturation and Synaptic Plasticity of Adult-Born Hippocampal Granule Cells

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Chronic treatments with selective serotonin reuptake inhibitors (SSRIs) have been shown to increase hippocampal neurogenesis. However, it is not known whether SSRIs impact the maturation and functional integration of newborn neurons. Here we examined the effects of subchronic and chronic fluoxetine on the structural and physiological properties of young granule cells. Our results show that doublecortin-positive immature neurons displayed increased dendritic arborization after chronic fluoxetine treatment. In addition, chronic but not subchronic fluoxetine elicited a decrease in the number of newborn neurons expressing immature markers and a corresponding increase in those expressing mature markers. These results suggest that chronic fluoxetine accelerates the maturation of immature neurons. We also investigated the effects of fluoxetine on a form of neurogenesis-dependent long-term potentiation (LTP) in the dentate gyrus. This form of LTP was enhanced by chronic fluoxetine, and ablation of neurogenesis with x-irradiation completely blocked the effects of chronic fluoxetine on LTP. Finally, we demonstrated that the behavioral effect of fluoxetine in the novelty-suppressed feeding test requires chronic administration and is blocked by x-irradiation. These results show that the effects of fluoxetine on LTP and behavior both require neurogenesis and follow a similar delayed time course. The effects of chronic fluoxetine on the maturation and functional properties of young neurons may therefore be necessary for its anxiolytic/antidepressant activity and contribute to its delayed onset of therapeutic efficacy.

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# A Systems Level Analysis of Transcriptional Changes in Alzheimer's Disease and Normal Aging

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Alzheimer's disease (AD) is a debilitating neurodegenerative disorder affecting millions of elderly individuals worldwide. Advances in the genetics of AD have led to new levels of understanding and treatment opportunities. Here, we used a systems biology approach based on weighted gene coexpression network analysis to determine transcriptional networks in AD. This method permits a higher order depiction of gene expression relationships and identifies modules of coexpressed genes that are functionally related, rather than producing massive gene lists. Using this framework, we characterized the transcriptional network in AD, identifying 12 distinct modules related to synaptic and metabolic processes, immune response, and white matter, nine of which were related to disease progression. We further examined the association of gene expression changes with progression of AD and normal aging, and were able to compare functional modules of genes defined in both conditions. Two biologically relevant modules were conserved between AD and aging, one related to mitochondrial processes such as energy metabolism, and the other related to synaptic plasticity. We also identified several genes that were central, or hub, genes in both aging and AD, including the highly abundant signaling molecule 14.3.3  $\zeta$  (*YWHAZ*), whose role in AD and aging is uncharacterized. Finally, we found that presenilin 1 (*PSEN1*) is highly coexpressed with canonical myelin proteins, suggesting a role for *PSEN1* in aspects of glial-neuronal interactions related to neurodegenerative processes.

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## Intron-3 Retention/Splicing Controls Neuronal Expression of Apolipoprotein E in the CNS

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Neuronal expression of apolipoprotein (apo) E4 may contribute to the pathogenesis of Alzheimer's disease (AD). In studying how apoE expression is regulated in neurons, we identified a splicing variant of apoE mRNA with intron-3 retention (apoE-I3). ApoE-I3 mRNA was detected in neuronal cell lines and primary neurons, but not in astrocytic cell lines or primary astrocytes, from humans and mice by reverse transcription (RT)-PCR. In both wild-type and human apoE knock-in mice, apoE-I3 was found predominantly in cortical and hippocampal neurons by *in situ* hybridization. Cell fractionation and quantitative RT-PCR revealed that over 98% of the apoE-I3 mRNA was retained in the nucleus without protein translation. In transfected primary neurons, apoE expression increased dramatically when intron-3 was deleted from a genomic DNA construct and decreased markedly when intron-3 was inserted into a cDNA construct, suggesting that intron-3 retention/splicing controls apoE expression in neurons. In response to excitotoxic challenge, the apoE-I3 mRNA was markedly increased in morphologically normal hippocampal neurons but reduced in degenerating hippocampal neurons in mice; apoE mRNA showed the opposite pattern. This apparent precursor-product relationship between apoE-I3 and apoE mRNA was supported by a transcriptional inhibition study. Thus, neuronal expression of apoE is controlled by transcription of apoE-I3 under normal conditions and by processing of apoE-I3 into mature apoE mRNA in response to injury.

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## White Matter Vulnerability to Ischemic Injury Increases with Age Because of Enhanced Excitotoxicity

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Stroke incidence increases with age and this has been attributed to vascular factors. We show here that CNS white matter (WM) is intrinsically more vulnerable to ischemic injury in older animals and that the mechanisms of WM injury change as a function of age. The mouse optic nerve was used to study WM function. WM function in older animals (12 months) was not protected from ischemic injury by removal of extracellular  $\text{Ca}^{2+}$  or by blockade of reverse  $\text{Na}^+/\text{Ca}^{2+}$  exchange, as is the case with young adults. Ischemic WM injury in older mice is predominately mediated by glutamate release and activation of AMPA/kainate-type glutamate receptors. Glutamate release, attributable to reverse glutamate transport, occurs earlier and is more robust in older mice that show greater expression of the glutamate transporter. The observation that WM vulnerability to ischemic injury is age dependent has possible implications for the pathogenesis of other age-related CNS conditions.

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