

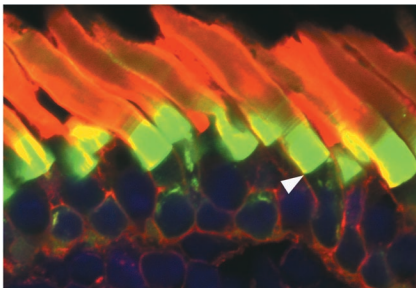
This Week in The Journal

● Cellular/Molecular

Targeting Proteins to the Outer Segment

Wenqin Luo, Nicholas Marsh-Armstrong, Amir Rattner, and Jeremy Nathans
(see pages 2623–2632)

Rods and cones are prototypic polarized cells with the protein machinery for phototransduction segregated in the outer segment (OS), whereas the basal side functions as a presynaptic nerve terminal. Maintaining this polarity in the face of rapid protein turnover in the OS requires an efficient sorting system. Rhodopsin is trafficked to the OS by a recently identified VXPX sequence at its C terminus. The importance of this sequence is underscored by human valine or proline mutations that cause one form of retinitis pigmentosa. Now Luo et al. report a similar localization signal, (V/I)XPX, in another OS protein, photoreceptor retinol dehydrogenase (prRDH). However prRDH, unlike rhodopsin, is mainly cytosolic; thus the authors looked to see how it might be retained in the OS. Using a series of green fluorescent protein (GFP) fusion proteins, they determined that fatty acylation of one or more cysteine residues in the C-terminal domain is necessary for membrane association and thus retention of the targeted protein in the OS.



The C-terminal segment from bovine photoreceptor retinol dehydrogenase, under the control of the zebrafish rhodopsin promoter, preferentially distributes to the basal region of the rod outer segment (GFP; green). The cell nuclei are stained dark blue, and the outer segments are stained red. See the article by Luo et al. for details.

▲ Development/Plasticity/Repair

Cortical Interneuron Development

Qing Xu, Inma Cobos, Estanislao De La Cruz, John L. Rubenstein, and Stewart A. Anderson
(see pages 2612–2622)

GABAergic interneurons can be subdivided based on their shape and connectivity as well as differential expression of markers such as parvalbumin, calretinin, and somatostatin. In this week's *Journal*, Xu et al. explore whether interneurons also originate from different sources. Many interneurons arise from the ventral telencephalon before migrating tangentially into the cortex. Thus the authors compared interneurons present in wild-type mice with those from mice deficient for the homeobox-containing genes *Nkx2.1*, in which the medial ganglionic eminence (MGE) does not form, or *Dlx1/2*^{−/−}, both of which have greatly reduced numbers of interneurons. *Nkx2.1*^{−/−} mice die at birth from lung defects. Thus the authors grew cultured neurons from the proliferative zones (PZ) of the subcortical telencephalon on cortical feeder layers and assayed interneuronal type by immunohistochemistry. Parvalbumin- and somatostatin-expressing interneurons were absent in *Nkx2.1*^{−/−} cells, whereas calretinin was nearly absent in *Dlx1/2*^{−/−} cells, consistent with distinct origins in the medial and caudal ganglionic eminence, respectively.

■ Behavioral/Systems/Cognitive

A Gut Feeling with CB1 and CCK

Galina Burdyga, Simon Lal, Andrea Varro, Rod Dimaline, David G. Thompson, and Graham J. Dockray
(see pages 2708–2715)

That cannabinoids cause an increase in appetite, the munchies, was appreciated long before endocannabinoids and their receptors, CB1 and CB2, were identified. This week, Burdyga et al. examine the role of the CB1 receptor as an enteric neuro-modulator. They show that CB1 receptors

are expressed by vagal sensory afferent neurons that carry appetitive signals between the gut and the brainstem. The same neurons expressed receptors for another appetite modulator, cholecystikinin (CCK) that acts as a gut satiety signal. CCK is released in the gut in response to fat or protein, and it now seems that it also actively regulates CB1 receptor expression. CB1 mRNA and protein were present at high levels in fasted rats when CCK levels were low. However, with refeeding or CCK delivery, CB1 was rapidly downregulated, suggesting that CCK may in part inhibit food intake in the peripheral nervous system by countering the action of endocannabinoids.

◆ Neurobiology of Disease

The Nuclear Envelope and Mutant TorsinA

Pedro Gonzalez-Alegre and Henry L. Paulson
(see pages 2593–2601)

DYT1, a disabling autosomal dominant form of dystonia, shares some common threads with other neurological disorders. Although the disease protein [TorsinA (TA)] and its mutation have been isolated, the dysfunction, and indeed the normal function, of the protein are unknown. TorsinA is a so-called AAA protein, which stands for ATPase associated with various cellular activities. Wild-type TA is distributed diffusely in the cytoplasm, consistent with endoplasmic reticulum (ER) localization, but mutant TA, due to a single glutamic acid deletion near the C terminus, is associated with intracellular membranous inclusions. Now Gonzalez-Alegre and Paulson report that the nuclear envelope rather than the ER may be the site of dysfunction in DYT1. To track the movements of mutant TA, the authors created an inducible PC12 cell model of the disorder. Mutant TA, but not wild-type TA, accumulated in the nuclear envelope, leading to formation of inclusions. These results add to several other studies implicating the nuclear envelope in neurological disorders.

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Cover picture: Enantioselective projection (output) neurons in the antennal (olfactory) lobe of the female moth *Manduca sexta*. Projection neurons with arborizations in the glomerulus depicted in green respond preferentially to (+)linalool, whereas those with arborizations in the glomerulus shown in red respond preferentially to (–)linalool. The cover shows a three-dimensional reconstruction of antennal lobe glomeruli and pictures of two projection neurons with opposite enantioselectivity. For details, see the article by Reisenman et al. in this issue (pages 2602–2611)

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Correction: In the article “Distinguishing between Directional Guidance and Motility Regulation in Neuronal Migration,” by Michael Ward, Corey McCann, Michael DeWulf, Jane Y. Wu, and Yi Rao, which appeared on pages 5170–5177 of the June 15, 2003 issue, it is incorrectly stated that mSlit1 was used in all experiments. The authors want to clarify that in fact, human Slit2 was used in all experiments.

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Inhibitory Control at a Synaptic Relay

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The mammalian medial nucleus of the trapezoid body (MNTB) harbors one of the most powerful terminals in the CNS, the calyx of Held. The mechanisms known to regulate this synaptic relay are relatively ineffective. Here, we report the presence of a remarkably robust and fast-acting glycinergic inhibitory system capable of suppressing calyceal transmission.

Evoked glycinergic IPSCs were relatively small in 2-week-old rats, an age by which calyceal maturation has reportedly neared completion. However, by postnatal day 25 (P25), glycinergic transmission had undergone a vigorous transformation, resulting in peak synaptic conductances as high as 280 nS. These are comparable with glutamatergic conductances activated by calyceal inputs. Decay kinetics for IPSCs were severalfold faster than for glycinergic synaptic events reported previously. At physiological temperatures in P25 rats, IPSCs decayed in ~1 msec and could be elicited at frequencies up to 500 Hz. Moreover, EPSPs triggered by glutamatergic signals derived from the calyx or simulated by conductance clamp were suppressed when preceded by simulated glycinergic IPSPs. The matching of excitatory transmission in the calyx of Held by a powerful, precision inhibitory system suggests that the relay function of the MNTB may be rapidly modified during sound localization.

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Constitutive Spinal Cyclooxygenase-2 Participates in the Initiation of Tissue Injury-Induced Hyperalgesia

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Inhibitors of the isozyme cyclooxygenase-2 (COX-2) represent an important advance in pain management, although where and when these inhibitors can exert their antihyperalgesic actions are not completely understood. Here we show that unlike many peripheral tissues in which COX-2 is only expressed in physiologically significant levels after tissue injury, in the normal rat lumbar spinal cord, the majority of neurons and radial glia constitutively express high levels of COX-2 protein. Immediately after peripheral tissue injury and before any measurable upregulation of COX-2 protein in peripheral tissue or spinal cord, inhibition of constitutively expressed spinal COX-2 reduced injury-induced activation of primary afferent neurons, activation of spinal neurons, and the mechanical and thermal hyperalgesia that normally occurs after peripheral tissue injury. The present data demonstrate that constitutively expressed spinal COX-2 plays an important role in the initial hyperalgesia that follows peripheral tissue injury. These results suggest that blocking constitutive spinal COX-2 before tissue injury may reduce the initial peripheral and central sensitization that occurs after tissue injury.

The Journal of Neuroscience, March 17, 2004 • 24(11):2727–2732

Anterograde Transneuronal Viral Tracing of Central Viscerosensory Pathways in Rats

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Previous studies demonstrated that strain H129 of herpes simplex virus-1 undergoes anterograde transneuronal transport in mice and primates after peripheral or central injection. In this study, H129 was used in rats to identify CNS regions that receive relayed viscerosensory inputs from the stomach wall. We also examined whether transneuronal viral transport in this model is exclusively anterograde. H129 or an established retrograde transneuronal viral tracer, pseudorabies virus (PRV), was injected into the ventral stomach wall in intact rats or in rats with previous subdiaphragmatic vagal sensory deafferentation. Rats were perfused with fixative 3–5 d later, and tissues were processed for immunocytochemical detection of transported virus. In intact rats, H129 was transported transneuronally via vagal and spinal viscerosensory neurons to postsynaptic target cells in the medullary dorsal vagal complex and thoracic dorsal horn, respectively, with subsequent transport to discrete regions of the medullary and pontine reticular formation, cerebellum, parabrachial nucleus, periaqueductal gray, thalamus, hypothalamus, amygdala, bed nucleus of the stria terminalis, and other central sites. Comparison of labeling patterns in intact and vagal deafferented rats indicated that H129 also produced first-order retrograde infection of autonomic neurons that project directly to virus injection sites, similar to PRV. Unlike PRV, however, H129 was not transported transneuronally in the retrograde direction from infected neurons to central sources of presynaptic input. We conclude that transneuronal transport of H129 occurs exclusively in the anterograde direction to reveal CNS regions that receive direct and relayed viscerosensory signals.

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Neural Stem Cells Show Bidirectional Experience-Dependent Plasticity in the Perinatal Mammalian Brain

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Many of the effects of prenatal stress on the endocrine function, brain morphology, and behavior in mammals can be reversed by brief sessions of postnatal separation and handling. We have tested the hypothesis that the effects of both the prenatal and postnatal experiences are mediated by negative and positive regulation of neural stem cell (NSC) number during critical stages in neurodevelopment. We used the *in vitro* clonal neurosphere assay to quantify NSCs in hamsters that had experienced prenatal stress (maternal restraint stress for 2 hr per day, for the last 7 d of gestation), postnatal handling (maternal–offspring separation for 15 min per day during postnatal days 1–21), or both. Prenatal stress reduced the number of NSCs derived from the subependyma of the lateral ventricle. The effect was already present at postnatal day 1 and persisted into adulthood (at least 14 months of age). Similarly, prenatal stress reduced *in vivo* proliferation in the adult subependyma of the lateral ventricle. Conversely, postnatal handling increased NSC number and reversed the effect of prenatal stress. The effects of prenatal stress on NSCs and proliferation and the effect of postnatal handling on NSCs did not differ between male and females. The findings demonstrate that environmental factors can produce changes in NSC number that are present at birth and endure into late adulthood. These changes may underlie some of the behavioral effects produced by prenatal stress and postnatal handling.

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Articles

CELLULAR/MOLECULAR

An Outer Segment Localization Signal at the C Terminus of the Photoreceptor-Specific Retinol Dehydrogenase

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Photoreceptor retinol dehydrogenase (prRDH) is a membrane-associated cytosolic protein that localizes to the outer segments (OS) of rods and cones. Here, we demonstrate that the C-terminal 16 amino acids of prRDH confer membrane association as well as cone and rod OS targeting on a linked green fluorescent protein. Membrane association in transfected 293 cells and in transgenic *Xenopus* photoreceptors is mediated by fatty acylation at one or more evolutionarily conserved cysteines within the prRDH C-terminal tail. In bovine OS, native prRDH is similarly acylated, and hydrolysis of this linkage releases the protein from the membrane. Efficient OS localization requires both membrane association and the prRDH sequence . . . (V/I)XPX at the extreme C terminus, which closely resembles the C-terminal sequence that targets opsin/rhodopsin to the OS. Taken together, these data imply that the C-terminal . . . (V/I)XPX sequence is a general OS localization signal that can function in the context of both integral and peripheral membrane proteins. This strategy for OS localization resembles those used for protein localization to mitochondria, peroxisomes, endosomes, and endoplasmic reticulum; in each case, a short N- or C-terminal sequence is shared among structurally diverse proteins that are targeted to the same subcellular destination.

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Vesicular Glutamate Transporter-Dependent Glutamate Release from Astrocytes

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Astrocytes exhibit excitability based on variations of their intracellular Ca²⁺ concentrations, which leads to glutamate release, that in turn can signal to adjacent neurons. This glutamate-mediated astrocyte–neuron signaling occurs at physiological intracellular Ca²⁺ levels in astrocytes and includes modulation of synaptic transmission. The mechanism underlying Ca²⁺-dependent glutamate release from astrocytes is most likely exocytosis, because astrocytes express the protein components of the soluble N-ethyl maleimide-sensitive fusion protein attachment protein receptors complex, including synaptobrevin 2, syntaxin, and synaptosome-associated protein of 23 kDa. Although these proteins mediate Ca²⁺-dependent glutamate release from astrocytes, it is not well understood whether astrocytes express functional vesicular glutamate transporters (VGLUTs) that are critical for vesicle refilling. Here, we find in cultured and freshly isolated astrocytes the presence of brain-specific Na⁺-dependent inorganic phosphate cotransporter and differentiation-associated Na⁺-dependent inorganic phosphate cotransporter that have recently been identified as VGLUTs 1 and 2. Indirect immunocytochemistry showed a punctate pattern of VGLUT immunoreactivity throughout the entire cell body and processes, whereas pharmacological inhibition of VGLUTs abolished mechanically and agonist-evoked Ca²⁺-dependent glutamate release from astrocytes. Taken together, these data indicate that VGLUTs play a functional role in exocytotic glutamate release from astrocytes.

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Metabotropic Glutamate Receptor Activation Regulates Fragile X Mental Retardation Protein and *Fmr1* mRNA Localization Differentially in Dendrites and at Synapses

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Fragile X syndrome is caused by the absence of the mRNA-binding protein Fragile X mental retardation protein (FMRP), which may play a role in activity-regulated localization and translation of mRNA in dendrites and at synapses. We investigated whether neuronal activity and glutamatergic signals regulate trafficking of FMRP and its encoding *Fmr1* mRNA into dendrites or at synapses. Using high-resolution fluorescence and digital imaging microscopy in cultured hippocampal neurons, FMRP and *Fmr1* mRNA were localized in granules throughout dendrites and within spines. KCl depolarization rapidly increased FMRP and *Fmr1* mRNA levels in dendrites. Metabotropic glutamate receptor (mGluR) activation, in particular mGluR5 activation, was necessary for localization of FMRP into dendrites. Blockade of either PKC or internal calcium prevented mGluR-dependent localization of both FMRP and *Fmr1* mRNA in dendrites. The activity-dependent localization of FMRP was not dependent on protein synthesis. Fluorescence recovery after photobleaching analysis of live neurons transfected with enhanced green fluorescent protein–FMRP revealed increased granule trafficking in response to KCl depolarization. In contrast to its dendritic localization, mGluR activation diminished FMRP, but not *Fmr1* mRNA, localization at synapses. These results demonstrate regulation of FMRP and *Fmr1* mRNA trafficking in dendrites and synapses in response to specific glutamatergic signals.

The Journal of Neuroscience, March 17, 2004 • 24(11):2648–2655

Electroconvulsive Seizures Regulate Gene Expression of Distinct Neurotrophic Signaling Pathways

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Electroconvulsive therapy (ECT) remains the treatment of choice for drug-resistant patients with depressive disorders, yet the mechanism for its efficacy remains unknown. Gene transcription changes were measured in the frontal cortex and hippocampus of rats subjected to sham seizures or to 1 or 10 electroconvulsive seizures (ECS), a model of ECT. Among the 3500–4400 RNA sequences detected in each sample, ECS increased by 1.5- to 11-fold or decreased by at least 34% the expression of 120 unique genes. The hippocampus produced more than three times the number of gene changes seen in the cortex, and many hippocampal gene changes persisted with chronic ECS, unlike in the cortex. Among the 120 genes, 77 have not been reported in previous studies of ECS or seizure responses, and 39 were confirmed among 59 studied by quantitative real time PCR. Another 19 genes, 10 previously unreported, changed by <1.5-fold but with very high significance. Multiple genes were identified within distinct pathways, including the BDNF–MAP kinase–cAMP–cAMP response element-binding protein pathway (15 genes), the arachidonic acid pathway (5 genes), and more than 10 genes in each of the immediate-early gene, neurogenesis, and exercise response gene groups. Neurogenesis, neurite outgrowth, and neuronal plasticity associated with BDNF, glutamate, and cAMP–protein kinase A signaling pathways may mediate the antidepressant effects of ECT in humans. These genes, and others that increase only with chronic ECS such as neuropeptide Y and thyrotropin-releasing hormone, may provide novel ways to select drugs for the treatment of depression and mimic the rapid effectiveness of ECT.

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The Orexin/Hypocretin System in Zebrafish Is Connected to the Aminergic and Cholinergic Systems

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The orexin/hypocretin (ORX) system is involved in physiological processes such as feeding, energy metabolism, and the control of sleep and wakefulness. The ORX system may drive the aminergic and cholinergic activities that control sleep and wakefulness states because of the ORX fiber projections to the aminergic and cholinergic cell clusters. The biological mechanisms and relevance of the interactions between these neurotransmitter systems are poorly understood. We studied these systems in zebrafish, a model organism in which it is possible to simultaneously study these systems and their interactions.

We cloned a zebrafish prepro-ORX gene that encodes for the two functional neuropeptides orexin-A (ORX-A) and orexin-B (ORX-B). The prepro-ORX gene of the zebrafish consisted of one exon in contrast to mammals. The sequence of the ORX-A peptide of the zebrafish was less conserved than the ORX-B peptide compared with other vertebrates. By using *in situ* hybridization and immunohistochemistry, we found that the organization of the ORX system of zebrafish was similar to the ORX system in mammals, including a hypothalamic cell cluster and widespread fiber projections. The ORX system of the zebrafish showed a unique characteristic with an additional putatively ORX-containing cell group. The ORX system innervated several aminergic nuclei, raphe, locus ceruleus, the mesopontine-like area, dopaminergic clusters, and histaminergic neurons. A reciprocal relationship was found between the ORX system and several aminergic systems. Our results suggest that the architecture of these neurotransmitter systems is conserved in vertebrates and that these neurotransmitter systems in zebrafish may be involved in regulation of states of wakefulness and energy homeostasis by similar mechanisms as those in mammals.

The Journal of Neuroscience, March 17, 2004 • 24(11):2678–2689

A Nonsense Mutation of the Sodium Channel Gene *SCN2A* in a Patient with Intractable Epilepsy and Mental Decline

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Mutations, exclusively missense, of voltage-gated sodium channel α subunit type 1 (*SCN1A*) and type 2 (*SCN2A*) genes were reported in patients with idiopathic epilepsy: generalized epilepsy with febrile seizures plus. Nonsense and frameshift mutations of *SCN1A*, by contrast, were identified in intractable epilepsy: severe myoclonic epilepsy in infancy (SMEI). Here we describe a first nonsense mutation of *SCN2A* in a patient with intractable epilepsy and severe mental decline. The phenotype is similar to SMEI but distinct because of partial epilepsy, delayed onset (1 year 7 months), and absence of temperature sensitivity. A mutational analysis revealed that the patient had a heterozygous *de novo* nonsense mutation R102X of *SCN2A*. Patch-clamp analysis of Na_v1.2 wild-type channels and the R102X mutant protein coexpressed in human embryonic kidney 293 cells showed that the truncated mutant protein shifted the voltage dependence of inactivation of wild-type channels in the hyperpolarizing direction. Analysis of the subcellular localization of R102X truncated protein suggested that its dominant negative effect could arise from direct or indirect cytoskeletal interactions of the mutant protein. Haploinsufficiency of Na_v1.2 protein is one plausible explanation for the pathology of this patient; however, our biophysical findings suggest that the R102X truncated protein exerts a dominant negative effect leading to the patient's intractable epilepsy.

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Carbonic Anhydrase Isoform VII Acts as a Molecular Switch in the Development of Synchronous Gamma-Frequency Firing of Hippocampal CA1 Pyramidal Cells

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Identification of the molecular mechanisms that enable synchronous firing of CA1 pyramidal neurons is central to the understanding of the functional properties of this major hippocampal output pathway. Using microfluorescence measurements of intraneuronal pH, *in situ* hybridization, as well as intracellular, extracellular, and K⁺-sensitive microelectrode recordings, we show now that the capability for synchronous gamma-frequency (20–80 Hz) firing in response to high-frequency stimulation (HFS) emerges abruptly in the rat hippocampus at approximately postnatal day 12. This was attributable to a steep developmental upregulation of intrapyramidal carbonic anhydrase isoform VII, which acts as a key molecule in the generation of HFS-induced tonic GABAergic excitation. These results point to a crucial role for the developmental expression of intrapyramidal carbonic anhydrase VII activity in shaping integrative functions, long-term plasticity and susceptibility to epileptogenesis.

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An Arginine Involved in GABA Binding and Unbinding But Not Gating of the GABA_A Receptor

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GABA_A receptor function can be conceptually divided into interactions between ligand and receptor (binding) and the opening and closing of the ligand-bound channel (gating). The relationship between binding, gating, and receptor structure remains unclear. Studies of mutations have identified many amino acid residues that contribute to the GABA binding site. Most of these studies assayed changes in GABA dose–response curves, which are macroscopic measures that depend on the interplay of many processes and cannot resolve individual microscopic transitions. Understanding the microscopic basis of binding and gating is critical, because kinetic transition rates predict how receptors will behave at synapses. Furthermore, microscopic rates are directly related to the molecular interactions underlying receptor function.

Here, we focused on a residue (β_2 -R207) previously identified as lining the GABA-binding site that, when mutated to cysteine, greatly reduces apparent GABA affinity and was predicted to affect both binding and gating. To better understand the role of β_2 -R207, we expressed $\alpha_1\beta_2$ and $\alpha_1\beta_2$ -R207C receptors in human embryonic kidney 293 cells and studied receptor kinetics using fast solution applications. The mutation accelerated deactivation by 10-fold, without altering desensitization in the presence of saturating GABA. Maximum open probability and single-channel open times were also unaltered by the mutation, but the GABA-binding rate was reduced eightfold. Therefore, the effects of this mutation in a predicted binding site residue are solely attributable to changes in GABA-binding and unbinding kinetics, with no changes in channel gating. Because β_2 -R207 stabilizes GABA in the binding pocket, it may directly contact the GABA molecule.

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A Novel p75 Neurotrophin Receptor-Related Protein, NRH2, Regulates Nerve Growth Factor Binding to the TrkA Receptor

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Nerve growth factor (NGF) functions as a ligand for two receptors, the TrkA tyrosine kinase receptor and the p75 neurotrophin receptor (p75^{NTR}). The Ig-like domains of Trk receptors and the cysteine-rich repeats of p75^{NTR} are involved in binding to the neurotrophins. Recently, a closely related gene to p75^{NTR} called neurotrophin receptor homolog-2 (NRH2) was identified; however, the function of NRH2 and its relevance to neurotrophin signaling are unclear. NRH2 contains a similar transmembrane and intracellular domain as p75^{NTR} but lacks the characteristic cysteine-rich repeats in the extracellular domain. Here we show that NRH2 is expressed in several neuronal populations that also express p75^{NTR} and Trk receptors. NRH2 does not bind to NGF; however, coimmunoprecipitation experiments demonstrate that NRH2 is capable of interacting with TrkA receptors. Coexpression of NRH2 with TrkA receptors resulted in the formation of high-affinity binding sites for NGF. These results indicate that a transmembrane protein related to p75^{NTR} is capable of modulating Trk receptor binding properties.

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Ischemic Preconditioning: Neuronal Survival in the Face of Caspase-3 Activation

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Apoptosis is an evolutionarily conserved process critical to tissue development and tissue homeostasis in eukaryotic organisms and, when dysregulated, causes inappropriate cell death. Global ischemia is a neuronal insult that induces delayed cell death with many features of apoptosis. Ischemic preconditioning affords robust protection of CA1 neurons against a subsequent severe ischemic challenge. The molecular mechanisms underlying ischemic tolerance are unclear. Here we show that ischemia induces pronounced caspase-3 activity in naive neurons that die and in preconditioned neurons that survive. Preconditioning intervenes downstream of proteolytic processing and activation of caspase-3 (a protease implicated in the execution of apoptosis) and upstream of the caspase-3 target caspase-activated DNase (CAD, a deoxyribonuclease that catalyzes DNA fragmentation) to arrest neuronal death. We further show that global ischemia promotes expression of the pro-survival inhibitor-of-apoptosis (IAP) family member cIAP, but unleashes Smac/DIABLO (second mitochondria-derived activator of caspases/direct IAP-binding protein with low pI), a factor that neutralizes the protective actions of IAPs and promotes neuronal death. Preconditioning blocks the mitochondrial release of Smac/DIABLO, but not the ischemia-induced upregulation of IAPs. In the absence of Smac/DIABLO, cIAP halts the caspase death cascade and arrests neuronal death. These findings suggest that preconditioning preserves the integrity of the mitochondrial membrane, enabling neurons to survive in the face of caspase activation.

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Presynaptic NMDA Receptors Modulate Glutamate Release from Primary Sensory Neurons in Rat Spinal Cord Dorsal Horn

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NMDA receptors have the potential to produce complex activity-dependent regulation of transmitter release when localized presynaptically. In the somatosensory system, NMDA receptors have been immunocytochemically detected on presynaptic terminals of primary afferents, and these have been proposed to drive release of substance P from central terminals of a subset of nociceptors in the spinal cord dorsal horn. Here we report that functional NMDA receptors are indeed present at or near the central terminals of primary afferent fibers. Furthermore, we show that activation of these presynaptic receptors results in an inhibition of glutamate release from the terminals. Some of these NMDA receptors may be expressed in the preterminal axon and regulate the extent to which action potentials invade the extensive central arborizations of primary sensory neurons.

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Interleukin-1 β Induces a Reactive Astroglial Phenotype via Deactivation of the Rho GTPase–Rock Axis

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The cytokine interleukin-1 β (IL-1 β) is critical to the formation of an astrocytic scar after CNS injury, but the mechanisms by which it induces a reactive phenotype remain unresolved. Here, we show that IL-1 β regulates the phenotype of astrocytes via deactivation of the Rho GTPase–Rho kinase (ROCK) pathway, which governs cellular morphology and migration via effects on F-actin and its interactions with focal adhesions, nonmuscle myosin, and microvillar adapter proteins of the ezrin–radixin–moesin (ERM) family. We found that IL-1 β induced cortical reorganization of F-actin and dephosphorylation of focal adhesion kinase, myosin light chain 2, and myosin phosphatase targeting subunit 1 in primary human astrocytes, and that all of these effects were mimicked by Rho–ROCK pathway blockade. We also found that IL-1 β conversely potentiated ERM phosphorylation, and that this effect was mediated via a Rho–ROCK-independent mechanism. Next, we used a rhotekin pulldown assay to confirm directly that IL-1 β deactivates Rho, and further demonstrated that a constitutively active Rho construct rescued astrocytes from developing an IL-1 β -induced reactive phenotype. These data implicate cytokine regulation of the Rho–ROCK pathway in the generation of a reactive astrogliosis, and we suggest that interventions targeted at this level may facilitate manipulation of the glial scar in inflammatory disorders of the human CNS.

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DEVELOPMENT/PLASTICITY/REPAIR

Origins of Cortical Interneuron Subtypes

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Cerebral cortical functions are conducted by two general classes of neurons: glutamatergic projection neurons and GABAergic interneurons. Distinct interneuron subtypes serve distinct roles in modulating cortical activity and can be differentially affected in cortical diseases, but little is known about the mechanisms for generating their diversity. Recent evidence suggests that many cortical interneurons originate within the subcortical telencephalon and then migrate tangentially into the overlying cortex. To test the hypothesis that distinct interneuron subtypes are derived from distinct telencephalic subdivisions, we have used an *in vitro* assay to assess the developmental potential of subregions of the telencephalic proliferative zone (PZ) to give rise to neurochemically defined interneuron subgroups. PZ cells from GFP⁺ donor mouse embryos were transplanted onto neonatal cortical feeder cells and assessed for their ability to generate specific interneuron subtypes. Our results suggest that the parvalbumin- and the somatostatin-expressing interneuron subgroups originate primarily within the medial ganglionic eminence (MGE) of the subcortical telencephalon, whereas the calretinin-expressing interneurons appear to derive mainly from the caudal ganglionic eminence (CGE). These results are supported by findings from primary cultures of cortex from *Nkx2.1* mutants, in which normal MGE fails to form but in which the CGE is less affected. In these cultures, parvalbumin- and somatostatin-expressing cells are absent, although calretinin-expressing interneurons are present. Interestingly, calretinin-expressing bipolar interneurons were nearly absent from cortical cultures of *Dlx1/2* mutants. By establishing spatial differences in the origins of interneuron subtypes, these studies lay the groundwork for elucidating the molecular bases for their distinct differentiation pathways.

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Formation of Projection Pathways from the Arcuate Nucleus of the Hypothalamus to Hypothalamic Regions Implicated in the Neural Control of Feeding Behavior in Mice

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The arcuate nucleus of the hypothalamus (ARH) is a critical component of forebrain pathways that regulate a variety of neuroendocrine functions, including an important role in relaying leptin signals to other parts of the hypothalamus. However, neonatal rodents do not lose weight in response to leptin treatment in the same way as do adults, suggesting that certain aspects of leptin signaling pathways in the hypothalamus may not be mature. We tested this possibility by using DiI axonal labeling to examine the development of projections from the ARH to other parts of the hypothalamus in neonatal mice, paying particular attention to the innervation of the paraventricular nucleus (PVH), the dorsomedial nucleus (DMH), and the lateral hypothalamic area (LHA), each of which have been implicated in the regulation of feeding. The results indicate that ARH projections are quite immature at birth and appear to innervate the DMH, PVH, and LHA in succession, within distinct temporal domains. The projections from the ARH to the DMH develop rapidly and are established by the sixth postnatal day (P6), whereas those to the PVH develop significantly later, with the mature pattern of innervation first apparent between postnatal day 8 (P8)–P10. Furthermore, the ability of leptin to activate Fos in the PVH, DMH, and LHA appears to be age-dependent and correlates with the arrival of ARH projections to each nucleus. Taken together, these findings provide new insight into development of hypothalamic circuits and suggest an anatomical basis for the delayed postnatal regulation of food intake and body weight by leptin.

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Enantioselectivity of Projection Neurons Innervating Identified Olfactory Glomeruli

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Projection neurons (PNs) with arborizations in the sexually dimorphic “lateral large female glomerulus” (latLFG) in the antennal lobe (AL) of the moth *Manduca sexta* previously were shown to respond preferentially to antennal stimulation with ($\pm$)linalool, a volatile compound commonly emitted by plants. In the present study, using intracellular recording and staining techniques, we examined the responsiveness of latLFG-PNs to the enantiomers, (+)linalool and (–)linalool and found that (1) latLFG-PNs are more responsive to antennal stimulation with (+)linalool than with (–)linalool, (2) PNs with arborizations in a glomerulus adjacent to the latLFG are preferentially responsive to (–)linalool, and (3) PNs with arborizations confined to other glomeruli near the latLFG are equally responsive to both enantiomers of linalool. Structure-activity studies showed that the hydroxyl group in this tertiary terpene alcohol is the key feature of the molecule determining the response of enantioselective PNs to linalool. In contrast, the responses of non-enantioselective PNs are less dependent on the alcoholic functionality of linalool. Our findings show that PNs innervating a uniquely identifiable glomerulus respond preferentially to a particular enantiomer of an odor substance. Moreover, PNs with arborizations in a glomerulus adjacent to the latLFG, although less sensitive than latLFG-PNs to linalool, respond preferentially to the opposite enantiomer, demonstrating that information about stimulus-absolute configuration can be encoded in different olfactory glomeruli.

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Changes in Excitability of Ascending and Descending Inputs to Cerebellar Climbing Fibers during Locomotion

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The inferior olive climbing fiber projection plays a central role in all major theories of cerebellar function. Therefore, mechanisms that control the ability of climbing fibers to forward information to the cerebellum are of considerable interest. We examined changes in transmission in cerebro-olivocerebellar pathways (COCs) and spino-olivocerebellar pathways (SOCs) during locomotion in awake cats ($n = 4$) using low-intensity electrical stimuli delivered to the contralateral cerebral peduncle or the ipsilateral superficial radial nerve to set up volleys in COCs and SOCs, respectively. The responses were recorded as evoked extracellular climbing fiber field potentials within the C1 or C3 zones in the paravermal cerebellar cortex (lobule Va–Vc). At most C1 and C3 zone sites, the largest COC responses occurred during the stance phase, and the smallest responses occurred during the swing phase of the ipsilateral forelimb step cycle. In marked contrast, SOC responses recorded at the same sites were usually largest during the swing phase and smallest during the stance phase. Because substantial climbing fiber responses could be evoked in all phases of the step cycle, the results imply that olivary neurons remain excitable throughout, and that the differences between SOCs and COCs in their pattern of step-related modulation are unlikely to have arisen solely through inhibition at the level of the inferior olive (e.g., by activity in the inhibitory cerebellar nucleo-olivary pathway). The different patterns of modulation also suggest that climbing fiber signals conveyed by COCs and SOCs are likely to affect information processing within the cerebellar cortical C1 and C3 zones at different times during locomotion.

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Expression of Cannabinoid CB1 Receptors by Vagal Afferent Neurons Is Inhibited by Cholecystokinin

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Both inhibitory (satiety) and stimulatory (orexigenic) factors from the gastrointestinal tract regulate food intake. In the case of the satiety hormone cholecystokinin (CCK), these effects are mediated via vagal afferent neurons. We now report that vagal afferent neurons expressing the CCK-1 receptor also express cannabinoid CB1 receptors. Retrograde tracing established that these neurons project to the stomach and duodenum. The expression of CB1 receptors determined by RT-PCR, immunohistochemistry and *in situ* hybridization in rat nodose ganglia was increased by withdrawal of food for ≥ 12 hr. After refeeding of fasted rats there was a rapid loss of CB1 receptor expression identified by immunohistochemistry and *in situ* hybridization. These effects were blocked by administration of the CCK-1 receptor antagonist lorglumide and mimicked by administration of CCK to fasted rats. Because CCK is a satiety factor that acts via the vagus nerve and CB1 agonists stimulate food intake, the data suggest a new mechanism modulating the effect on food intake of satiety signals from the gastrointestinal tract.

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Evidence for Gaze Feedback to the Cat Superior Colliculus: Discharges Reflect Gaze Trajectory Perturbations

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Rapid coordinated eye–head movements, called saccadic gaze shifts, displace the line of sight from one location to another. A critical structure in the gaze control circuitry is the superior colliculus (SC) of the midbrain, which drives gaze saccades by relaying cortical commands to brainstem eye and head motor circuits. We proposed that the SC lies within a gaze feedback loop and generates an error signal specifying gaze position error (GPE), the distance between target and current gaze positions. We investigated this feedback hypothesis in cats by briefly stopping head motion during large ($\approx 50^\circ$) gaze saccades made in the dark. This maneuver interrupted intended gaze saccades and briefly immobilized gaze (a plateau). After brake release, a corrective gaze saccade brought the gaze on goal. In the caudal SC, the firing frequency of a cell gradually increased to a maximum that just preceded the optimal gaze saccade encoded by the position of the cell and then declined back to zero near gaze saccade end. In brake trials, the activity level just preceding a brake-induced plateau continued steadily during the plateau and waned to zero only near the end of the corrective saccade. The duration of neural activity was stretched to reflect the increased time to target acquisition, and firing frequency during a plateau was proportional to the GPE of the plateau. In comparison, in the rostral SC, the duration of saccade-related pauses in fixation cell activity increased as plateau duration increased. The data show that the cat's SC lies in a gaze feedback loop and that it encodes GPE.

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Neurochemical and Behavioral Alterations in Glucocorticoid Receptor-Impaired Transgenic Mice after Chronic Mild Stress

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Mice (GR-i) bearing a transgene encoding a glucocorticoid receptor (GR) antisense RNA under the control of a neuron-specific neurofilament promoter were used to investigate the effects of a 4 week chronic mild stress (CMS) on the hypothalamo–pituitary–adrenocortical (HPA) axis and the serotonergic system in a transgenic model of vulnerability to affective disorders. GR-i mice showed a decrease in both GR-specific binding (hippocampus and cerebral cortex) and GR mRNA levels [hippocampus, cerebral cortex, and dorsal raphe nucleus (DRN)] as well as a deficit in HPA axis feedback control (dexamethasone test) compared with paired wild-type (WT) mice. In the latter animals, CMS exposure caused a significant decrease in both GR mRNA levels and the density of cytosolic GR binding sites in the hippocampus, whereas, in the DRN, GR mRNA levels tended to increase. In contrast, in stressed GR-i mice, both GR mRNA levels and the density of GR binding sites were significantly increased in the hippocampus, cerebral cortex, and DRN. Electrophysiological recordings in brainstem slices and [γ -³⁵S]GTP-S binding measurements to assess 5-HT_{1A} receptor functioning showed that CMS exposure produced a desensitization of DRN 5-HT_{1A} autoreceptors in WT, but not in GR-i, mice. In addition, CMS was found to facilitate choice behavior of WT, but not GR-i, mice in a decision-making task derived from an alternation paradigm. These results demonstrate that impaired GR functioning affects normal adaptive responses of the HPA axis and 5-HT system to CMS and alters stress-related consequences on decision-making behaviors.

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Spinal G-Protein-Gated K⁺ Channels Formed by GIRK1 and GIRK2 Subunits Modulate Thermal Nociception and Contribute to Morphine Analgesia

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G-protein-gated potassium (K⁺) channels are found throughout the CNS in which they contribute to the inhibitory effects of neurotransmitters and drugs of abuse. Recent studies have implicated G-protein-gated K⁺ channels in thermal nociception and the analgesic action of morphine and other agents. Because nociception is subject to complex spinal and supraspinal modulation, however, the relevant locations of G-protein-gated K⁺ channels are unknown. In this study, we sought to clarify the expression pattern and subunit composition of G-protein-gated K⁺ channels in the spinal cord and to assess directly their contribution to thermal nociception and morphine analgesia. We detected GIRK1 (G-protein-gated inwardly rectifying K⁺ channel subunit 1) and GIRK2 subunits, but not GIRK3, in the superficial layers of the dorsal horn. Lack of either GIRK1 or GIRK2 was correlated with significantly lower expression of the other, suggesting that a functional and physical interaction occurs between these two subunits. Consistent with these findings, GIRK1 knock-out and GIRK2 knock-out mice exhibited hyperalgesia in the tail-flick test of thermal nociception. Furthermore, GIRK1 knock-out and GIRK2 knock-out mice displayed decreased analgesic responses after the spinal administration of higher morphine doses, whereas responses to lower morphine doses were preserved. Qualitatively similar data were obtained with wild-type mice after administration of the G-protein-gated K⁺ channel blocker

tertiapin. We conclude that spinal G-protein-gated K^+ channels consisting primarily of GIRK1/GIRK2 complexes modulate thermal nociception and mediate a significant component of the analgesia evoked by intrathecal administration of high morphine doses.

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Nitric Oxide Inhibits the Rate and Strength of Cardiac Contractions in the Lobster *Homarus americanus* by Acting on the Cardiac Ganglion

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The lobster heart is synaptically driven by the cardiac ganglion, a spontaneously bursting neural network residing within the cardiac lumen. Here, we present evidence that nitric oxide (NO) plays an inhibitory role in lobster cardiac physiology. (1) NO decreases heartbeat frequency and amplitude. Decreased frequency is a direct consequence of a decreased ganglionic burst rate. Decreased amplitude is an indirect consequence of decreased burst frequency, attributable to the highly facilitating nature of the synapses between cardiac ganglion neurons and muscle fibers (although, during prolonged exposure to NO, amplitude recovers to the original level by a frequency-independent adaptation mechanism). NO does not alter burst duration, spikes per burst, heart muscle contractility, or amplitudes of synaptic potentials evoked by stimulating postganglionic motor nerves. Thus, NO acts on the ganglion, but not on heart muscle. (2) Two observations suggest that NO is produced within the lobster heart. First, immunoblot analysis shows that nitric oxide synthase (NOS) is strongly expressed in heart muscle relative to other muscles. Second, L-nitroarginine (L-NA), an NOS inhibitor, increases the rate of the heartbeat (opposite to the effects of NO). In contrast, the isolated ganglion is insensitive to L-NA, suggesting that heart muscle (but not the ganglion) produces endogenous NO. Basal heart rate varies from animal to animal, and L-NA has the greatest effect on the slowest hearts, presumably because these hearts are producing the most NO. Thus, because the musculature is a site of NOS expression, whereas the ganglion is the only intracardiac target of NO, we hypothesize that NO serves as an inhibitory retrograde transmitter.

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Dopamine Release in Response to a Psychological Stress in Humans and Its Relationship to Early Life Maternal Care: A Positron Emission Tomography Study Using [¹¹C]Raclopride

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Mesolimbic dopamine is thought to play a role in the processing of rewards. However, animal studies also demonstrate dopamine release in response to aversive stressful stimuli. Also, in animal studies, disruptions of the mother–infant relationship have been shown to have long-lasting effects on the mesolimbic dopamine system and the hypothalamic–pituitary–adrenal axis. We therefore investigated dopamine release in response to stress in human subjects, considering the relationship to early life parental care. We screened 120 healthy young college students for parental care in early life using a combination of telephone interviews and questionnaires. Five students from the top end and five students from the bottom end of the parental care distribution were then invited for a positron emission tomography study using [¹¹C]raclopride and a psychosocial stress task. The psychosocial stressor caused a significant release of dopamine in the ventral striatum as indicated by a reduction in [¹¹C]raclopride binding potential in the stress versus resting condition in subjects reporting low parental care. Moreover, the magnitude of the salivary cortisol response to stress was significantly correlated with the reduction in [¹¹C]raclopride binding in the ventral striatum ($r = 0.78$), consistent with a facilitating effect of cortisol on dopamine neuron firing. These data suggest that aversive stressful events can be associated with mesolimbic dopamine release in humans, and that the method presented here may be useful to study the effects of early life events on neurobiological stress systems.

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Organic Cation Transporter 3 (Slc22a3) Is Implicated in Salt-Intake Regulation

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Organic cation transporters (OCTs) are carrier-type permeases known to participate in general detoxification functions in peripheral tissues. Previous *in vitro* studies have suggested that OCTs ensure Uptake₂, a low-affinity, corticosteroid-sensitive catecholamine removal system, which was characterized initially in sympathetically innervated tissues. Although the presence of both Uptake₂-like transport and most OCT subtypes has also been demonstrated in the brain, the physiological role of this family of transporters in CNS remained totally unknown. In the present work, we show that the OCT3 transporter is found throughout the brain and highly expressed in regions regulating fluid exchange, including circumventricular organs such as area postrema and subfornical organ (SFO), and in other structures implicated in the sensing of changes in blood osmolarity and regulation of salt and water ingestion. OCT3/Slc22a3-deficient mice show an increase in the level of ingestion of hypertonic saline under thirst and salt appetite conditions, as well as alterations of the neural response in the SFO after sodium deprivation, as monitored by Fos immunoreactivity. This work demonstrates that the presence of OCT3 is critical for the balanced neural and behavioral responses to environmentally induced variations in osmolarity and provides for the first time physiological evidence of the importance of OCTs for CNS function.

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Aberrant Cellular Behavior of Mutant TorsinA Implicates Nuclear Envelope Dysfunction in DYT1 Dystonia

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Torsion dystonia-1 (DYT1) dystonia, the most common inherited form of dystonia, is caused by a three base pair deletion that eliminates a single amino acid from the disease protein, torsinA. TorsinA is an "AAA" protein thought to reside in the endoplasmic reticulum (ER), yet both its cellular function and the basis for neuronal dysfunction in DYT1 remain unknown. A clue to disease pathogenesis is the fact that mutant, but not wild-type, torsinA forms membranous inclusions in cell culture. To explore the pathobiology of DYT1 dystonia, we generated PC12 neural cell lines that inducibly express wild-type or mutant torsinA. Although in this model torsinA displays some properties consistent with ER localization, mutant torsinA also accumulates in the nuclear envelope (NE), a structure contiguous with cytoplasmic ER. Consistent with this, membranous inclusions formed by mutant torsinA are shown to derive not from the ER, as thought previously, but from the NE. We demonstrate further that torsinA forms different disulfide-linked complexes that may be linked functionally to subcellular localization in the NE versus cytoplasmic ER. Despite mutant TA accumulation in NE structures, nucleocytoplasmic transport of a reporter protein was unaffected. These findings, together with parallel studies failing to demonstrate perturbation of ER function, implicate the NE as a primary site of dysfunction in DYT1. DYT1 dystonia can be added to the growing list of inherited neurological disorders involving the NE.

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3' Untranslated Region in a Light Neurofilament (NF-L) mRNA Triggers Aggregation of NF-L and Mutant Superoxide Dismutase 1 Proteins in Neuronal Cells

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The pathogenesis of neurodegenerative diseases is believed to involve abnormal aggregation of proteins, but the mechanisms initiating protein aggregation are unclear. Here we report a novel phenomenon that could be instrumental in triggering protein aggregation in neurodegenerative diseases. We show that the 3' untranslated region (3' UTR) of a light neurofilament (NF-L) transcript enhances the reactivity of its own translated product and leads to loss of solubility and aggregation of NF-L protein and to coaggregation of mutant superoxide dismutase 1 (SOD1) protein. Full-length mouse NF-L cDNAs, with and without NF-L 3' UTR, were fused to the C terminus of a green fluorescent protein (GFP) reporter gene, and the GFP-tagged NF-L proteins were examined in transfected Neuro2a cells. The GFP-tagged NF-L protein expressed from the transgene containing NF-L 3' UTR, but not from the transgene lacking NF-L 3' UTR, colocalizes with endogenous heavy neurofilament protein and, at high-level expression, leads to loss of solubility and aggregation of GFP-tagged NF-L protein. Aggregation of GFP-tagged NF-L protein triggers coaggregation and loss of solubility of coexpressed DsRed-tagged mutant (G93A) SOD1 protein but not wild-type SOD1 protein. Deletional mutagenesis maps the RNA sequence causing aggregation of GFP-tagged NF-L protein to the proximal 45 nucleotides of NF-L 3' UTR. This is the site of a major destabilizing element in NF-L RNA and binding site for RNA-binding proteins. Our findings support a working model whereby NF-L RNA, or cognate RNA-binding factors, enhances the reactivity of NF-L protein and provides a triggering mechanism leading to aggregation of NF-L and other proteins in neurodegenerative diseases.

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